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Rumen effective degradability of amino acids from soybean meal corrected for microbial contamination

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Abstract — Rumen degradation kinetics and effective degradability of individual amino acids, total analysed amino acids (TAA) and crude protein (CP) of soybean meal were measured on four rumen-cannulated wethers using the nylon bag technique. Microbial contamination of the incubated residues was corrected using a continuous ^{15}N intraruminal infusion and isolated solid associated bacteria as a reference sample. TAA showed a lower soluble fraction (14.9 vs. 20.8%; $P < 0.01$), a similar insoluble-degradable fraction (79.0 vs. 79.2%) and a higher degradation rate (11.5 vs. 8.4%·h^{-1}; $P < 0.05$) than CP. As a consequence, effective degradability was similar for TAA and CP (74.7 vs. 75.7%). Degradability values of individual amino acids varied moderately (range: ±6% of TAA degradability). Valine, isoleucine, leucine, alanine, aspartic acid and tyrosine showed significantly lower degradability than TAA, while the opposite effect was observed for histidine, threonine and glutamic acid. Degradability of individual amino acids was related to their soluble fraction ($r = 0.877$; $P < 0.001$).

amino acids degradability / soybean meal / microbial contamination

Résumé — **Dégradabilité ruminale, corrigée par la contamination microbienne, des acides aminés du tourteau de soja.** Les cinétiques de dégradation ruminale et la dégradabilité théorique des acides aminés individuels, des acides aminés totaux analysés (AAT) et des matières azotées totales (MAT) du tourteau de soja ont été établies par la méthode des sachets de nylon sur quatre béliers fistulés dans le rumen. Les résultats ont été corrigés en fonction de la contamination microbienne des résidus, moyennant l'infusion en continu de ^{15}N dans le rumen et l'emploi comme référence d'un échantillon de bactéries adhérentes isolées du rumen. Les AAT ont été moins solubles que les MAT

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(14,9 vs. 20,8 % ; $P < 0,01$), pendant que la fraction insoluble potentiellement dégradable a résultée similaire (79,0 vs. 79,2 %). Au contraire, le taux de dégradation a été supérieur pour les AAT (11,5 vs. 8,4 %·h^{-1} ; $P < 0,05$). Comme conséquence, les valeurs de la dégradabilité théorique ont été similaires (74,7 vs. 75,7 %). La dégradabilité des acides aminés individuels a montré une variabilité modérée (range de variation : ±6 % de la dégradabilité des AAT). La valine, l'isoleucine, la leucine, l'alanine, l'acide aspartique et la tyrosine ont présenté une dégradabilité significativement plus faible que celle des AAT, pendant que l'effet contraire a été montré pour l'histidine, la thréonine et l'acide glutamique. La dégradabilité des acides aminés individuels a été reliée avec sa fraction soluble ($r = 0,877$; $P < 0,001$).

dégradabilité des acides aminés / tourteau de soja / contamination microbienne

1. INTRODUCTION

Current systems of protein evaluation for ruminants have described the supply of true protein that can be absorbed from the small intestine. In order to increase accuracy in meeting the protein demands of high-productive ruminants, these systems are under revision, to establish food evaluation in terms of individual amino acids [19, 28]. Therefore, an important step in the development of these systems is the knowledge of the amino acid composition of undegraded feed protein, which, in fact, is scarce and subjected to controversy [28, 31]. The nylon bag technique is widely used for predicting ruminal degradability of dietary-N in ruminant feeds, being recognised as the official method in most of the current protein systems [10]. This technique is also the most currently used to study amino acid degradation. However, true degradation values are always biased by the microbial contamination of incubated feed residues [4, 14].

Soybean meal (SBM) is the most commonly utilised protein concentrate in ruminant diets. Therefore, available information concerning rumen degradation of their amino acids is abundant. Most published results only refer to a few incubation times [5, 8, 13, 16, 20, 27, 32]. However, studies that measured degradability values are limited [4, 9, 30], and only that of Harstad and Prestløken [9] provided full information about degradation. The aim of the present experiment was to measure the in situ degradation characteristics and the effective degradability of amino acids from SBM, using ^{15}N as a microbial marker for correcting microbial contamination of feed residues.

2. MATERIALS AND METHODS

2.1. Animals and feeding

Nylon bag incubations were carried out with four rumen-cannulated sheep (63.1 ± 0.90 kg LW) receiving a mixed diet of 2:1 chopped vetch-oat hay: pellet concentrate mixture. The chemical and raw composition of these feeds has previously been published [26]. This diet was offered at 40 g DM·kg^{-1} LW$^{0.75}$ in six meals per day (every 4 h).

2.2. Experimental procedures

Nylon bags (pore size 46 μm, reference 120T, Tissages Tissues Techniques, France) of 11 × 7 cm (inner dimensions) made by heat-sealing were filled with approximately 3 g (air-dry basis) of a commercial SBM sample ground through a 2 mm screen. The bags were incubated in two incubation series, on different days, for periods of 2, 4, 8, 16 and 24 h. All the bags of each incubation series were placed simultaneously in the rumen just before animals were offered the first meal of the morning (9:00 h). The specifications of the bags and incubations are in agreement with those of the INRA

standard method [15]. After collecting bags from the rumen, they were washed with tap water and stored frozen. Once defrosted, bags were washed three times for 5 min in a turbine washing machine, then the bags were freeze-dried prior to DM, nitrogen (N), and amino acid analysis. An additional set of three bags was reserved for zero incubation that involved the washing procedure without prior rumen incubation.

Microbial contamination of the incubated residues was determined by labelling ruminal micro-organisms with ^{15}N by continuous intraruminal infusion of ammonium sulphate (80 mg $N \cdot d^{-1}$, 50 atoms %) starting five days before the start of the incubation trial up until the end of the trial. At this moment, representative samples of rumen content were obtained for solid associated bacteria (SAB) isolation. The methodology employed is described in detail by Rodríguez et al. [26]. Isolated SAB samples were freeze-dried and analysed for DM, N, amino acids, and N isotopic proportions ($^{15}N/N$). This last determination was also carried out for the bag residues. Microbial contribution of DM and N-content of bag residues was determined as follows:

$$\text{microbial N \%} = \frac{^{15}\text{N abundance in residue} - {}^{15}\text{N abundance in SBM}}{^{15}\text{N abundance in SAB} - {}^{15}\text{N abundance in SBM}} \times 100$$

$$\text{microbial DM \%} = \text{microbial N \%} \times \left(\frac{\text{\%N in residue}}{\text{\%N in SAB}}\right).$$

The microbial content of individual amino acids in the residues was calculated from this last value and from their concentration (on a DM basis) in SAB. The ^{15}N abundance in SBM was determined on the zero incubation samples because only the insoluble fraction is subjected to microbial contamination.

The pattern of amino acid or CP (N × 6.25) disappearance (corrected for microbial contamination) with the incubation time was described for each animal using the Ørskov and McDonald model [21]. Effective degradability (D) was estimated, according to the method of the same authors, using the rumen particulate outflow rate (k_p) determined in each animal for the concentrate included in the diet labelled with ytterbium. Description of the method and results (mean ± SE = 3.61 ± 0.095%·h^{-1}) have previously been published [26].

2.3. Analytical

SBM was analysed for DM, ash, CP (N Kjeldahl × 6.25) and ether extract (EE) [1], and neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) [24]. Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were performed by Kjeldhal analysis on NDF and ADF residues, respectively. Nitrogen and ^{15}N enrichment of the bag residues and microbial samples were analysed by the Kjeldhal method and mass spectrophotometry (VG PRISM II, IRMS linked in series to a Dumas-style N analyser 1 108 Carlo ERBA), respectively. Amino acids were determined in bacteria, SBM and bag residues by high-pressure liquid chromatography in reverse phase after previous derivatization, following the technique of Jones et al. [11], but using a fluorescence detector only. Proline, cystine and tryptophan were not determined due to technical limitations.

2.4. Statistical analysis

Degradation kinetics of CP and total and individual amino acids were fitted using a non-linear regression programme. Degradation values of each amino acid were compared with those obtained for total analysed amino acids (TAA) using the paired t-test. The same procedure was used to compare the latter values vs. those of CP degradation. Finally, possible relations between degradation parameters were studied by correlation analysis of data. All analyses were performed using the statistical software SAS v 6.12 [29].

3. RESULTS

Chemical characterisation of the SBM sample gave the following values (g·kg^{-1} DM): OM = 928, EE = 10.4, CP = 551, NDF = 89.2, ADF = 56.7, ADL = 9.3. The proportions (%) of NDIN and ADIN in total N were 2.02, and 0.43, respectively. Their concentration of TAA was 90 g/16 g N. The amino acid proportions of SBM are shown in Table I, together with those of SAB samples. Mean values of the CP content and of TAA concentration in SAB samples were 440 g·kg^{-1} DM and 81.8 g/16 g N, respectively.

Degradation parameters and effective degradability of CP and amino acids obtained from corrected microbial disappearance data are presented in Table II. Mean values of microbial contamination of incubated residues were 1.11, 1.93, 3.81, 6.22, and 6.94 g bacterial DM per 100 g DM and 0.78, 1.41, 2.71, and 5.32 g bacterial N per 100 g N for 2, 4, 8, 16 and 24 h, respectively. Some constraints were necessary to fit degradation kinetics. Thus for CP, methionine, and lysine the total degradable fraction ($a + b$) was assumed to be 100% in all animals. This restriction was also adopted in animal 2 for the remaining amino acids (except for aspartic acid, glutamic acid, isoleucine, valine, and threonine) and for TAA.

Table I. Amino acid proportions (g per 100 g AA) of soybean meal (SBM) and of solid associated bacteria (SAB) of the rumen content.

Item	SBM	SAB
Arginine	6.72	4.28
Histidine	2.62	1.32
Isoleucine	4.55	6.53
Leucine	7.64	7.99
Lysine	6.44	7.99
Methionine	1.10	2.20
Phenylalanine	5.13	5.62
Threonine	6.20	5.85
Valine	4.71	5.70
Alanine	4.54	6.15
Aspartic acid	12.5	14.3
Glutamic acid	24.6	16.4
Glycine	4.19	5.21
Serine	5.21	5.20
Tyrosine	3.91	5.30

For aspartic acid, the disappearance value at zero incubation time was negative (–11.5%). Consequently, its soluble fraction was assumed to be 0%. The variability of this fraction between individual amino acids was high (from 0 to 38.4%). Differences between amino acids for their potential degradable fraction were also observed, with values ranging from 53.7% to 88%. A negative correlation (r = –0.922; $P < 0.001$) was observed between both fractions. Therefore, the extent of degradation was similar between amino acids. Thus all the values were above 90%, except for valine (88%) and aspartic acid (86.5%). Values of the degradation rates (k_d) of amino acids showed a great variability. Indeed, the maximum value (17.7%·h^{-1}, aspartic acid) was three times higher than the minimum value (6%·h^{-1}, methionine). Effective degradability values of individual amino acids from SBM presented a moderate variability, ranging from –6% to +6% of the value of TAA degradability (74.7%). Valine, isoleucine, aspartic acid, leucine, alanine, and tyrosine showed a significant lower effective degradability than TAA, while the opposite effect was observed for threonine, histidine and glutamic acid. The differences of degradability between amino acids were mainly related to differences in their soluble fraction, since there was a significant correlation between the two parameters (r = 0.877; $P < 0.001$).

When rumen degradation kinetics of TAA and CP were compared, TAA showed a lower ($P < 0.01$) solubility than CP (14.9 vs. 20.8%, respectively), while there was no difference in the insoluble potentially degradable fraction (79.0 vs. 79.2%). On the contrary, the fractional rate of degradation was higher ($P < 0.05$) for TAA than

Table II. Degradation parameters and effective degradability (*D*) of crude protein (CP), total analysed amino acids (TAA) and individual amino acids of soybean meal.

Item	*a* (%)	SED	*b* (%)	SED	*a* + *b* (%)	SED	k_d (%·h^{-1})	SED	*D* (%)	SED
Arginine	18.0	0.51**	81.6	2.07	99.5	2.44	9.0	0.67*	75.8	0.82
Histidine	24.7	0.28***	72.0	1.42*	96.6	1.53	10.4	0.52	77.6	0.32**
Isoleucine	4.1	0.25***	85.8	1.47*	90.0	1.35	13.1	0.50	71.1	0.31**
Leucine	8.5	0.16***	88.0	1.69*	96.6	1.58	9.5	0.42*	71.9	0.51*
Lysine	23.1	0.84**	76.9	2.49	100.0	2.70	8.4	0.66*	76.4	1.05
Methionine	38.4	1.09***	61.6	2.84**	100.0	2.70	6.0	0.73**	76.2	0.90
Phenyalanine	13.3	0.42*	84.3	2.49	97.6	2.30	9.6	0.50*	74.0	0.59
Threonine	36.5	0.21***	53.7	1.93***	90.2	2.13	14.4	1.01	79.3	0.55**
Valine	5.4	0.19***	82.6	1.53	88.0	1.51*	13.8	0.58*	70.8	0.38**
Alanine	13.0	0.42*	82.5	1.20	95.5	1.42	9.7	0.28**	72.6	0.64*
Aspartic acid	0.0	0.38***	86.5	1.12**	86.5	1.49*	17.7	0.52**	71.8	0.27**
Glutamic acid	21.3	0.20***	73.4	1.02*	94.6	0.85	12.2	0.26	77.6	0.36**
Glycine	17.0	0.73	80.2	2.57	97.2	2.41	9.3	0.58*	73.8	0.63
Serine	9.9	1.32*	83.6	1.84	93.5	1.05	11.4	0.47	72.4	0.96
Tyrosine	13.7	0.28*	81.8	1.84	95.5	1.62	10.1	0.40*	73.2	0.40*
TAA[†]	14.9	0.91**	79.0	2.25	93.9	2.70	11.5	0.82*	74.7	0.78
CP	20.8		79.2		100.0		8.4		75.7	

a: soluble fraction; *b*: insoluble potentially degradable fraction; k_d: degradation rate.
SED: standard error of the difference vs. TAA. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
[†] Values of SED are calculated vs. CP.

for CP (11.5 vs. 8.4%·h^{-1}). Values of effective degradability were similar for both fractions (74.7 vs. 75.7% for TAA and CP, respectively) as a consequence of the opposite differences found for the soluble fraction and for the fractional degradation rate.

4. DISCUSSION

For SBM, $a + b$ values higher than 100% are often observed for CP in the literature, when this restriction is not introduced. In the present work, these limitations cannot be derived from an insufficient incubation time, because the values of the degradation parameters and the effective degradability of CP are close to those obtained from other series of bags incubated together with these samples and through an additional time of 48 h, which were dried at 80 °C for 48 h and destined to further analysis [22, 25]. As in other works [3, 23], the microbial contamination of SBM residues was low. Contamination values are affected by the treatment of the incubated feed residues, that includes freezing and subsequent washing of bags, which involves the detachment of part of the adherent microbes [6]. The difference between the apparent and corrected CP degradability was low (75.2 vs. 75.7%, ESD = 0.05; $P = 0.002$), which is in agreement with other results [3, 18, 25]. Consequently, the amino acid corrections for microbial contamination are also little for SBM. Nevertheless, the differences between SAB and SBM in terms of the proportions of some amino acids (methionine, histidine, and glutamic acid) in the proteins are important (Tab. I), and, therefore, microbial correction could have more importance.

The fact that the soluble fraction observed for TAA (14.9%) was lower than that for CP (20.8%) can be attributed to the higher solubility of the non-protein nitrogen feed compounds. Thus Pérez et al. [22] observed a soluble fraction of 44.2% for the puric bases (representative of nucleic acids) of this same sample. Additional evidence of this hypothesis was the significant increase ($P < 0.05$) observed in the concentration of amino acids between the original feed and its insoluble fraction at zero time (90.0 vs. 95.8 g/16 g N; ESD = 0.77). The similar potentially degradable fraction (b) obtained for TAA and CP is in agreement with the results of Harstad and Prestløkken [9] and it shows that this fraction should be mainly composed of true protein. The degradation rate of this fraction recorded to be higher for TAA than for CP seems to indicate a faster degradation of proteins. This effect could be associated with the nitrogenous compounds bound to the fibre fraction. These compounds will be slowly degraded and, if they were proteins, they could be resistant to hydrolysis and therefore not detected in the chromatography analysis. Nevertheless, the proportion of NDIN for SBM is low, and therefore, its possible effect should be limited. Furthermore, this difference should be derived from the stronger fitting constraints assumed for CP than for TAA, which reduce the degradation rate values. As a consequence of the offsetting effects observed for the solubility and the degradation rate, the degradability values for CP and TAA were similar, which shows that the degradability of true protein of SBM should not be different to that of the total nitrogen compounds. The CP degradability value was somewhat higher than the mean values of 62% and 65% recorded, respectively, by Verite et al. [33] and the National Research Council [17], but it is close to those of Aufrere et al. [2], and England et al. [7].

The differences recorded between amino acids for the soluble fraction as well as for the degradation rate should be derived from important differences in the amino acid composition of the different SBM proteins. Nevertheless, the present results show that the most important differences should be between the soluble fraction and the insoluble proteins. However, the importance of this effect on the composition of the undegraded protein could be reduced by this outflow

from the rumen with the liquid phase of a part of the soluble proteins. Nevertheless, Aufrère et al. [2] estimated that for the feed this outflow is negligible. The insoluble protein fraction of SBM is basically composed of two kinds of globulins: conglycinins and glycinins. In this manner, the very lower degradation rate observed for methionine is in agreement with the methionine concentration which is 3 to 4 times higher in glycinins than in conglycinins [12]. Glycinins are more slowly degraded in the rumen than conglycinins [2]. Because soybean protein is generally deficient in sulphur amino acids, the glycinin content of SBM becomes highly valuable for ruminants from a nutritional perspective.

The results of Susmel et al. [30] and Harstad and Prestløkken [9] showed lower degradability values for CP (54% and 52.2%, respectively) and TAA (50% and 53.3%, respectively) than those of the present work. In spite of these lower degradability values, the variability between individual amino acids (in relation to the value of TAA) was, respectively, 2.5 and 2 times higher than in our study. These differences are partly derived from k_p values that were used. Thus, in both cited works, animals were feeding at maintenance levels of 1.5 and 1 and values of 7 and 8%·h^{-1} of k_p were used, respectively, whereas in the present study a mean value of 3.61 was established using a 1.1 maintenance level. On the contrary, Varvikko et al. [32], after incubations at 0, 5, 12 and 24 h, observed that variations of the amino acid profile of SBM were small, which is consistent with the present results, in which the differences observed for some amino acids were not excessive. The results of Harstad and Prestløkken [9] support the effects observed in the present study for valine, leucine, isoleucine, alanine, glutamic acid and tyrosine and only show the opposite effect for threonine. In the present study, the way of variations of individual amino acid degradability in relation to the value of TAA are in agreement with the results of Susmel et al. [30], except for threonine and aspartic acid. Cozzi et al. [4] only studied the essential amino acids and recorded an apparent higher degradability for histidine, arginine and lysine in agreement with the present results. In the same manner, the degradation of branched-chain amino acids, lower than that of TAA showed in this study was also observed in many works [5, 9, 13, 20, 27] carried out with punctual incubations.

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