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Original article

The significance of the degradation products of rapeseed meal proteins in the rumen according to different meal processing techniques

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Abstract – The extent to which rumen soluble nitrogen can contribute to the intestinal protein flow is unknown. Therefore, a study was carried out to assess simultaneously the kinetics of: 1) protein disappearance from rumen bags; 2) the amount of various N products in the rumen fluid contents (total nitrogen (Nt), ammonia N (NH3-N), non-ammonia nitrogen (NAN), true protein); and 3) electrophoretical characteristics of the protein in feeds, bag residues and ruminal fluids. Measurements were made on four sheep fed successively with five diets: hay alone (basal) or hay plus one of four rapeseed oilmeals (60% hay, 40% meals). Oilmeal batches originated from different heating and solvent extraction processes: control at 60 °C (T60°), commercial (TC), and experimental at 90 °C (T90°) or 130 °C(T130°). They differed in their effective protein degradability as assessed by an in situ method which gave results of 0.83, 0.69, 0.39, 0.42 respectively for T60°, TC, T90° and T130°. In the rumen fluid, the Nt and NAN contents peaked 1 h after feeding and then decreased rapidly (for the 7 h post-feeding). The NAN peak level was generally higher for highly degradable oilmeals (0.56 mg/g and 0.36 mg/g respectively in diets containing T60° and TC) than for low-degradable oilmeals (0.15 mg/g and 0.23 mg/g) in diets containing T90° and T130°. NH3-N was fairly high (0.3 mg/g) whatever the oilmeal, due to the high CP content of the diet (21%) and showed only small variations in the post-feeding hours. At peak time, the NAN/Nt ratios in rumen fluid were 0.61, 0.52, 0.30 and 0.42 mg/g respectively in diets containing T60°, TC, T90° and T130°. The true protein-N was roughly the same and low whatever the time, for the diets containing T90° and T130°, but true-protein-N was 0.30 mg/g 1 h after feeding for T60° and TC. Using electrophoresis (SDS-PAGE), feed proteins had similar characteristics among oilmeals, though extraction yields differed widely: 70%, 52%, 21% and 19% respectively for T60°, TC, T90° and T130°. The main proteins contained in the oilmeals are 2S and 12S, which were degraded in nylon bags in the rumen at various degradation rates. Similar proteins were found in the bag residues for various times apparently related to degradability: up to 8 h, 16 h, 24 h and 24 h respectively for T60°, TC, T90° and T130°. They were also observed in rumen fluid for 1 h and 2 h post-feeding for T60° and TC respectively. Therefore, it appeared that there could be some rumen outflow of solubilized protein. Here, whatever the oilmeal, this outflow would represent

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5 to 7% of the amount of degraded protein (assuming the passage rate of rumen fluid in sheep to be 0.07 h^{-1}). New protein systems could therefore underestimate the potential protein by-pass unless their calibration is based on the duodenal protein flow (such as the Inra system). (© Elsevier / Inra)

rapeseed meal / technological processes / nitrogen degradation / rumen fluid composition / electrophoresis

Résumé – Importance des produits de dégradation dans le rumen des protéines du tourteau de colza suivant les traitements technologiques subis. L'importance du flux d'azote solubilisé dans le rumen pouvant contribuer au flux d'azote intestinal est mal connu. C'est pourquoi une étude a été conduite pour estimer simultanément en cinétique : 1) la disparition des protéines des sachets de nylon déposés dans le rumen ; 2) la teneur en différentes fractions azotées du jus de rumen (azote total (N_t), azote ammoniacal (NNH_3), azote non ammoniacal (NAN), N protéique) ; 3) la caractérisation des protéines des aliments, des résidus de sachets et du jus de rumen par électrophorèse. Les mesures ont été effectuées sur quatre moutons nourris successivement avec cinq régimes : foin seul (régime de base), foin plus un des quatre tourteaux dans la proportion 60 % de foin, 40 % de tourteau. Les tourteaux étudiés, issus d'un même lot de graines, avaient subi des traitements technologiques différents lors de la cuisson et de de la désolvantation : un tourteau témoin (T60°), un tourteau commercial (TC), et deux tourteaux expérimentaux, chauffés à 90 °C (T90°) et 130 °C (T130°). Leur dégradabilité théorique estimée à partir de la méthode des sachets de nylon était respectivement de : 0,83 ; 0,69 ; 0,39 ; 0,42, pour T60°, TC, T90°, T130°. Dans le jus de rumen, les teneurs en Nt et NAN sont élevées 1 h après le repas et diminuent rapidement (jusqu'à 7 h après le repas). Le pic de NAN est plus élevé pour les tourteaux les plus dégradables (0,56 mg/g et 0,36 mg/g respectivement pour les rations contenant T60° et TC) que pour les moins dégradables (0,15 mg/g et 0,23 mg/g dans les rations contenant T90° et T130°). En raison de la teneur élevée en azote de la ration, la concentration en NNH_3 reste élevée (0,3 mg/g) pour tous les tourteaux quel que soit le temps de la cinétique. 1 h après le repas, la teneur en NAN dans le jus de rumen est maximale soit de 0,61 ; 0,52 ; 0,30 et 0,42 mg/g respectivement pour les rations contenant T60°, TC, T90° et T130°. La teneur en N protéique est faible pour T90° et T130° mais on observe un pic (0,30 mg/g) 1 h et 2 h après le repas pour T60° et TC respectivement. Les résultats des électrophorèses indiquent que les protéines ont les mêmes caractéristiques pour les différents tourteaux bien que les pourcentages d'extraction soient très différents (70 %, 52 %, 21 %, 19 %, respectivement pour T60°, TC, T90° et T130°). Les protéines majeures sont des protéines 2S (de poids moléculaire 8 000 à 10 000) et 12S (de poids moléculaire 18 000 à 30 000). Ces mêmes protéines sont trouvées dans les résidus de sachets jusqu'à des temps plus ou moins longs selon leur dégradation mesurée par la méthode des sachets (jusqu'à 8 h, 16 h, 24 h et 24 h respectivement pour T60°, TC, T90° et T130°). On trouve également des protéines dans le jus de rumen 1 h et 2 h après le repas pour T60° et TC. Ces résultats confiment que des protéines solublisées des sachets ruminaux peuvent échapper à la dégradation dans le rumen. Quel que soit le tourteau étudié, la proportion de NAN par rapport aux protéines dégradées (calculées à partir de la dégradation en sachets de nylon) est de 5 à 7 %.(pour un taux de sortie de la phase liquide chez le mouton de 0,07 h^{-1}). Les nouveaux systèmes d'évaluation de la valeur azotée peuvent sous-estimer les protéines *by-pass* à moins qu'ils ne soient calibrés sur des flux duodénaux comme dans le système Inra. (© Elsevier / Inra)

tourteau de colza / traitement technologique / dégradation de l'azote / composition du jus de rumen / électrophorèse

1. INTRODUCTION

The true protein value of a diet for ruminants depends largely on the extent to which feed proteins escape rumen degradation. In most modern protein systems, rumen by-pass of proteins is assessed from the nylon bag method [48].

However, several shortcomings of this method have been identified [19, 34]. For example, the assumption that N disappearing from bags would be completely degraded to ammonia N and could not, therefore, contribute to the protein by-pass is questionable.

The purpose of this study was to check whether solubilized feed N products could outflow from rumen before being degraded to ammonia. Four rapeseed meals produced from the same seed batch and submitted to different technological treatments were studied.

2. MATERIALS AND METHODS

2.1. Feeds

The study was carried out on four different rapeseed meals prepared from the same Eurol type seed batch, under the trituration conditions described below:

– eight n-hexane washes of the flaked seeds at a temperature below 60 °C, followed by solvent extraction at 80 °C with steam injection (direct extraction process minimizing the heating of the seeds (T60°));

– heat treatment at 90 °C, followed by solvent extraction at 105 °C, with steam injection at 50 kg/h (conventional industrial process TC);

– heat treatment at 90 °C, followed by solvent extraction at 108 °C, with steam injection at 120 kg/h (T90°);

– heat treatment at 130 °C, followed by solvent extraction at 120 °C, with steam injection at 80 kg/h (T130°).

These triturations were carried out in the Cetiom pilot plant (Pessac, France).

2.2. Animals and experimental design

Four ruminally cannulated Texel sheep were used in the experiment. The rapeseed meal given to the sheep was the same as the one whose degradability had been studied in nylon bags. The dry matter intake was limited to 40 g per kg of metabolic weight per day.

The experiment had five experimental periods of animal feeding using successively one of the four rapeseed meals (with hay) or with hay alone (last period). Each period had a 2-week adaptation phase and 2 weeks for measurements. The first period was for measuring the in situ degradation kinetics of the rapeseed meal, and the second period for the kinetic sampling of ruminal fluids, which were carried out for all animals.

2 3. In situ degradation

Nitrogen degradability was measured using the nylon bag procedure, as described by Michalet-Doreau et al. [32]. About 3 g of the samples (8 mm mesh) were weighed into bags. The sample was then incubated in the rumen of the four fistulated sheep fed a 40% rapeseed meal diet. The rapeseed meal in the diet was the same as used in the nylon bag. Incubation periods were 2, 4, 8, 16, 24, 48 h for T60° and TC and 2, 4, 8, 16, 24, 48, 72 h for T90° and T130°. After the bags were removed from the rumen, they were kept at –15 °C until being analyzed. Prior to analysis, the bags were defrosted, then rinsed with cold water until the water ran clear. The bags were then beaten for 7 min in a 'stomacher' [29], washed again and finally dried at 60 °C for 48 h. The solubility of the nitrogen without incubation in the rumen (0 h) was determined by soaking bags containing the samples in warm water (40 °C) for 2 h, then drying them as before.

2.4. Ruminal fluid sampling

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. 150 mL of ruminal fluid were taken and muslin-filtered, then centrifuged for 5 min at 120 *g* in order to remove nutritional particles and protozoa. 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 sulfosalycilic acid (400 g/L) and separated after centrifugation (20 000 *g* for 10 min).

2.5. Analyses

The granulometry of the rapeseed meal was carried out according to Grenet [17]. 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 sulfosalycilic acid) were determined using the Kjeldahl method. The protein contents of the ruminal fluid N content values were obtained from the difference, and the NH3-N values were determined from the acid supernatant (after precipitation with sulfosalycilic acid) according to the Conway method [10].

Enzymatic degradability for the rapeseed meals was measured at time 1 h (DE1) [1].

The cell-wall contents (NDF and ADF) were determined for the feeds using the Van Soest [44] and Van Soest and Wine [45] methods, modified by Giger et al. [15] and Dorléans et al. [13], 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.

2.6. Protein separation procedures

The procedure of Bhatty et al. [6] was used. Feed proteins were extracted with 10% NaCl (w/v). The extract was centrifuged at 20 °C, 36 000 *g* for 20 min. The supernatant was dialyzed for 92 h with distilled water. The globulins were precipitated and separated from the albumins. After centrifuging, the albumins were precipitated in TCA 20% (w/v), then the proteins were solubilized in the extraction solution.

Purified proteins and proteins from the ruminal fluid were precipitated with sulfosalycilic acid (40 g/100 mL), centrifuged (10 min at 20 000 *g*) and washed with ether ethanol (v/v) before extraction.

2.7. Electrophoresis

The rapeseed meals and their residues after in situ degradation were fractionated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the modified procedure of Laemmli [21]. The proteins in the residues were extracted in an extraction solution (1 M Tris-HCl, pH 6.8, glycerol, SDS, pyronin, β-mercaptoethanol and distilled water) in the proportion of 750 µL per 1 mg nitrogen (based on the Kjeldahl method). The gels (80–100 mm) consisted of a stacking gel containing 125 mM Tris-HCl (pH 6.8) plus 3.75% acrylamide (30% C = 5%) and 1% SDS (0.1 g/mL), polymerized with 0.5% ammonium persulphate (15%), 0.094% tetramethylethylendiamine approximately 30 mm high, layered over a separating gel. The separating gel contained 15% acrylamide-bisacrylamide (30% C = 5%), 37.5% 1 M Tris-HCl (pH 8.8) and 1% SDS (0.1 mg/mL), polymerized with 0.5% ammonium persulphate (0.15 g/mL) and 0.083% tetramethyl-ethylendiamine. Electrophoresis was carried out for approximately 1.5 h at 200 V. The gels were then stained with a 0.2% solution of Coomassie brilliant blue R in 10% acetic acid (w/v) and 25% 2-propanol (w/v) for 30 min at 20 °C to detect the protein band. This was followed by de-staining with a solution of 10% acetic acid (v/v) and 35% ethanol (w/v) for 45 min.

The protein subunit molecular masses were determined with a standard protein solution consisting of lactalbumin (M_r 14 200), trypsin inhibitor (M_r 20 100), trypsinogen (M_r 24 200), carbonic anhydrase (M_r 29 000); glyceraldehyde-3-phosphate deshydrogenase (M_r 36 000), ovalbumin (M_r 45 000) and bovine plasma albumin (M_r 66 000).

2.8. Calculations and statistical analysis

The in situ DM and N disappearances in hay and rapeseed meals were fitted to the model of Orskov and McDonald [33] using a non-linear regression procedure [35]: $\%N \text{ degraded} = a + b\,(1\text{-}\exp^{-ct})$.

The effective degradability of nitrogen Deg(N) was calculated as $\text{Deg (N)} = a + (bc)/(c + kp)$ assuming $kp = 0.06\ h^{-1}$ [20].

The same model was used to calculate the effective degradability of dry matter (Deg(DM)).

The various degradability parameters for the nylon bags were analyzed by a variance analysis [35] according to the following factorial model:

$$Y = M + Ai + Tj + Eij$$

where M is the overall average, Ai the animal effect, Tj the rapeseed meal effect; and Eij the residual error.

Since no significant difference appeared between the two measurement days for the parameters measured in the ruminal fluid, the average values of the two measurement days were analyzed following the same factorial model.

3. RESULTS

3.1. Chemical analyses

The chemical composition of the feeds is shown in *table I*. The contents in NDF, ADF, NDIN, ADIN were similar for T60° and TC but much higher for T90° and T130°.

The average diameters of the rapeseed meal particles were 0.25 mm, 0.35 mm, 0.36 mm and 0.24 mm respectively for T60°, TC, T90° and T130°.

3.2. In situ study (*table II*)

All degradation parameter values for N and DM were similar for T90° and T130° and significantly different from T60°; the values for TC were intermediate. For both T90° and 130°, the degradability rate was very low. Deg values for N and DM were higher for T60° than for TC and both were higher than T90° and T130° respectively.

Table I. Chemical composition of the rapeseed meals and hay.

	T60°	TC	T90°	T130°	Control hay
Nt (% DM)	5.77	5.93	5.93	6.10	1.25
DE1 (% Nt)	66.26	38.14	15.37	12.24	
NDF(% DM)	29.19	30.06	41.54	48.66	65.00
ADF (% DM)	21.77	20.62	25.68	29.69	40.12
NDIN (% Nt)	11.23	14.37	37.60	52.25	40.04
ADIN (% Nt)	8.45	7.62	20.10	25.39	9.30

DM, dry matter; Nt, total nitrogen.

Table II. In situ degradation parameters for rapeseed meals.

	T60°	TC	T90°	T130°
DM				
a	0.310^{a}	0.265^{b}	0.217^{c}	0.211^{c}
b	0.482^{a}	0.606^{b}	0.680^{c}	0.684^{c}
c	0.354^{a}	0.103^{b}	0.041^{b}	0.036^{b}
Deg(DM)	0.717^{a}	0.644^{b}	0.486^{c}	0.468^{c}
N				
a	0.348^{a}	0.248^{b}	0.102^{c}	0.094^{c}
b	0.553^{a}	0.694^{b}	0.898^{c}	0.906^{c}
c	0.424^{a}	0.108^{b}	0.029^{b}	0.034^{b}
Deg(N)	0.826^{a}	0.693^{b}	0.391^{c}	0.422^{c}

DM, dry matter; N, nitrogen. a, rapidly degraded fraction (%); b, slowly degraded fraction (%); c, rate of degradation (h^{-1}); Deg, degradability (%) a+bc/c+k; different subscripts in a same line correspond to a significant difference ($P < 0.05$).

For T90° and T130° the fitting model was imperfect because the sum of the soluble fraction and the potentially degraded nitrogen fraction was close to 100 even though the percentage of residual nitrogen at 72 h was about 7% Nt. Inversely, for T60° and TC, the asymptote was reached respectively at 16 h and 24 h.

However, Deg (N) was significantly higher for T60° and TC because the (a) fractions were higher, whereas the (b) fractions were lower and had a higher degradation rate (c), particularly for T60°.

3.3. Ruminal fluid composition: Ntotal (Nt), non ammonia N (NAN), ammonia N (NH3 N), protein nitrogen (protein-N)

NAN is the difference between Nt and NH3-N. It therefore consists of amino acids, peptides and protein-N (true protein). For all diets and the various nitrogen fractions, (Nt, NH3-N, NAN protein-N) (*figures 1, 2, 3, 4*) the concentrations observed in the ruminal fluid were higher at time 0 h than at time 7 h. The Nt, and NAN contents in the ruminal fluid were highest 1 h after feeding and diminished gradually until 7 h after feeding. For all sampling times, the various nitrogen fractions were the lowest ($P < 0.05$) for the hay ration and the highest for the ration containing T60°. For diets containing T90° and T130°, the Nt, NH3-N, NAN contents of the ruminal fluid did not show any significant differences between them ($P > 0.05$) for each sampling time. They were lower than those for the ration containing T60° and TC. One hour after feeding, the ruminal fluid for the T60° ration had a Nt content (0.926 mg/g) nearly twice as high as for diets containing T90° (0.508 mg/g) and T130° (0.546 mg/g). This ratio decreased to 1.3 times at time 7 h. At peak time, NAN/Nt ratios in rumen fluid were 0.61, 0.52, 0.30 and 0.42 respectively for T60°, TC, T90° and T130°.

The ruminal fluid NH3-N contents reached a maximum 4 h after feeding for the ration containing T60°, 2 h after feeding for those containing T130° and TC and 1 h after feeding for the ration containing T90°. It reached its minimum 7 h after feeding for all diets. This pattern was not the same as that of Nt. For Nt, the concentrations varied much less between sampling times and the kinetic profiles were different. In contrast with results obtained for Nt content, the ruminal fluid from the ration containing T60° had only a significantly higher NH3-N content than T90° and T130° at times 2 h, 4 h and 7 h, and was not significantly different from the TC ration at times 2 h and 7 h.

The NH3-N/Nt ratio in the ruminal fluid was only 39% at time 1 h, and 50.5% at time 2 h for the ration containing T60°, 47% at time 1 h and 60.4% at time 2 h for the ration containing TC, while at time 7 h it was 75% for both diets. On the other hand, the NH3-N contents in the ruminal fluid for diets containing T90° and T130° were 60% to 70% Nt, and showed little variation throughout the sampling period.

On average, the true protein contents (*figure 4*) were low and only the ruminal fluid from the diets containing T60° and TC had high amounts of protein-N at 1 h and 2 h after feeding (respectively 34.8% Nt and 24.7% Nt at 1 h after feeding, and 31.5% Nt, 19.2% Nt at 2 h after feeding). These amounts were significantly different from the ones found in the ruminal fluid from diets containing rapeseed meals T90° and T130° ($P < 0.05$) for which the true protein contents were very low (about 7% Nt). For diets containing T60° and TC, the true protein contents found in ruminal fluid decreased to 7% Nt at time 7 h.

3.4. Electrophoresis

For rapeseed meals, the yield of protein N (as percentage of initial N) obtained by the extraction procedure was 70%, 52%, 21% and 19% respectively for T60°, TC, T90° and T130°. Rapeseed meal proteins consisted of a water soluble albumin fraction with 8000 to 10 000 molecular mass and a globulin fraction soluble in saline solutions made up of polypeptides with 18 000 to 20 000 and 28 000 to 30 000 molecular mass. These same proteins were found in all meals, whether or not they had been treated.

In the bags, residual proteins were observed up to 8 h (*figure 5*) for T60°, 16 h for TC (results not shown) and 24 h for T90° and T130° (results not shown). Protein fractions (12S and 2S) disappeared at the same time for T60° and TC, while the 8000 molecular mass fractions disappeared first for T90° and T130°. At time 0 (before feeding) proteins in the ruminal fluid could not be found by electrophoresis whereas, for the T60° and TC, proteins with a

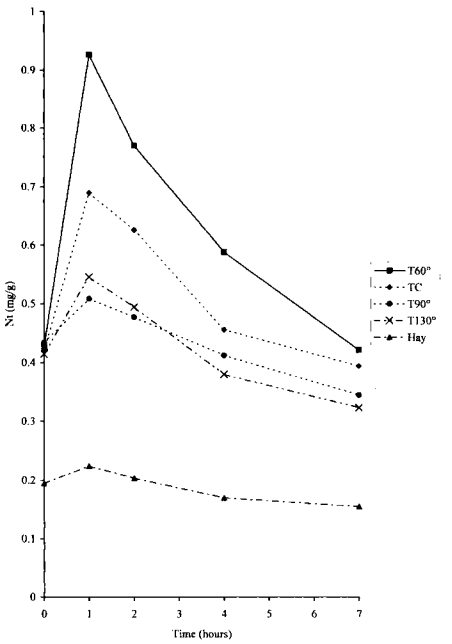


Figure 1. Evolution of the Nt content in rumen fluid for rations containing T60°, TC, T90°, T130° and hay alone, before the morning feed (T0h) and 1 h, 2 h, 4 h and 7 h after feeding.

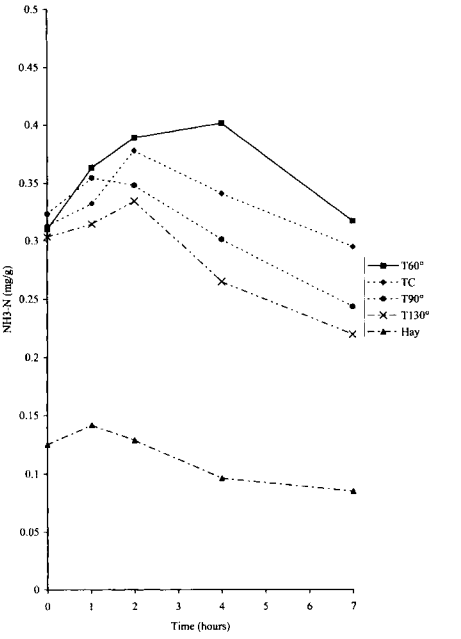


Figure 2. Evolution of the NH3-N content in rumen fluid for rations containing T60°, TC, T90°, T130° and hay alone, before the morning feed (T0h) and 1 h, 2 h, 4 h and 7 h after feeding.

10 000 to 30 000 molecular mass were observed 1 h and 2 h after feeding (*figure 5*).

4. DISCUSSION

4.1. Chemical composition and degradability

The amounts of cell-wall contents for T60° and TC were normal for rapeseed meal [4, 20, 36, 43]. The increased NDF and ADF contents in T90° and T130° were fully explained by the increase in their NDIN and ADIN fractions. The non N cell wall contents were similar for all rapeseed meals: 24–27% for (NDF-NDIN × 6.25) and 18–19% for (ADF-ADIN × 6.25).

There were no significant Deg(N) variations between T90° and T130°, although we might have expected a lower degradability for T130°. Indeed, for T130°, the cell-wall N content was higher, the DE1 value was lower and the sum of the cooking and solvent extraction temperatures was higher than for T90°. The lower mean particle size for T130° (0.24 mm) compared to T90° (0.36 mm) could explain the higher degradability for T130° due to greater particle losses in the bags. Furthermore, the finer particles might facilitate microbial enzymatic degradation in the rumen.

The low-heat treated rapeseed meal (T60°) had a very high Deg(N) as well as a very high (a) fraction and (c) degradation

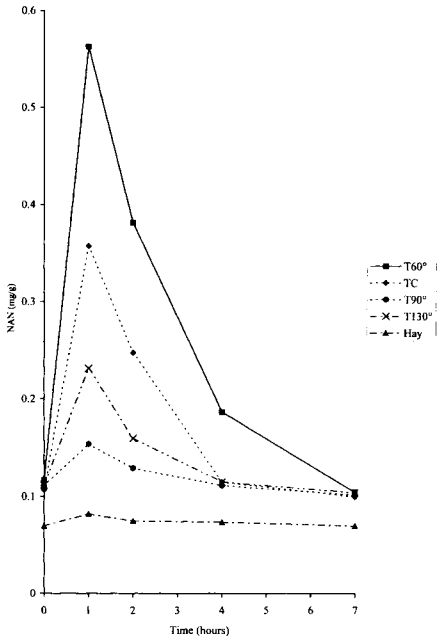


Figure 3. Evolution of the non-ammonia nitrogen (NAN) content in rumen fluid (peptides-N + protein-N + amino acids-N) for rations containing T60°, TC, T90°, T130° and hay alone, before the morning feed (T0h) and 1 h, 2 h, 4 h and 7 h after feeding.

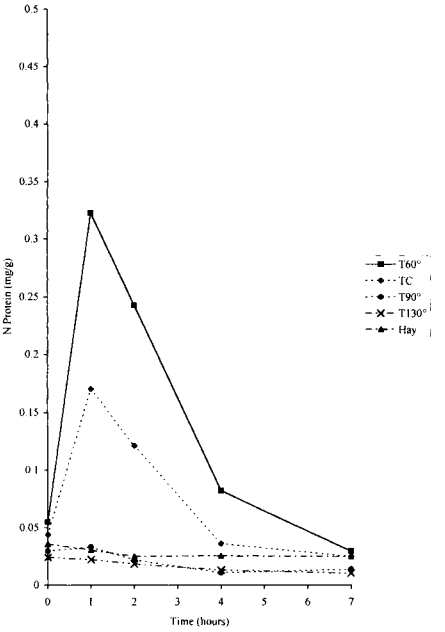
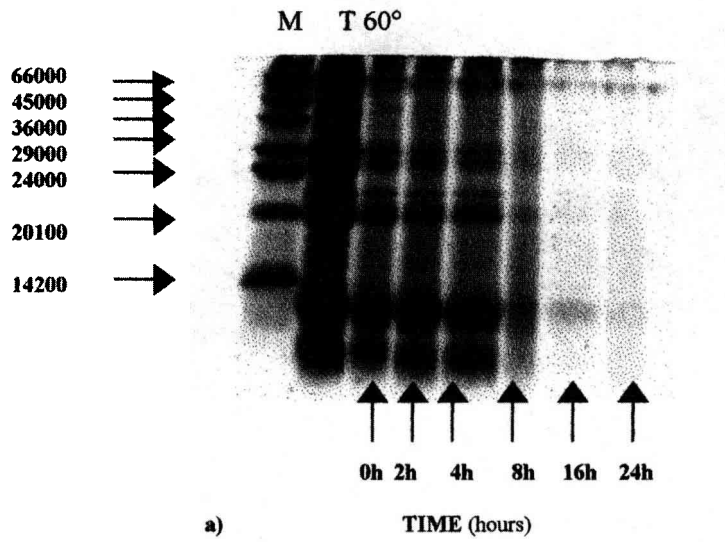


Figure 4. Evolution of protein-N content in rumen fluid for rations containing T60°, TC, T90°, T130° and hay alone before the morning feed (T0h) and 1 h, 2 h, 4 h and 7 h after feeding.

rate. The Deg(N) value obtained for TC (0.69) was similar to other published results [11, 18, 20, 24, 47]. The Deg(N) for TC, which was higher for T90° and T130°, was consistent with the sum of temperatures undergone in the heating of the seeds and solvent extraction, and therefore lower than for T90° and T130°.

Heat treatment reduced the Deg(N) for T90° (0.39) and T130° (0.42). The Deg(N) decreased by around 0.30, and this is higher than results of Bertilson et al. [5] (0.20 decrease). In agreement with observations made by Subuh et al. [42], our results showed a significant reduction in the immediately soluble fraction as a result of the heat treatment (*table II*). Michalet-Doreau and Evrard [31] have also shown that raising the heating temperature and steam injection during solvent extraction decreases degradability in the rumen considerably. This decrease can be more or less significant depending on the processing techniques [11, 22, 27], whereas seed extrusion was shown to be inefficient in the work of Deacon et al. [12]. Technological processes, and particularly heat, may modify protein structure. The 3D structure may be altered. Heating also involves Maillard reactions between carbohydrates and the free amino groups in the proteins to form covalent bonds which are resistant to enzyme hydrolysis (for reviews see [41, 46]). This structural change in the feed may also inhibit the fixation of proteolytic bacteria [26].

The amount of protein extracted from the meals with the extraction solution



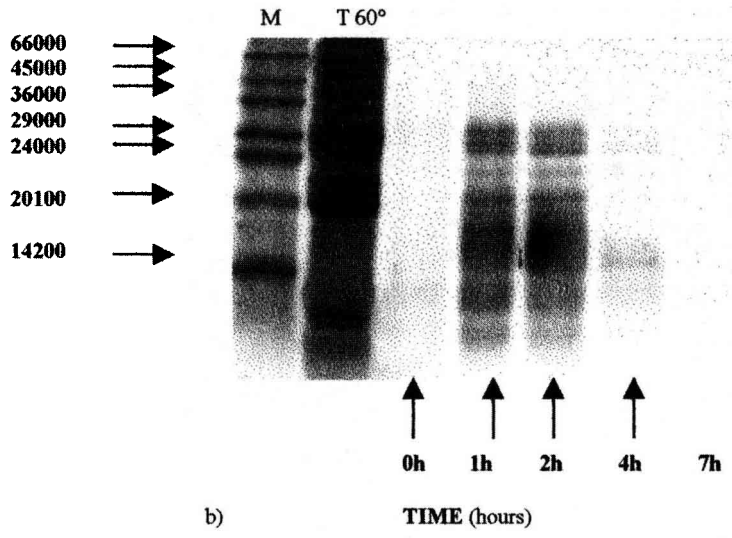


Figure 5. SDS gel electrophoresis of T60° showing residues after in situ incubation for 0 h, 2 h, 4 h, 8 h, 16 h and 24 h in the rumen (**a**) and proteins in the rumen fluid for sheep receiving a ration of hay and T60°, before the meal (0 h) and 1 h, 2 h, 4 h and 7 h after feeding (**b**). M, molecular mass markers from Sigma.

(before electrophoresis protein separation) was low for T90° and T130°. Girault [16] explains these differences by the more or less significant denaturation of the proteins caused by the heat treatment during meal making. The sodium dodecyl sulfate (SDS) used in the extraction solution before protein separation by electrophoresis, also present in the neutral detergent solution (NDS) used in Van Soest's fractionation, cannot solubilize the covalent bonds that might have formed between glucose and lysine (Schiff base) during the heat treatment and solvent extraction process. Proteins linked to the NDF residue and whose nitrogen content was about 50% Nt for T90° and T130° were not extracted with SDS.

In agreement with Bhatty et al. [6] and Schwenke [38], our results showed that rapeseed proteins are mostly made up of 12S globulin or cruciferin, containing several molecular mass subunits (18 500, 21 100, 26 800, 31 200 molecular mass) and 1.7S, 2S albumin or napin (8000 and 10 000 molecular mass). The last two are made up of two polypeptide chains linked by disulfide bonds [23]. For all the meals, whether experimental or not, the residual proteins of the samples for the four rapeseed meals incubated in the nylon bags degraded at different rates, as might be expected from the Deg(N) results. Furthermore, the various protein fractions for T60° and TC disappeared at the same time, while, for other rapeseed meals [2] and for T90° and T130°, the 8000 molecular mass protein was rapidly degraded. As indicated previously, the amount of extracted proteins was very low for T90° and T130°.

4.2. Nitrogen forms in the ruminal fluid

The higher Nt, NH3-N and true protein contents in ruminal fluid before the first meal compared to 7 h after were due to the smaller size of the rumen pool before the meal, as we found elsewhere (unpublished results). Sehgal and Makkar [39] have also showed this.

The concentration of different nitrogen fractions present in the rumen at a given time (particularly NH3-N, small size peptides) is a balance between dietary proteolysis, microbial synthesis use, absorption through the rumen wall, endogenous nitrogen flow, and transit to the small intestine.

The high crude protein content in the ration (approximately 195 g/kg DM) explained the high Nt contents measured in the ruminal fluid. The high Deg(N) and the greater immediately soluble fraction (a fraction) for T60° compared with the other meals explained the high amount of total nitrogen measured in the ruminal fluid from the ration containing T60°at time 1 h after feeding. The variation in Nt content in the ruminal fluids for diets containing T90° and T130° was much in smaller, but these meals had lower Deg(N) and were degraded more slowly.

According to Satter and Slyter [37], a minimum concentration of 5 to 8 mg/100 mL NH3-N is necessary to obtain optimum microbial growth. With all the diets containing the rapeseed meals, NH3-N concentration in ruminal fluid was much higher than this value. Thus, it may be that bacterial activity was not altered much in the different rapeseed meals used in the ration.

For diets containing T90° and T130°, ruminal fluid nitrogen was mainly as NH3-N (60% to 70% of total N). The NH3-N content in ruminal fluid, however, was lower than this for the diets containing T60° or TC. For the diets containing T60° and TC, the NH3-N content was higher but, in the 2 h following the meal, it represented a much lower proportion Nt, as there was a transient accumulation of proteins which was confirmed by the electrophoresis results (*figure 5*). Spencer et

al. [40], using rapeseed protein and pea proteins, Messman and Weiss [30], using soluble protein in fish and blood meal, and Mc Naab et al. [28], using rubisco, have also shown that these proteins could be solubilized inside the rumen without being immediately degraded as was suggested in the model from Fox et al. [14]. However, the electrophoresis results did not show any protein in the ruminal fluid at time 4 h after feeding. In some other in vitro studies, different values were found. For example, Spencer et al. [40] found some up to 24 h, and Messman and Weiss [30], up to 20 h.

The amino acids and peptide contents in the ruminal fluid, calculated by difference, were highest for all diets 1 h after feeding and only represented 25% of Nt for diets containing T60°, TC and T90°, but 38% Nt for the ration containing T130°. Rumen microbes use peptides rapidly and incorporate them efficiently into their proteins. However, if the rate of released peptides exceeds the rate of which peptides are hydrolysed or incorporated by microorganisms, peptides can accumulate and can then be detected in the ruminal fluid [9]. According to these authors, these peptides could escape degradation in the rumen and be absorbed in the intestine or partly absorbed through the rumen and the omasum [25, 49]. The results obtained in this study for T130° disagreed with those obtained by Broderick and Wallace [7], and Broderick and Craig [8], who observed an accumulation of peptides in vitro only when feed proteins degraded rapidly (casein).

In order to estimate the consequences in terms of nutrition, it is important to quantify the extent to which NAN in ruminal fluid can contribute to the intestinal protein flow. From our results, for each of the rapeseed meals, we estimated this proportion of NAN, taking into account the nitrogen fraction from the hay (in the hay diet). The volume of rumen (6 L) we retained was estimated from the measurements made on the same sheep receiving a ration made up of 60% hay and 40% soya meal. The fractional passage rate for liquids was estimated at 0.07 h^{-1} for an ingestion rate of 1 kg/day according to measurements carried out by Baumont et al. [3]. Feed proteins arriving into the intestine (PIA), estimated from the Deg(N) actually measured in the nylon bags were calculated taking into account a fractional rate of outflow from the rumen of 0.06 h^{-1}. (PIA (g/kg DM) = 1.11 × CP(1-Deg(N)) with CP (g/kg DM) [48].

The ratio between the amount of NAN × 6.25 in ruminal fluid able to escape degradation in the rumen and the amount of PIA, enabled us to calculate the amount of PIA that was under-estimated solely based on measuring Deg(N) in the bags. This amount is shown in *table III*.

Table III. Ratio between the amount of non-ammonia nitrogen (NAN) liable to escape degradation in the rumen and the PIA (dietary proteins arriving into the intestine) or degraded protein, for the various rapeseed meals.

	T60°	TC	T90°	T130°
Deg (N)	0.826	0.693	0.391	0.422
NAN × 6.25 (g/kg)	22.4	14.9	10.0	10.7
PIA(g/kg)	69.6	126.2	250.5	243.6
NAN × 6.25/PIA (%)	32.1	11.8	4.0	4.4
(CP × Deg (N)(g/kg)	297.7	256.6	144.9	160.4
NAN × 6.25/(CP × DEG (N))(%)	7.5	5.8	6.9	6.7

It was approximately 32% for T60°, 12% for TC and 4% for T90° and T130°.

Nevertheless, whatever the oilmeal, the NAN × 6.25 able to escape degradation represented 5.5 to 7.5% of the amount of degraded protein (Deg(N)×CP).

5. CONCLUSION

For the four rapeseed meals studied, the degradability of total nitrogen assessed through the in situ method differed: 0.83, 0.69, 0.39 and 0.42 respectively for T60°, TC, T90°, and T130°. The main proteins were 12S globulin or cruciferin and 1.7S albumin or napin. The proteins in the nylon bag residues disappeared at the same time for T60° and TC, and more slowly for T90° and T130° except for the 8000 molecular mass protein fraction. Some soluble proteins could escape degradation in the rumen when the rapeseed meals were degraded rapidly (T60° and TC). The nitrogen forms present in the ruminal fluid were proportionally different depending on the sampling times, and on the meal being studied. For the low heat meals (TC and T60°), 1 h and 2 h after feeding, we observed a balance between NH3-N, protein N, peptides and amino acids, whereas for T90° and T130°, 70% would be made up of NH3-N and the remaining part of amino acids and peptides, except at 1 h for T130°, where 57% was NH3-N and 38% as amino acids and peptides. Whatever the oilmeal, the solubilized NAN represented 5.5 to 7.5% of the degraded protein. In addition, true digestibility (dr) of NAN in the small intestine, which is essentially formed of peptides and amino acids was close to 1, even though, the dr of rapeseed meal PIA would be close to 0.80 [48].

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