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Original article

In situ intestinal digestibility of dry matter and crude protein of cereal grains and rapeseed in sheep

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Abstract — The ruminal degradation and intestinal digestibility (ID) of dry matter (DM) and crude protein (CP) of different feed samples were measured in two trials by using nylon bag and rumen outflow rate techniques in three wethers cannulated in the rumen and in the duodenum. In trial 1, three samples of grains of wheat, barley, and corn treated by cooking (TW, TB, and TC, respectively) were studied together with a sample of untreated corn grains (CG) of different origin. In trial 2, these studies were carried out on a sample of rapeseed (RS) and on a mix of this same sample and rapeseed meal (in proportions 70: 30) treated by cooking (TR). In both trials, the animals were fed at the same intake level (40 g DM·kg^{-1} LW$^{0.75}$) with 2:1 (DM basis) forage to concentrate diets. Rumen degradation rates of DM were high in the treated cereals (between 11.0 and 14.2%·h^{-1}) and low in the CG (6.35%·h^{-1}), whereas for CP these rates were low in all cereals. For DM, in all cereals, ID decreased linearly as the ruminal incubation time increased. The values of intestinal effective digestibility (IED), calculated from these functions and from the rumen outflow, were respectively: 86.4, 62.1, 51.5, and 67.9%. For CP, ID was unaffected by the ruminal incubation time in corn samples, whereas in TW and TB a reduction of these values was only observed for the time of 48 h. The values of IED of CP for CG, TW, TB and TC were: 82.6, 88.9, 82.5, and 91.6%, respectively. Rumen degradation rates of the RS and TR samples were 8.35 and 8.23%·h^{-1} for DM and 12.0 and 9.59%·h^{-1} for CP. In RS, the ID of DM and CP showed a downward trend with an increase of the ruminal incubation time, as modelled according to an exponential function. This same trend was observed for TR after a lag period estimated at 7.53 and 6.51 h for DM and CP, respectively. The values of IED of RS and TR were respectively 56.5 and 50.8% for DM and 71.9 and 80.1% for CP. These same results were also determined by a simplified method using a sample pooled to be representative of the rumen outflow of undegraded feed. The respective values for RS and TR were 54.8 and 51.6 for DM and 65.8 and 78.9% for CP. This method seems to be a promising technique to estimate IED, although more studies are needed to improve its accuracy.

cereal / rapeseed / intestinal effective digestibility / in situ

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1. INTRODUCTION

The current rationing systems for ruminants, based on the estimation of the total amount of amino acids absorbed in the intestine, need suitable estimates of intestinal digestibility (ID) of the rumen undegraded protein of feedstuffs. The obtention in an extensive form of these data is not possible through in vivo essays, since this is a complex and time-consuming process, requiring the application of regression or infusion techniques. On the contrary, the mobile nylon bag technique, based on the incubation of rumen pre-incubated feed samples in bags that transit between the duodenum and the ileum or the anus, is a simple and adaptable method that can be used in systematic studies. The main limitation of this method is derived from the variation of the ID values with the rumen pre-incubation time in many types of feeds [1, 2], as a consequence of the changes in the concentrations of undigestible nitrogenous compounds in the small intestine caused by the rumen microbial actions. This limitation can be avoided by the estimation of the intestinal effective digestibility (IED) of the feed protein from measurements with mobile bags weighed according to the rumen outflow of undegraded protein [1, 3, 4]. Nevertheless, this methodology is laborious, since incubations with mobile bags should be carried out for different times of rumen pre-incubation. Therefore, the characterisation of feedstuffs is of interest to limit the application of this technique only to feeds subject to important variations of ID with the rumen pre-incubation time. In this work, we grouped together the results of two essays carried out for this purpose on grains and seeds. In addition, the accuracy of a simplification of this technique, based on the incubation in mobile nylon bags of rumen undegraded samples pooled in accordance with the feed rumen outflow, was studied.

2. MATERIALS AND METHODS

2.1. Tested feeds

In two different trials, a total of six samples of grains and seeds were examined to determine their rumen degradability and their ID using in situ techniques. In trial 1, three samples of hydrothermal treated cereals: wheat (TW), barley (TB) and corn (TC) and an untreated corn grain (CG) of a

Table I. Chemical composition (g·kg^{-1} DM) of tested samples.

Items	CG	TW	TB	TC	RS	TR
Organic matter	983	980	977	991	954	934
Crude protein	85.7	127	103	77.9	221	261
Ether extract	–	–	–	–	389	313
Neutral detergent fibre	137	153	174	129	245	266
Acid detergent fibre	42.8	40.9	47.9	38.5	115	144
Acid detergent lignin	12.2	14.3	11.6	8.9	45.4	55.5
NDIN[1]	8.7	11.3	19.5	10.2	8.7	13.5
ADIN[1]	3.5	4.3	14.3	11.8	5.0	4.5

[1] Percentage of total nitrogen.
CG: corn grain; TW: treated wheat grain; TB: treated barley grain; TC: treated corn grain; RS: rapeseed; TR: treated rapeseed.

different origin were studied. In trial 2, a sample of rapeseed (RS) and an other of treated rapeseed (TR) were studied. The hydrothermal treatment of cereal grains included the following steps: cracking, cooking for 1 h (mean temperature and moisture: 98 ºC and 22–25%), flaking and drying. As a consequence of the high fat content of rapeseed, this feed was mixed with a whole rapeseed meal in proportions of 70% RS and 30% rapeseed meal, before the treatment, indicated above, except that flaking was substituted by treatment in an expander at high pressure. The chemical composition of these feeds is given in Table I.

2.2. Animals and diets

Three Manchega wethers fitted with rumen cannulas were used in trial 1 to measure rumen degradability. Then, these animals were provided with duodenal cannulas and used to determine the ID of the cereal grains. In trial 2, the same animals were employed to perform both measures. The animals were fed 2:1 forage to concentrate diets, starting 15 days before the experimental periods. The level of DM intake was fixed at 40 $g \cdot kg^{-1}$ $LW^{0.75}$. In trial 1, the forage was good quality lucerne hay and the diet was distributed in two equal meals (at 9.00 and 17.00 h). In trial 2, the forage was a mix (1:1 on DM) of corn silage and Italian ray-grass silage and the diet was distributed in six equal meals (every 4 h), starting at 9:00 h. A concentrate with a content ($g \cdot kg^{-1}$ DM) of 192 CP and 297 neutral detergent fibre (NDF) was used in both trials. The respective concentrations of CP and NDF in the diets were 209 and 352 in trial 1 and 116 and 527 in trial 2.

2.3. Rumen incubations

In both trials, nylon bags with a pore size of 46 μm and 11 × 7 cm (inner dimensions) were filled with approximately 3 g (air-dry basis) of the different samples (grounded to pass a 2 mm screen). The bags were incubated in the rumen of each animal for periods of 2, 4, 8, 16, 24 and 48 h. Two series of incubation with duplicate bags were performed for each feed. At each series of incubation, all the bags were placed simultaneously in te rumen just before the sheep were offered their first meal in the morning. After collecting from the rumen, the bags were washed with tap water and stored at –20 ºC. After thawing, the bags were washed three times for 5 min in a turbine washing machine. The same procedure was applied to two series of two bags of each feed to obtain the zero hours value. For each sheep and incubation time, one bag of each incubation series was oven dried for 48 h at 80 ºC and analysed for DM and nitrogen. The other bag was stored at –20 ºC, freeze-dried and reserved for intestinal digestibility measurements.

Ruminal disappearance of DM or CP were fitted for each animal to the exponential model of Ørskov and McDonald [5], except the CP disappearance from TC, which showed a sigmoid trend and was fitted to the model described by Van Milgen and Baumont [6]. The ruminal effective degradability (RED) of all the samples was estimated by the general procedure proposed by Ørskov and McDonald [5] using rumen outflow rate (k_p) values determined for the diet concentrate. In TC, this estimation was solved by a numerical integration. The concentrate was washed to eliminate the soluble components and marked by immersion with 10 mg $Yb \cdot g^{-1}$ of feed as described by González et al. [7]. To determine k_p values, a pulse dose (40 g) of labelled concentrate was fed to each animal immediately before the first daily meal. In trial 1, a total of 20 samples of faeces were obtained from the rectum of each animal, the first sample before supplying the marker and the remainder between 12 and 120 h afterwards. In trial 2, the pre-dosed sample and 20 post-dosed samples (collected between

1 and 82 h) were obtained through the duodenal cannula. These samples were dried, milled and analysed for Yb. The pattern of Yb concentrations in the faeces or in the duodenal digesta over time was described for each animal by fitting to the model of Dhanoa et al. [8] and rate constants derived from the decreasing phase of concentrations were used as k_p values for all the samples.

2.4. Intestinal digestibility

In both trials, the freeze-dried residues of each tested sample were pooled for each incubation time and the resulting samples were analysed for DM and nitrogen. Then, a total of 6 sub-samples of about 200 mg of each pooled sample were weighed into mobile nylon bags with an approximately round shape (∅ ≈ 3 cm). Two bags of each pooled sample were introduced through the duodenal cannula into the small intestine of each fistulated wether and recovered from the faeces. In both trials, 6 bags per sheep per day were inserted at random, at a rate of one bag every 15 min. The bags were then conditioned, stored, washed, dried, and weighed as described above and destined intact for nitrogen analysis. Blanks containing a known weight of nylon were used to correct the nitrogen content of the nylon material. The intestinal disappearance (ID) of undegraded material (DM or CP) was calculated as the amount lost from the bag divided by the amount in the bag before the intestinal passage. Then, the evolutions of the ID values with the rumen pre-incubation time were fitted for each animal to different functions in relation to feed to determine IED by the method proposed by González et al. [1]. To obtain the effective proportion digested in the intestine of a feed fraction, these authors propose to consider together the functions which describe (1) the variation of its ID with the rumen incubation time, and (2) the outflow of undegraded material from the rumen. When rumen degradation is described by a simple exponential equation, the rumen outflow of any feed constituent is described considering that undegraded material is defined by $u = r + b\,e^{-kd\,t}$, and that the rumen outflow is defined by $f = 1 - e^{-kp\,t}$. In the first equation, the constant r represents the undegradable fraction and b represents the non-soluble degradable component, which is degraded at a constant fractional rate k_d per unit time. Thus, the corrected outflow rate from the rumen undegraded material is $u\,(\mathrm{d}f/\mathrm{d}t)$. Therefore, the corrected rate of digested feed in the intestine can be obtained as $\mathrm{ID}u\,(\mathrm{d}f/\mathrm{d}t)$, and their cumulative proportion up to time t ($D_{i(t)}$) can be derived from:

$$D_{i(t)} = \int_0^t \mathrm{ID}u \frac{\mathrm{d}f}{\mathrm{d}t}\,\mathrm{d}t. \qquad (1)$$

In the work of González et al. [1] ID was only fitted to an exponential function:

$$\mathrm{ID} = s + h\mathrm{e}^{-k_i t}. \qquad (2)$$

In this model, the value h+s represents the ID of the insoluble feed fraction, whereas the asymptotic value s corresponds to the ID of the fraction that was apparently undegradable in the rumen. The constant k_i is a rate of decrease of the ID, derived from the sample enrichment in undigestible compounds with the extent of rumen degradative actions. When the expression (1) is applicated to equation (2), and considering that as the time from feeding increases, the fraction of feed flowing into the intestine tends to zero, the proportion of feed digested in the intestine tends to [1]:

$$D_i = s\left(r + \frac{bk_p}{k_d + k_p}\right) + hk_p\left(\frac{r}{k_p + k_i} + \frac{b}{k_d + k_p + k_i}\right). \qquad (3)$$

For some feeds, a lag period (t_0) before the reduction of ID values was observed. In these cases, equation (2) was established considering only the rumen incubation

times in which the reduction of ID was evident. Then, t_0 estimations were obtained by solving this equation particularized for ID as the average of the disappearance values included in the lag period. D_i values were calculated as the sum of two fractions: before and after t_0. The first fraction was determined as the product of the constant ID value by the rumen outflow until t_0, which can be obtained by the equation:

$$\varnothing_{until\ t_0} = r(1 - e^{-k_p t_0}) + \frac{bk_p}{k_d + k_p}(1 - e^{-(k_d + k_p)t_0}). \tag{4}$$

The second fraction was obtained by equation (3), but replacing the r and b values by their respective fractions remaining in the rumen at the time t_0, calculated by the expressions:

$$r_{for\ t=t_0} = re^{-k_p t_o}, \tag{5}$$

$$b_{for\ t=t_0} = be^{-(k_d + k_p)t_o}. \tag{6}$$

In other feeds the evolution of ID with increasing pre-incubation times showed a linear decrease and was fitted to the equation:

$$\mathrm{ID} = g - k_i t. \tag{7}$$

When applied at $t = 0$, it is shown that the value g of this model represents the ID of the total insoluble feed fraction ($b + r$), whereas the constant k_i is the rate of decrease of ID produced by the sample enrichment in undigestible compounds with the progression of the rumen degradative actions. The application of expression (1) to the model of equation (7) leads to:

$$D_{i(t)} = \int_0^t (g - k_i t) \cdot (r + b\mathrm{e}^{-k_d t}) k_p \mathrm{e}^{-k_p t} \mathrm{d}t44 \tag{8}$$

and, finally to:

$$D_i = r\left(g - \frac{k_i}{k_p}\right) + \frac{bk_p}{k_d + k_p}\left(g - \frac{k_i}{k_d + k_p}\right). \tag{9}$$

Finally, in all cases IED can be obtained as:

$$\mathrm{IED} = \frac{D_i}{(1 - \mathrm{RED})}. \tag{10}$$

In trial 2, the IED of DM and the CP of rapeseed samples were also estimated by using a simplified method, based on the incubation in mobile nylon bags of samples pooled from the undegraded residues of rumen incubations in accordance with the feed outlow from the rumen. To prepare these samples, the pooled residues of rumen incubation at 0, 2, 4, 8, 16, 24, and 48 h were considered representative up to times of 1, 3, 6, 12, 20, 36, and 60 h, respectively. Then, the rumen outflow of feed DM in these intervals was established according to equation (4) and these values were used to determine the weight proportion of each residue. Prior to and after the intestinal passage, these samples were managed as indicated above.

2.5. Chemical analyses

Tested samples were analysed for DM, ash, ether extract, and CP (Kjeldahl N × 6.25) by AOAC methods [9] and for neutral detergent fibre (NDF) [10], acid detergent fibre (ADF), and acid detergent lignin (ADL) [11]. Insoluble nitrogen in neutral detergent (NDIN) and in acid detergent (ADIN) solutions was determined by Kjeldahl analysis of the NDF and ADF residues, respectively. Rumen incubation residues and intestinal mobile nylon bags were also analysed for nitrogen by the Kjeldahl method. Samples of duodenal content or faeces collected for transit studies were analysed for ytterbium by atomic absorption spectrometry as described by González et al. [7].

2.6. Statistical methods

The different kinetics associated with the employed models were fitted using a

non-linear regression model. The results of ID for each tested feed were analysed considering the animals and rumen pre-incubation times as factors in the variance analysis before modelling these data. The means for the different rumen incubation times were compared by a *t*-test. All the statistical analyses were performed using the Statistical Analysis System for Windows software, version 6.12 (SAS Institute Inc., Cary, NC, USA).

3. RESULTS AND DISCUSSION

3.1. Rumen degradation

Mean values from both trials of degradation kinetic parameters and RED of DM and CP are shown in Tables II and III, respectively. Estimates of RED are based on k_p values of 2.50 and 4.93%·h^{-1} recorded for the same concentrate in trials 1 and 2, respectively, which were different at $P < 0.02$ (standard error of the mean (S.E.M.) = 0.388). Several authors [12–14] have obtained similar values of k_p using samples taken from the faeces or the duodenum. The differences between both diets in the concentration of NDF and in the degradation rate of their forages – grasses are degraded more slowly in the rumen than legumes [15, 16] – justifies the higher k_p values observed in trial 2. The outflow rate of particles from the rumen is conditioned mainly by the rumen fill, which is basically associated with fibrous particles. So, an increase in this fill is associated with higher rumen pressure, rumination activity, and rumen motility (contractile and propulsive movements) that lead to a higher evacuation through the reticulo-omasal orifice [17].

The degradation characteristics observed for treated cereals showed high values for the degradation rate (between 11.0 and 14.2%·h^{-1}) and RED (around 82%) of DM and, inversely, low degradation rates of CP. These results show a high rumen availability of starch, its main component, and a resistance to the degradation of proteins, which are the purpose of these treatments. The effect of hydrothermal treatments on

Table II. Degradation kinetics and rumen effective degradability (RED) of dry matter of the tested feeds.

Trials and feeds	*a*	*b*	*r*	k_d	RED
		(%)		(%·h^{-1})	(%)
Trial 1					
CG	21.9	77.2	0.9	6.35	77.0
TW	40.3	52.0	7.7	11.0	82.4
TB	40.7	49.2	10.1	14.2	81.8
TC	35.6	56.0	8.4	12.9	81.6
Trial 2					
RS	13.6	81.1	5.3	8.35	64.6
TR	31.4	59.2	9.4	8.23	68.3

a, *b* and *r* represent soluble, non-soluble degradable and undegradable fractions, respectively; k_d: fractional degradation rate of fraction *b*.
For other abbreviations see Table I.

Table III. Degradation kinetics and rumen effective degradability (RED) of crude protein of the tested feeds.

Trials and feeds	*a*	*b*	*r*	k_d	RED
		(%)		(%·h^{-1})	(%)
Trial 1					
CG	24.5	75.5	0.0	4.89	74.2
TW	20.2	79.8	0.0	4.35	70.5
TB	21.5	78.5	0.0	4.69	72.2
TC[1]	23.6	74.2	2.2	–	61.2
Trial 2					
RS	19.9	74.1	6.0	12.0	72.4
TR	31.1	65.8	3.1	9.59	74.4

[1] Mean values of k_0 and k_∞ from the model of Van Milgen and Baumont [6] were 0.65 and 10.0 (%·h^{-1}), respectively. For abbreviations see Tables I and II.

rumen degradation is especially important in corn [18], as a consequence of its high content of corneous starch, which agrees with the present results. The RED of the CP of CG was high, although this value is in the range indicated by the National Research Council [19] for 11 samples of corn grain: 48 ± 18% (mean ± S.D.). The high RED values of this sample and those of treated cereals were conditioned by the slow rumen transit observed in this trial (k_p = 2.50%·h^{-1}). Bacha et al. [20], on a sample (n = 20) of 8 different cereals, obtained from the Spanish market, observed RED values of CP higher than those recorded in other data sources [19, 21, 22]. The lowest differences were found for corn and sorghum, which are mainly imported.

The differences between RS and TR samples included the effects of both the seed treatment and its previous mix with rapeseed meal. For this reason any statistical comparison between both samples was intended. The TR sample showed a higher soluble fraction than RS, both for DM and CP. Deacon et al. [23] observed this same behaviour of both fractions with the extrusion of this seed. In the present results, this fact could be associated with the reduction of fat concentration (Tab. I), as a consequence of the mix with rapeseed meal. In the same way, the higher undegradable fraction of DM recorded in TR is in agreement with the increase in fibre content of this sample produced by the inclusion of rapeseed meal. Degradation rates of DM were similar for both samples, whereas for CP a lower value was observed for TR (9.59 vs. 12.0%·h^{-1}), in accordance with the effects produced by heat in this treatment and in those for the obtention of rapeseed meal. The results of RED were slightly lower in RS than in the TR sample, both for DM (64.6 vs. 68.3%) and for CP (72.2 vs. 74.4%).

3.2. Intestinal digestibility

The effect of the rumen pre-incubation time on the ID of undegraded DM or CP of cereals is shown in Table IV. Residues of ruminal incubation at 48 h of CG were too small to prepare the respective sample and consequently the ID was not determined for this time.

Table IV. Effect of rumen pre-incubation time (RPT) on intestinal digestibility (%) of dry matter and crude protein of the cereal samples.

	Dry matter				Crude protein			
RPT (h) Feeds:	CG	TW	TB	TC	CG	TW	TB	TC
0	90.2[a]	89.0[a]	87.9[a]	87.7[a]	83.1	92.0[a]	86.4[a]	88.7
2	88.9[a]	90.3[a]	84.4[a]	85.5[ab]	78.2	95.6[a]	89.0[a]	92.2
4	85.5[ab]	86.3[a]	83.9[a]	88.9[a]	80.6	94.4[a]	90.0[a]	93.7
8	86.7[ab]	83.5[a]	73.4[b]	83.8[b]	87.3	93.2[a]	89.3[a]	92.5
16	86.7[ab]	65.8[b]	54.8[c]	70.9[c]	86.3	91.9[a]	86.0[a]	92.3
24	83.0[b]	53.9[c]	52.7[c]	65.1[d]	79.5	90.5[a]	85.6[a]	90.4
48	–	25.9[d]	21.0[d]	39.6[e]	–	48.0[b]	30.2[b]	91.3
M.S.E.	1.79	2.80	2.25	1.13	2.76	1.92	2.75	1.56
Fitting parameters[1] *and effective values of intestinal digestion*								
g (%)	89.2	91.4	85.9	89.6				
k_i	0.24	1.39	1.40	1.03				
R^2	0.49	0.97	0.96	0.96				
Di (%)	19.9	10.9	9.4	12.5	21.3	26.2	22.9	35.5
IED (%)	86.4	62.1	51.5	67.9	82.6	88.9	82.5	91.6
UF (%)	3.13	6.67	8.83	5.91	4.49	3.27	4.87	3.26

[1] Parameters obtained by fitting the equation ID = $g - k_i\, t$, where g: ID of insoluble crude protein; k_i: slope of decrease of the ID; *Di*: intestinal digested fraction; IED: effective intestinal digestibility; UF: whole tract undigested fraction; M.S.E.: mean standard error; other abbreviations see Table I.
[a,b,c,d,e] Values with different superscripts in the same column are significant at $P < 0.05$.

A linear decrease of the ID of DM with the rumen pre-incubation time was observed in all cereal samples. The mean values of the parameters of equation (7) as well as the respective values of D*i* and IED of DM are also shown in Table IV. These results show that the progressive ruminal degradative actions can influence gut digestibility values. Consequently, the method proposed by González et al. [1] is based on the integration of the functions which describe the evolution over time of the rumen outflow of undegraded material and the variation with the rumen residence time of the intestinal digestibility. These authors indicated that this method remains valid irrespective of the forms of the basic functions employed (u, f and ID), as shown in this paper for D_i estimation when ID decreased linearly (equation 9). These decreases showed the progressive enrichment in fibre of feed particles with the extent of rumen degradation. On this basis, the differences recorded for k_i can be explained by the differences among samples for fibre concentration (NDF; Tab. I) and for the rumen degradation rate of DM (k_d, Tab. II), as the main factors affecting the fibre accumulation in the ruminal-digested samples. In this way, the biggest difference both for k_i and k_d was observed between CG and the treated cereals. The low proportions of DM digested in the intestine in treated cereals (between 9.4 and 12.5% vs. 19.9% in CG)

agree with its faster degradation and its higher rumen digestion. These differences were also shown by the IED values (86.4% in CG vs. 62.1, 51.5, and 67.9% in TW, TB, and TC, respectively). Estimations of undigested DM do not disagree with the usual values of apparent digestibility observed for cereals, considering that a part of the endogenous materials have been removed by the washing procedure. So, mobile nylon bags were thawed before washing, which promotes the partial detachment of adherent microbes from feed particles by the cold [24].

The effects of rumen pre-incubation time on the ID of CP (Tab. IV) were limited to TW and TB, in which an important reduction was observed at 48 h of rumen residence time. The low variation observed in all cereals agreed with the low k_d values (Tab. III), which should not promote an important accumulation of undigestible nitrogen compounds in the undegraded CP. The high values of ID observed indicates a low content of CP unavailable in the gut, especially for the treated cereals, in spite of its higher contents in fibre-bound nitrogen (NDIN and ADIN). These compounds may be degraded to different extents in the rumen by the combined actions of cellulolytic and proteolytic microbial enzymes. In addition, some authors [25, 26] have shown that an important part of NDIN of heat-treated feeds is solubilised in the gut. Estimations of IED of undegraded CP in the corn samples were obtained as mean ID values. This approach is not valid in TW and TB due to the decrease of ID at 48 h of rumen pre-incubation time. In these samples, D_i estimations were obtained by weighing in accord with the rumen outflow of undegraded CP determined by equation (4). Up to 24 h of rumen pre-incubation, the mean of the ID values was employed, whereas for the remaining fraction of outflow, the mean between this last value and that of 48 h was used. Finally, the values of D_i were derived from the IED values in accord with equation (10). Values of D_i of CP for treated cereals, which were higher than those of the CG sample, showed the change of the digestion site from the rumen to the intestine, as a consequence of the low rumen degradation rate of CP produced by the hydrothermal treatment. Consequently, the values of IED were high, even for TB, which was similar to that of CG. The present values showed that the ID of CP varied with the type of cereal because of differences in the rumen degradation characteristics of its CP. Verité et al. [22] estimated ID values of 95% for undegraded CP of corn and wheat and of 85% for barley. The protein value of treated cereals was not only enhanced by the shift of the protein digestion site, but also by the high microbial grow associated with its high DM degradability.

The effects of rumen incubation time on ID were evident in the rapeseed samples (Tab. V). The lower initial ID values of DM observed in TR rather than in RS may be attributed to the higher fibre content of TR, but also to the higher soluble DM fraction of this sample, which increased comparatively this concentration. The results of the ID of CP of De Boer et al. [27] for rapeseed meal showed a variation from 90.6 to 37.3% between 0 and 24 h of rumen incubation. Similar results of Liu et al. [28] varied from 92 to 65%. In agreement with our work, the differences between these results are also in accordance with the extent of rumen degradation of CP, which was higher in the first study. The evolution of ID of DM and CP of rapeseed samples was fitted to equation (2). Nevertheless, the existence of a lag period (t_0) for ID was observed for TR both for DM and CP, and for DM in RS only in one animal (Tab. V). The proportions of DM digested in the gut and the resultant IED were higher in the RS sample (20.0 and 56.5%, respectively) – with lower levels of rumen digestion and fibre – than in the TR sample (16.1 and 50.8%, respectively). This behaviour was similar to that observed in the cereals. The value of IED of the CP of the TR

sample was slightly higher than that of the RS sample (80.1 vs. 71.9%) as a consequence of the higher transit of insoluble by-pass CP thus allowing for the lower CP degradation rate in the rumen. This same behaviour has been observed in different feeds [1, 3, 4, 26]. Our results were higher than the value of 60% indicated by Verité et al. [22] for rapeseed, but closer to that reported for rapeseed meal (80%).

The estimations of IED of DM obtained by the simplified method from the samples pooled weighing with the DM rumen outflow were similar to those obtained mathematically from the ID evolution with the rumen pre-incubation time both for RS (54.8 vs. 56.5%, $P = 0.156$), and for TR (51.6 vs. 50.8%, $P = 0.560$). A similar behaviour was observed for CP in TR (78.9 vs. 80.1%, $P = 0.591$), but a difference of some

Table V. Effect of rumen pre-incubation time (RPT) on intestinal digestibility (ID; %) of dry matter and crude protein of rapeseed samples.

		Dry matter		Crude protein	
RPT (h)	Feeds:	RS	TR	RS	TR
0		81.7[a]	60.3[a]	90.2[a]	88.5[a]
2		74.3[a]	59.7[a]	88.4[ab]	88.2[a]
4		64.8[b]	60.9[a]	83.3[b]	86.9[a]
8		61.5[b]	60.5[a]	74.9[c]	84.1[a]
16		37.0[c]	35.8[b]	54.8[d]	66.3[b]
24		22.8[d]	29.9[c]	41.0[e]	58.7[c]
48		8.5[e]	17.6[d]	22.4[f]	29.7[d]
M.S.E.		2.71	1.83	1.68	1.91
Fitting parameters[1] and effective values of intestinal digestion					
t_0 (%)		0.70	7.53		6.51
s (%)		1.50	17.6	0.00	6.00
h (%)		78.8	44.2	89.8	81.8
k_i (%·h^{-1})		5.37	9.24	2.99	2.97
Di (%)		20.0	16.1	19.8	20.5
IED (%)					
– Integration method		56.5	50.8	71.9*	80.1
– Simplified method		54.8	51.6	65.8	78.9
M.S.E.		0.56	0.78	0.99	1.41
UF (%)		15.4	15.6	7.7	5.1

[1] Parameters obtained by fitting the equation ID = $s + he^{-k_i t}$. Where, $s+h$: ID of insoluble feed crude protein; s: ID of rumen undegradable crude protein; k_i: rate of decrease of the ID; t_0 = lag time. For other abbreviations see Tables I and IV.

[a,b,c,d,e] Values with different superscripts in the same column are significant at $P < 0.05$.

* Pair of values differed at $P < 0.05$.

importance was recorded in RS (65.8 vs. 71.9%, $P = 0.048$). The possibility of errors in sample preparation and analyses was higher in the simplified method, which is based on only one pooled sample. In addition, the variability between bags within the same animal is high. In this way, the accuracy of this technique should be reinforced with the preparation of more than one pooled sample and the incubation of more mobile bags. Therefore, more studies are needed to improve this promising technique. So, the use of these pooled samples is a simple and fast method, which reduces the experimental effort and spares the complex mathematical calculations needed in the original method. Consequently, this simplification should be useful in order to develop more complex studies as those of intestinal availability of amino acids, which will be too expensive by using the original technique. Nevertheless, this simplified method does not provide information about the variation of ID with the rumen residence time of particles, which is useful for the study of the factors affecting intestinal digestion.

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