Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Treated Soybean Meal Products

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ABSTRACT

Four lactating dairy cows equipped with ruminal and duodenal cannulas were used to determine the impact of different methods of treating soybean meal (SBM) on the ruminal degradability and intestinal digestibility of crude protein and AA. Solvent-extracted SBM (SE), expeller SBM (EP), lignosulfonate SBM (LS), and heat and soyhulls SBM (HS) were incubated in the rumen in nylon bags for 48, 24, 16, 8, 4, 2, and 0 h according to National Research Council (2001) guidelines. Additional samples of each SBM product were also incubated for 16 h in the rumen; the residues from these bags were transferred to mobile bags, soaked in pepsin HCl, and then used for determination of intestinal digestibility in situ or in vitro. Treatment of SBM (EP, LS, HS) protected the crude protein and AA from ruminal degradation, increasing rumen undegradable protein from 42% in SE to 68% in EP. Kinetic analysis of crude protein and AA degradation in the rumen revealed that, compared with LS and HS, EP exhibited slower rates of degradation but a shorter lag phase and a higher proportion of soluble protein. For all SBM products, the pattern of ruminal degradation, at 16 h of incubation, was characterized by extensive degradation of Lys and His, whereas Met and the branched-chain AA were degraded to the least extent. Estimates of intestinal digestibility of AA and crude protein were lower when measured in vitro than in situ; the magnitude of the difference between the 2 methods was greater (25%) with treated SBM products than with SE (10%). The availability of essential and nonessential AA was consistently greater (30%) with treated SBM than with SE. Among the treated SBM products, 4 essential AA (Ile, Leu, Phe, and Val) showed differences in availability, with values consistently lower for HS than for LS. This study showed that, based on in situ measures, heat and chemical treatment of SBM enhanced AA availability, and that compared with HS, EP and LS

had a higher potential to enhance the AA supply to the small intestine of high-producing dairy cows.

Key words: soybean meal, amino acid, rumen degradation, intestinal digestibility

INTRODUCTION

To optimize the amount of absorbable AA for high-producing dairy cows, one of the diet formulation objectives is to provide adequate amounts of RUP (Schwab, 1995). Much research has been conducted on rumen degradability of both plant and animal protein supplements (Erasmus et al., 1994; Cozzi et al., 1995; Ceresnáková et al., 2002). However, the European Union and North American regulations on the use of animal protein supplements in ruminant diets (Hasha, 2002; FDA, 2004) are likely to promote even more research into the use of plant protein concentrates. Soybean meal (SBM) is the most commonly used protein supplement for dairy cattle in North America (Statistics Canada, 2003; USDA-NASS, 2004), and among oilseed meals, it has the highest content of essential AA (EAA; NRC, 2001). For these reasons, SBM continues to be extensively studied as a source of AA for high-producing dairy cattle (Ipharraguerre and Clark, 2005).

Soybean meal has been treated in various ways to enhance the quantity of RUP, and several commercial sources of treated SBM are available for use in the diets for dairy cattle. The heat-generating expeller process is a conventional method of extracting oil from soybeans; this process results in an increased proportion of RUP from SBM (Liu, 1999) and does not require the use of organic solvents. Treatment of SBM with lignosulfonate is an alternative method to increase RUP. The process involves a chemical reaction with sulfite liquors, which are by-products of wood pulp processing. The method takes advantage of the nonenzymatic browning reaction, which results in reduced ruminal degradability of the protein (Cleale et al., 1987a,b; Can and Yilmaz, 2002). Sulfite liquors from paper mills can be a source of environmental pollution under industrial conditions that do not allow for recovery of sulfites (Smook, 1982). Therefore, the sustainabil-

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ity of the use of sulfite liquors in the manufacture of rumen-protected SBM products may be in doubt.

The use of heat and soyhulls is yet another industrial method for protecting SBM protein from ruminal degradation (Heitritter et al., 1998). This method also involves nonenzymatic browning, but it may be more environmentally acceptable than the lignosulfonate method because it uses a natural ingredient (soyhulls). However, published information on the heat and soyhulls method is lacking, and scientific studies are needed to evaluate its efficacy. According to Waltz and Stern (1989), treatment of SBM can increase the supply of AA to the duodenum of ruminants by 40 to 70%, and Bateman (2005) and Ipharraguerre and Clark (2005) have concluded that an increase in RUP can increase milk yield. Based on an exhaustive analysis of data from 35 research publications, Bateman et al. (2005) reported that, although DMI is a major source of variation in RUP supply to the small intestine, still other unknown factors influence the apparent RUP content of feeds. Therefore, any increase in the understanding of the relative merits of protein protection methods would improve the accuracy of predicting RUP and intestinal AA supply from SBM.

In addition to furthering knowledge of RUP from treated SBM, research is needed on AA availability from these products to improve current models for protein nutrition in dairy cattle (NRC, 2001; Rulquin et al., 2001b). These models rely on the assumption that the AA composition or profile of RUP is identical to that of the original feed, and that the effective degradability of individual EAA is similar to that of the protein in the original feed. However, González et al. (2000) and Ceresnáková et al. (2002) have reported that the AA composition of RUP from SBM can be modified during passage through the rumen. Based on studies in Europe (Rulquin et al., 2001a,b), models for AA nutrition of dairy cattle could under- or overestimate duodenal availability of AA by 13%, depending on the AA under consideration. According to Bateman et al. (2001), however, North American models (including the NRC model) for dairy cattle nutrition can result in an error as high as 50% in predicting the flow of EAA to the small intestine.

Published research dealing with the kinetics of ruminal degradation and intestinal availability of individual AA from rumen-protected SBM products is quite limited (Ipharraguerre and Clark, 2005). Furthermore, the impact of SBM treatment on intestinal digestion of AA does vary. For example, studies conducted with SBM treated with lignosulfonate or formaldehyde have shown no effects on intestinal digestion of AA (O'Mara et al., 1997; Harstad and Prestløkken, 2000; Prestløkken and Rise, 2003). However, when SBM was

Table 1. Ingredients and chemical composition of the basal diet fed during the in situ experiment

Item	Amount, % of DM
Ingredient	
Red clover-timothy silage	33.0
Corn silage	32.0
High-moisture shelled corn	21.0
Solvent-extracted soybean meal	11.0
Vitamin-mineral supplement ¹	3.0
Chemical composition	
DM	40.0
CP	16.5
Fat	3.1
NDF	38.6
ADF	25.7
Ash	8.2

¹10.8% Ca; 5.5% P; 3.5% Mg; 13.2% Na; 1.4% K; 2.1% S; 370,000 IU/kg of vitamin A; 65,000 IU/kg of vitamin D₃; 1,800 IU/kg of vitamin E.

treated with heat, there was a reduction in intestinal digestibility of Lys, Arg, and His in the protein escaping ruminal degradation (Demjanec et al., 1995; Ceresnáková et al., 2002); the reduction in intestinal digestibility of individual EAA ranged from 5 to 15%, suggesting that the response of individual AA to heat treatment is also variable. To improve existing models for a metabolizable AA system for dairy cattle, additional knowledge is required concerning intestinal availability of individual AA in common feeds.

This study was undertaken to evaluate the impact of multiple methods of treating SBM on ruminal degradability and intestinal digestibility of CP and AA and to compare methods in situ and in vitro to determine the intestinal digestibility of AA.

MATERIALS AND METHODS

Four multiparous Holstein cows, with an average BW of 649 (± 46.3) kg and 158 (± 28.7) DIM at the start of the experiment, were used for the study. Animal care procedures followed the guidelines of the Canadian Council on Animal Care (CCAC, 1993), and the protocol was approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Center in Sherbrooke, Québec (Agriculture and Agri-Food Canada). Animals were housed in tie stalls with water freely available. The TMR (Table 1) was fed ad libitum twice daily, at 0800 and 1600 h, and the animals were milked daily at 0900 and 2000 h.

Four types of SBM supplements were investigated: solvent-extracted SBM (**SE**; ADM Agri-Industries, Windsor, Ontario), expeller SBM (**EP**; SoyPlus; West Central, Ralston, IA), lignosulfonate-treated SBM (**LS**; Surepro; Land O'Lakes Purina Feed LLC, Saint Paul,

MN), and heat-treated SBM with soyhulls (HS; Aminoplus; Ag Processing Inc., Omaha, NE). The EP treatment involved feeding cracked whole soybeans into expeller presses with a central revolving shift. The pressure created within the press causes the oil to be mechanically extracted from the soybeans and temperatures to heat up to a maximum of 163°C (Liu, 1999). The LS method involved treatment of SBM with 8 to 25% (wt/wt) sulfite liquor from hardwood or softwood processing; the product was then heated at 95°C for 1 h, resulting in "early" Maillard reactions (Friedman, 1996) with xylose. The HS treatment involved mixing SBM with soyhulls (10:1 wt ratio), adding water to achieve 30 to 50% moisture, and then further cooking at 95°C to obtain a final moisture content of 12 to 16% (wt/wt).

The cows were equipped with ruminal (Bar Diamond, Parma, ID) and closed T-shaped duodenal cannulas (Berzins Vet Laboratory Ltd., Edmonton, Alberta, Canada) for in situ measurements of ruminal degradability and intestinal digestibility. Ruminal incubations in situ started after 1 wk of adaptation to the diet. Samples of each SBM product were milled through a 2-mm screen and subsamples (4 g) were then placed in nitrogen-free polyester bags (9 × 18 cm) with a pore size of $50 \pm 15 \mu\text{m}$ (R1020 Ankom products; Ankom, Fairport, NY); the ratio of sample size to surface area of each bag was 12.3 mg/cm^2 (NRC, 2001). Samples of each SBM product were incubated, in duplicate, in the rumen of each cow for 48, 24, 16, 8, 4, 2, and 0 h. Therefore, there were 2 replicates per cow for each time point of incubation. The bags were inserted in reverse order of incubation period, such that they could all be removed at the same time (NRC, 2001). Before incubation, the bags were soaked in water (39°C) for 20 min, attached to a stainless-steel weight, and placed in the ventral sac of the rumen. Once the bags were removed from the rumen, they were immersed in 20-L buckets containing cold water, then washed in an automatic washing machine (5 × 1-min wash, 2-min spin) until the rinse water was clear. The bags (with residues) were then frozen.

The same 4 cows were used to ruminally incubate additional samples of each SBM product to estimate intestinal disappearance of AA and CP. Four samples of each feed were placed in the rumen of each cow and incubated for 16 h. At the end of the incubation period, the contents of the bags from each cow were weighed, pooled by feed type, and transferred to nitrogen-free polyester bags (3.5 × 5.5 cm, pore size $50 \pm 15 \mu\text{m}$; R510 Ankom products; Ankom). All bags were placed in a 0.1 N HCl solution containing pepsin (1 g/L; Sigma P7000; Sigma, Oakville, Ontario, Canada), and incubated for 1 h at 39°C to mimic abomasal digestion. The bags from each cow, for each SBM product, were handled as

follows: 3 were randomly chosen and frozen for subsequent determination of acid-pepsin losses; 5 were randomly chosen for measurement of in vitro intestinal digestibility according to the 3-step procedure described by Calsamiglia and Stern (1995); the remaining 5 bags were used to estimate intestinal digestibility using the mobile nylon bag technique (Hvelplund and Weisbjerg, 2000). This latter procedure involves introducing the mobile bags, via the duodenal cannula, into the small intestine of the respective cows at the rate of 1 bag every 30 min. Upon recovery from feces, the mobile bags were washed in an automatic washing machine (5 × 1-min wash, 2-min spin) until the rinse water was clear; the bags (with residues) were then frozen.

The frozen residues from all bags used in the ruminal and intestinal incubation (in vitro or in situ) studies were freeze-dried to minimize nitrogen losses. At each time point, the respective residues were then pooled by feed type, for each of the 4 cows (1 replicate), and used for subsequent chemical analyses.

Analytical Methods

The DM content of bag residues was determined by freeze-drying; the DM content of feed ingredients and TMR was analyzed using a forced-air oven maintained at 60°C for 48 h. Dried samples of the TMR and SBM products were ground to pass a 1-mm screen and analyzed for ash and analytical DM with a thermogravimetric analyzer (model TGA-601; Leco Corporation, St. Joseph, MI). Fat was determined by gravimetric analysis using Isco SFX 3560 supercritical fluid extraction (Isco Inc., Lincoln, NE) without cosolvent modifiers for extraction of phospholipids. Analyses of NDF and ADF were performed according to the methods of Van Soest et al. (1991) using the Ankom system (Ankom 200 fiber analyzer; Ankom) with heat-stable α -amylase and without sodium sulfite. Nitrogen was determined by thermal conductivity (TruSpec v1.10 nitrogen determinator; Leco Corporation). Nitrogen fractions, defined according to the Cornell Net Carbohydrate and Protein System, were determined on SBM products using the methods of Licitra et al. (1996).

To analyze AA, samples were ground to pass a 0.5-mm screen; SBM products as well as bag residues were acid-hydrolyzed with 6 N phenol-HCl for 24 h at 110°C (AOAC, 2000), and AA concentrations of the hydrolysates were determined by the isotope dilution method (Calder et al., 1999). Briefly, 2 mL of the hydrolysate was diluted with 3 mL of ultrapure water, and 1 mL of this solution was then combined with 200 μL of a mixture of labeled AA (^{13}C and ^{15}N AA isotope standards; CDN Isotopes, Pointe-Claire, Quebec; Cambridge Isotope Laboratories Inc., Andover, MA), which

served as an internal standard. The solution was eluted through a poly-prep chromatography column (resin 100–200 mesh H; Bio-Rad, Hercules, CA), then derivatized with *N*-(*tert*-butyldimethylsilyl)-*N*-methyltrifluoroacetamide and dimethylformamide 1:1 (394882, 27.0547; Sigma-Aldrich) according to the method of Calder and Smith (1988). Amino acids were quantified using GC-MS (Hewlett-Packard Model GC6890-MS5973; Agilent Technologies, Wilmington, DE) and a mass selective detector (Hewlett-Packard, Palo Alto, CA). The AA Met, Cys, Arg, and Pro were analyzed separately by subjecting the samples to performic acid oxidation, followed by HCl hydrolysis (AOAC, 2000); these 4 AA were analyzed with a Biochrom 20 AA analyser (Amersham Pharmacia Biotech, Piscataway, NJ). A study of rumen kinetics was not performed for Met, Cys, Arg, and Pro because the sample size was insufficient to allow for their analyses at all time points of rumen incubation. Tryptophan was not analyzed because it cannot be determined under conditions of acid hydrolysis.

Calculations and Statistical Analyses

Data from rumen-undegraded residues were corrected for particle loss according to Hvelplund and Weisbjerg (2000). Rumen degradability of CP and AA was calculated according to the model of Ørskov and McDonald (1979) and Denham et al. (1989). Rumen degradation (p) was estimated by the iterative least-squares nonlinear procedure (PROC NLIN) of SAS (SAS Institute, 2001), which yielded the equation parameters a , b , c , and L , each of which is defined below. Akaike's information criterion (Akaike, 1973) was used to assess the relative performance of the model, with and without a lag phase. A model that included the lag phase was accepted, and is described based in the following equation:

$$\begin{aligned} \text{Given : } p &= a + b(1 - e^{-ct}); \\ \text{if } t \leq L : p &= a, \\ \text{if } t > L : p &= a + b[1 - e^{-c(t-L)}], \end{aligned}$$

where p is rumen degradation (%); a is the water-soluble fraction (%); b is the degradable fraction (%); c is the degradation rate of fraction b (h^{-1}); t is time (h); and L is the lag phase (h).

Effective degradability (ED) was calculated according to the following equation (Denham et al., 1989), assuming a rumen passage rate (k) of 0.08 h^{-1} :

$$\text{ED (\%)} = a + [bc/(c + k)]e^{-kL}.$$

Availability of AA was calculated as follows, based on the procedure of Berthiaume et al. (2000):

$$\text{AA availability (\% of original feed)} =$$

$$(100 - \% \text{ effective degradability})$$

$$\times (\% \text{ intestinal disappearance in situ}) \div 100.$$

Predicted values for a , b , c , L , and ED, as well as estimates of intestinal disappearance and availability, were analyzed using a randomized complete block design with the MIXED procedure of SAS (SAS Institute, 2001). The following model was adopted, with feed and cow as fixed and random effects, respectively:

$$Y_{ij} = \mu + F_i + c_j + e_{ij},$$

where Y_{ij} is the value of the variable studied on the i th feed, for the j th cow; μ is the overall mean; F_i is the fixed effect of the i th feed ($i = 1$ to 4); c_j is the random effect of the j th cow ($j = 1$ to 4); and e_{ij} is random error.

Preplanned orthogonal contrasts were designed to test: 1) untreated vs. treated SBM products; 2) the effect of heat vs. the effect of chemical processing of SBM; and 3) the difference between the 2 chemical methods of SBM treatment. The contrasts are summarized as follows: 1) SE vs. EP, LS, HS; 2) EP vs. LS, HS; and 3) LS vs. HS.

A concordance correlation coefficient was calculated to measure agreement between in situ and in vitro measures of intestinal disappearance (Lin, 1989, 1992, 2000). The criterion for declaring an effect to be statistically significant was predetermined at the 5% level; between 5 and 10% level of probability, values were considered as expressing a tendency.

RESULTS AND DISCUSSION

The chemical composition of the SBM studied is presented in Table 2. The CP content of the SBM products showed very little deviation from 50%. The concentrations of NPN and soluble protein were numerically lower in the treated SBM products than in SE. The EP, LS, and HS products were all exposed to higher temperatures during the respective processing methods, and heat denaturation of feed proteins is known to reduce their solubility (Liu, 1999). Compared with the NRC (2001) model, NDF, ADF, neutral detergent insoluble CP (**NDICP**), and acid detergent insoluble CP (**ADICP**) were higher for SE, EP, and LS. A possible explanation is the fact that sodium sulfite was not used in the analysis of fiber, a recommendation made by Van Soest et al. (1991). The increases in NDICP and ADICP for the treated SBM products were also the result of exposure to heat (EP) and chemical reactions (LS, HS) during processing (Demjanec et al., 1995; McKinnon et al., 1995). An increase in NDICP reflects an increase

Table 2. Chemical composition and AA content of soybean meal subjected to different methods of treatment

Item	Treatment ¹			
	SE	EP	LS	HS
CP, % of DM	51.8	47.8	49.2	51.1
NPN, % of CP	20.2	16.5	17.1	17.8
Soluble CP, % of CP	29.0	18.6	13.9	15.6
Neutral detergent insoluble CP, % of CP	4.0	29.5	38.6	29.3
Acid detergent insoluble CP, % of CP	1.8	8.2	7.6	3.6
NDF, % of DM	11.5	29.5	33.0	31.0
ADF, % of DM	6.3	13.8	12.3	10.1
Fat, ² % of DM	0.9	4.2	1.0	0.8
Ash, % of DM	7.3	7.1	7.8	7.2
Essential AA, % of DM				
His	1.26	1.13	1.23	1.29
Ile	2.08	2.10	2.15	2.23
Leu	3.81	3.67	3.69	3.87
Lys	2.99	2.58	2.67	2.89
Met	0.69	0.58	0.66	0.66
Phe	2.44	2.36	2.40	2.49
Thr	1.98	1.90	1.93	2.00
Val	2.12	2.16	2.20	2.28
Nonessential AA, % of DM				
Ala	2.15	2.08	2.07	2.17
Arg	3.64	3.24	3.51	3.64
Cys	0.69	0.56	0.64	0.63
Glx ³	7.74	7.25	7.18	7.94
Gly	2.05	2.08	2.08	2.14
Pro	2.05	1.85	1.95	2.07
Ser	2.57	2.45	2.45	2.56
Tyr	1.81	1.78	1.79	1.80

¹Treatments: SE = solvent-extracted; EP = expeller; LS = lignosulfonate; HS = heat and soyhulls.

²Excludes phospholipids.

³Glx = Glu plus Gln.

in the feed protein fraction that is slowly degraded in the rumen (Mustafa et al., 2000), whereas an increase in ADICP is an indication of heat-damaged protein, which leads to reduced protein digestibility (Faldet et al., 1992; Can and Yilmaz, 2002). The concentration of ADICP in the treated SBM products was higher than that in SE; the maximum value observed here was 8.2% (EP). In studies of the effects of heat treatment on ruminant digestion of CP, no adverse effects on intestinal disappearance were observed for ADICP between 5.1 and 7.0% (% CP) for canola meal (McKinnon et al., 1995) or up to 10.7% ADICP for roasted SBM (Demjanec et al., 1995; Schroeder et al., 1995).

The contents of NDF and ADF were also higher in the treated products than in SE; these increases are likely to be artifacts of the increases in NDICP and ADICP (Van Soest and Mason, 1991). A similar impact of heat treatment on the protein and fiber fractions was found for SBM (Demjanec et al., 1995). Results of the AA analysis of the SBM products (Table 2) revealed that the AA composition of SE was comparable to that reported by Degussa (Degussa Feed Additives, 1996); however, values for EP and LS were lower than those reported by the NRC (2001). Given that no information has been published on the AA composition of HS, the

data provided here would be quite useful in formulating diets for dairy cattle. Both EP and LS contained relatively low concentrations of His, Lys, and Arg, suggesting binding and cross-linking reactions involving these AA as a result of the treatment method (Adrian, 1974; Gerrard, 2002); this would reduce their release upon acid hydrolysis (Mauron, 1981).

Rumen Kinetics

Particle losses contributed to the disappearance of nitrogen from the rumen bags and this source of nitrogen disappearance was higher for EP (15.1% of feed N) than for the other SBM products. The estimates of particle loss were 10.4, 10.0, and 6.9% for SE, LS, and HS, respectively (data not shown).

Parameter estimates of CP and AA rumen degradation are presented in Table 3. The effective degradation of CP was higher ($P < 0.001$) for SE compared with treated SBM products. This observation was similar to that reported by Waltz and Stern (1989) and Maiga et al. (1996). When SE was compared with treated SBM products, ruminal degradation of CP decreased from 58% to approximately 35%. Ljøkjel et al. (2000) reported a similar reduction (from 63 to 28%) in ruminal degra-

Table 3. Effects of different methods of treatment of soybean meal on the kinetics of ruminal degradation¹ of CP and essential AA

Item ^{2,3}	Treatment ⁴				SEM, n = 4	Contrasts		
	SE	EP	LS	HS		SE vs. others	EP vs. LS, HS	LS vs. HS
ED, ⁵ %								
CP	58.3	32.2	38.4	35.4	4.53	<0.001	0.320	0.586
His	66.8	31.7	37.6	36.8	4.59	<0.001	0.206	0.873
Ile	61.9	37.7	34.8	47.9	3.82	<0.001	0.156	0.003
Leu	63.2	37.8	35.7	44.9	4.18	<0.001	0.360	0.025
Lys	61.9	34.0	34.5	36.4	3.91	<0.001	0.656	0.653
Phe	63.7	37.7	35.6	45.4	4.28	<0.001	0.338	0.022
Thr	57.1	32.4	36.4	38.5	4.25	0.003	0.316	0.715
Val	61.0	37.9	31.7	45.5	3.07	<0.001	0.829	0.008
L, h								
CP	2.0	2.2	9.2	6.5	2.99	0.224	0.115	0.516
His	4.1	2.1	8.1	6.8	3.28	0.645	0.135	0.754
Ile	6.1	7.6	8.0	16.3	4.82	0.135	0.119	0.035
Leu	6.4	7.7	8.7	14.8	4.36	0.064	0.053	0.027
Lys	3.1	2.3	6.6	6.9	2.56	0.412	0.098	0.924
Phe	6.4	7.8	8.8	14.9	4.33	0.057	0.052	0.024
Thr	1.5	2.6	8.7	8.5	3.57	0.245	0.168	0.965
Val	5.9	7.5	6.5	15.3	4.21	0.207	0.243	0.033
a, %								
CP	9.9	8.4	7.7	6.4	1.46	0.098	0.354	0.487
His	10.4	5.9	6.3	6.8	1.54	0.036	0.707	0.771
Ile	9.2	9.2	5.1	8.7	0.99	0.074	0.013	0.004
Leu	10.1	9.7	5.1	8.4	1.41	0.058	0.018	0.031
Lys	10.7	7.8	5.0	6.4	1.61	0.020	0.156	0.431
Phe	10.2	9.5	5.3	8.6	1.39	0.064	0.041	0.038
Thr	7.1	8.1	5.5	6.7	2.22	0.900	0.399	0.675
Val	9.0	9.2	3.9	8.3	0.90	0.079	0.009	0.005
b, %								
CP	87.0	88.5	88.4	90.4	1.52	0.203	0.607	0.337
His	85.6	91.0	90.2	90.2	1.54	0.020	0.614	0.999
Ile	88.5	86.5	91.9	88.9	1.14	0.633	0.017	0.092
Leu	87.4	85.9	90.7	89.3	1.48	0.469	0.030	0.480
Lys	85.8	89.1	92.0	90.7	1.64	0.011	0.149	0.436
Phe	86.9	86.0	91.7	88.7	1.58	0.232	0.017	0.116
Thr	89.3	88.6	91.5	90.3	2.38	0.724	0.345	0.693
Val	85.5	85.6	93.1	88.9	2.08	0.157	0.049	0.174
c, h ⁻¹								
CP	0.105	0.030	0.044	0.038	0.0012	<0.001	0.363	0.706
His	0.188	0.032	0.044	0.041	0.0331	0.004	0.771	0.946
Ile	0.111	0.041	0.039	0.062	0.0076	<0.001	0.131	0.013
Leu	0.117	0.041	0.042	0.055	0.0075	<0.001	0.116	0.049
Lys	0.122	0.034	0.039	0.041	0.0065	<0.001	0.193	0.648
Phe	0.120	0.040	0.040	0.056	0.0079	<0.001	0.154	0.039
Thr	0.102	0.031	0.042	0.044	0.0068	<0.001	0.138	0.773
Val	0.125	0.042	0.034	0.057	0.0085	<0.001	0.680	0.083

¹Rumen degradation estimated using the in sacco technique.²Model parameters of rumen degradability (p) according to Ørskov and McDonald (1979) and Denham et al. (1989): If lag time L = 0 : p = a + b(1 - e^{-ct}); if lag time L > 0 : if t ≤ L : p = a, and if t > L : p = a + b[1 - e^{-c(t-L)}].³Methionine rumen degradation kinetics not determined.⁴Treatments: SE = solvent-extracted; EP = expeller; LS = lignosulfonate; HS = heat and soyhulls.⁵ED = Effective degradability, a + [bc/(c + k)]e^{-kL} (Denham et al., 1989), estimated with a rate of passage (k) = 0.08 h⁻¹.

gradation of protein by heating SBM to 130°C. When heat (150°C) was combined with xylose treatment of SBM (3% of total SBM, DM basis), ruminal degradation of CP was reduced from 68 to 35% (Can and Yilmaz, 2002). When SBM was heat treated, as was the case with

EP, LS, and HS, the protein underwent denaturation, racemization, and cross-linking reactions (Rhee and Rhee, 1981; Friedman et al., 1984); this renders the protein less susceptible to microbial enzymes and leads to a reduction in ruminal degradation (Cleale et al.,

1987a; Wallace, 1994). The results of this study are consistent with previous reports, and confirm the concept that heat and chemical treatment of SBM protects the protein from ruminal degradation.

Among the treated SBM products (EP, LS, HS), there were no differences in the rate of protein degradation or in effective degradability. The estimate of protein ED reported for EP was similar to that reported by Waltz and Stern (1989) and Maiga et al. (1996), although neither lag phase nor correction for particle losses was considered in these studies. The estimate of CP degradation for LS was also comparable to that reported by Harstad and Prestløkken (2000), although they observed a higher proportion of soluble protein in their SBM products.

Table 3 shows the results of ruminal degradation of EAA. Estimates of ED and rates of ruminal degradation for all EAA were higher ($P < 0.01$) in SE than in the treated SBM products; the SE product also contained larger ($P < 0.05$) quantities of AA in the soluble fraction. Among the treated SBM products, only the branched-chain AA (BCAA) and Phe showed significant differences ($P < 0.05$) in effective degradability. When EP was compared with LS and HS, the differences were mainly due to a shorter lag phase ($P < 0.10$) for some EAA (Leu, Lys, and Phe), and higher solubility (a) for the BCAA and Phe ($P < 0.05$). One explanation is that the induction of mild nonenzymatic bonding with carbohydrates alters the polarity and net charges of the AA side groups, thereby affecting the solubility of the protein (Liu, 1999). Compared with HS, the LS treatment of SBM significantly ($P < 0.05$) decreased effective degradability of the BCAA and Phe. The degradation of the HS SBM product was characterized by a longer lag phase (L; $P < 0.05$) but a higher soluble fraction (a; $P < 0.05$) and higher rate of degradation (c; $P < 0.05$) for these EAA. It is reasonable to expect differences between LS and HS, because during the treatment process, the carbohydrate (CHO) added to LS is xylose, whereas in HS the main CHO added is pectin. Both types of CHO are involved in the "early" Maillard reactions, but the resulting linkages are more stable with simple CHO such as xylose, compared with insoluble CHO such as pectin (Adrian, 1974; Rhee and Rhee, 1981). This would result in a higher degree of protection against microbial degradation for some EAA in LS.

The present study provides novel information on the effective degradability of EAA in different rumen-protected SBM products; such information would be useful to improve current models for predicting the duodenal supply of AA in dairy cattle (NRC, 2001; Rulquin et al., 2001). For example, to determine the AA in RUP, the NRC (2001) uses the multivariate regression approach to adapt the AA submodel to measured data. However,

the independent variables utilized to construct the final predicting equations still assume that the AA composition of RUP is identical to that of the CP in the original feed; the model also assumes that the rate of ruminal degradation of CP is the same as that of the individual EAA.

Within each SBM product, the effective degradability of the individual EAA was variable (Table 3). The estimates of ED for BCAA and Phe in EP and HS were higher than values for the other EAA; furthermore, the ED for BCAA and Phe was not constant across SBM products. Other studies have also reported differences in AA composition of the RUP from SBM compared with the original feed protein (Crooker et al., 1987; González et al., 2000). The present study, with several rumen-protected products, confirms that not only are there differences in the effective degradation between CP and EAA, but also that there are differences in ED among the individual EAA. Using a single ED value for BCAA and Phe, for instance, and one based on the CP of original feed CP effective degradation would lead to errors in the calculation of EAA available for absorption.

Throughout the entire study, no corrections were made for bacterial contamination of the nylon bags. The implication of not correcting for bacterial contamination is that the residues in the bag may not reflect the actual AA profile of the RUP. In a review of factors affecting in situ ruminal degradation of protein, Nocek (1985) concluded that it was unnecessary to correct for bacterial contamination in protein supplements such as SBM. Varvikko (1986) compared the AA composition of RUP in rumen bag residues with and without correction for microbial contamination. The results showed that microbial contamination had no significant influence on the AA composition of RUP from protein supplements. It is therefore unlikely that, in the present study, the AA profile of the RUP was influenced by bacterial contamination.

The results of AA degradation after 16 h of ruminal incubation are presented in Table 4. These data were collected to assess the impact of SBM treatment on the pattern of ruminal degradation of AA at a specific time point. Values for the SE product revealed that His and Lys were degraded to the greatest extent; 79% of the His and 77% of the Lys in SE were degraded, whereas the sulfur AA (Met and Cys) and the BCAA (Ile, Val, and Leu) were degraded the least. Among the treated SBM products, His and Lys were also degraded to a greater extent than were the other EAA. These findings contrast with those of Maiga et al. (1996) and Prestløkken and Rise (2003), who reported slower release of Lys and His in EP and LS-treated SBM than in SE SBM. According to Gerrard (2002), Lys and His are very reactive AA, and this makes them more suscep-

Table 4. Effects of different methods of treatment of soybean meal on the extent of CP and AA disappearance at 16 h of rumen incubation¹

Item	Treatment ²				SEM, n = 4	Contrasts		
	SE	EP	LS	HS		SE vs. others	EP vs. LS, HS	LS vs. HS
CP, %	70.2	37.8	34.9	30.0	1.57	<0.001	<0.001	<0.001
Essential AA, %								
His	78.5	35.2	32.9	31.7	5.56	<0.001	0.244	0.662
Ile	74.4	31.7	29.4	26.3	6.56	<0.001	0.288	0.452
Leu	74.8	32.5	29.2	26.6	5.85	<0.001	0.180	0.481
Lys	77.3	36.2	30.9	30.3	5.65	<0.001	0.049	0.838
Met	68.7	32.2	28.7	28.1	1.67	<0.001	0.092	0.809
Phe	76.0	32.0	27.8	26.5	5.68	<0.001	0.110	0.689
Thr	75.1	33.8	29.3	28.0	5.55	<0.001	0.088	0.703
Val	74.2	32.5	28.2	26.6	6.65	<0.001	0.168	0.693
Nonessential AA, %								
Ala	74.6	32.5	28.8	27.4	5.58	<0.001	0.130	0.666
Arg	74.7	38.5	35.9	29.9	1.37	<0.001	0.008	0.013
Cys	67.7	39.2	33.8	28.6	1.84	<0.001	0.005	0.066
Glx ³	71.8	38.3	36.8	27.0	1.31	<0.001	0.002	0.004
Gly	75.5	34.4	30.5	28.9	5.36	<0.001	0.088	0.578
Pro	68.1	32.6	29.8	24.9	3.09	<0.001	0.187	0.275
Ser	75.8	34.3	28.4	25.8	5.42	<0.001	0.029	0.440
Tyr	74.1	31.4	27.6	24.7	5.77	<0.001	0.106	0.407

¹Percentage of rumen degradation of the treatments at 16 h of incubation (in situ technique).²Treatments: SE = solvent-extracted; EP = expeller; LS = lignosulfonate; HS = heat and soyhulls.³Glx = Glu plus Gln.

tible to degradation. Steric hindrance of the side chain of the BCAA as well as their hydrophobicity reduces the accessibility of microbial enzymes (Finley, 1985; Liu, 1999); this would explain the relatively slow rates of degradation of the BCAA. Studies by Crooker et al. (1987) and Van Straalen et al. (1997) with SE SBM have also shown slower degradation of Met and Cys compared with other AA. The treatment of SBM did not alter the pattern of degradation at 16 h; those AA with a greater extent of degradation in SE also reported higher degradation in treated sources of SBM (EP, LS, and HS).

Intestinal Disappearance

Estimates of intestinal disappearance of CP and AA in situ (mobile bag technique) and in vitro (3-step, enzymatic procedure) are presented in Table 5. The estimates of in situ digestion of CP and AA in SE and LS are similar to those reported by Ljøkjel et al. (2000) and Prestløkken and Rise (2003) for SE and LS-treated SBM. However, the values obtained for SE are lower than those reported by O'Mara et al. (1997), but these authors did not perform the pepsin-HCl step during the procedure. Significant differences in digestion of individual EAA were detected among the SBM products. For example, in situ digestion of His was lower ($P < 0.05$) with SE than with the other SBM products, but its digestion did not differ ($P > 0.10$) among the

treated SBM products. Lysine digestion in situ was lower ($P < 0.05$) for EP compared with LS and HS, but there was no difference ($P > 0.10$) in Lys digestion between these 2 chemical methods of SBM treatment. Despite such statistical differences, the values for AA intestinal disappearance in situ never differed by more than 3% across or within SBM products. Therefore, there may be no biologically relevant differences (<3%) in the in situ measures of intestinal AA disappearance among the treated SBM products.

The estimate of in vitro digestion of CP in SE (88%) is similar to that reported by Calsamiglia and Stern (1995) and by Maiga et al. (1996); however, the values for LS and EP are lower than those reported by these authors. Stern et al. (1997) reported values for in vitro intestinal CP digestion of LS that are similar to those reported here, but measurements on EP were not made. Differences in intestinal digestion among studies may be due to differences in the sources of SBM products. There are no previously published data on intestinal digestion of CP or AA in HS, but in vitro digestion of CP in HS was not significantly ($P > 0.10$) different from that of the other treated SBM products. In vitro digestion of all EAA and most of the NEAA was significantly greater ($P < 0.05$) with SE than with the treated SBM products. There were no significant differences in in vitro digestion of EAA between EP and LS or HS, although values for Ile, Lys, and Phe tended ($P < 0.10$) to be greater with HS than with LS. Among the treated

Table 5. Effects of different methods of treatment of soybean meal on intestinal disappearance of CP and AA in situ and in vitro¹

Item	Treatment ²				SEM, n = 4	Contrasts			Treatment				SEM, n = 4	Contrasts		
	SE	EP	LS	HS		SE vs. others	EP vs. LS, HS	LS vs. HS	SE	EP	LS	HS		SE vs. others	EP vs. LS, HS	LS vs. HS
	(In situ ³ disappearance)								(In vitro ⁴ disappearance)							
CP, %	98.5	98.4	99.1	98.9	0.12	0.010	<0.001	0.078	87.5	76.7	74.3	79.4	3.21	0.003	0.968	0.147
Essential AA, %																
His	97.6	98.1	98.9	98.5	0.36	0.025	0.143	0.434	87.6	72.6	74.8	84.0	3.60	0.022	0.151	0.088
Ile	99.3	99.3	99.6	99.4	0.09	0.050	0.007	0.016	89.5	72.8	73.6	83.6	2.96	0.002	0.094	0.020
Leu	99.2	99.3	99.6	99.4	0.09	0.007	0.021	0.013	86.7	67.3	67.5	86.7	2.80	<0.001	0.144	0.028
Lys	98.5	98.7	99.3	99.0	0.17	0.008	0.022	0.217	97.8	84.4	82.2	87.8	2.50	<0.001	0.817	0.092
Met	100.0	100.0	100.0	100.0	—	—	—	—	85.7	76.6	71.2	76.5	3.87	0.036	0.568	0.354
Phe	99.0	99.3	99.6	99.2	0.11	0.015	0.510	0.027	87.7	69.9	69.3	87.7	2.23	<0.001	0.158	0.013
Thr	98.8	99.1	99.5	99.2	0.14	0.017	0.154	0.170	90.1	73.9	73.7	82.7	3.10	0.003	0.241	0.059
Val	99.0	99.0	99.5	99.1	0.13	0.077	0.017	0.054	92.3	74.5	76.0	80.4	3.17	0.001	0.315	0.303
Nonessential AA, %																
Ala	99.0	99.1	99.5	99.2	0.15	0.031	0.036	0.125	89.8	71.7	72.6	80.3	2.48	<0.001	0.112	0.036
Arg	99.7	99.8	99.8	99.8	0.137	0.287	0.793	0.955	80.1	78.1	60.5	67.2	8.31	0.102	0.053	0.141
Cys	98.5	100.0	99.5	100.0	0.37	0.009	0.533	0.290	90.5	83.9	75.5	81.6	4.18	0.065	0.323	0.326
Glx ⁵	99.1	99.4	99.6	99.5	0.15	0.015	0.356	0.489	88.0	74.1	71.5	76.8	3.91	0.013	0.995	0.364
Gly	97.3	97.8	98.7	98.4	0.40	0.015	0.054	0.464	90.7	73.8	75.1	84.9	2.59	0.001	0.062	0.017
Pro	100.0	100.0	100.0	100.0	—	—	—	—	82.5	67.0	63.4	75.9	3.91	0.012	0.582	0.043
Ser	98.0	98.4	99.1	98.7	0.32	0.030	0.172	0.433	90.2	67.6	68.7	74.6	2.87	<0.001	0.226	0.131
Tyr	98.2	98.0	99.0	98.4	0.18	0.070	0.004	0.041	94.8	80.0	73.4	85.3	5.01	0.007	0.887	0.064

¹Performed on rumen degradation residues after 16 h incubation in situ.²Treatments: SE = solvent-extracted; EP = expeller; LS = lignosulfonate; HS = heat and soyhulls.³Intestinal disappearance (mobile bag technique), % of 16-h rumen residues.⁴In vitro digestion (Calsamiglia and Stern, 1995), % of 16-h rumen residues.⁵Glx = Glu plus Gln.

SBM products, in vitro disappearance of EAA ranged from 65% for Leu to 82% for Lys; this variation in in vitro digestion of EAA and NEAA was much greater than that observed for in situ digestion of the SBM products. To our knowledge, this is the first report of in vitro intestinal digestion of AA; therefore, more research will be required to confirm this variability of in vitro intestinal digestion of AA in rumen-protected SBM products.

Estimates of intestinal digestion of CP and AA obtained in situ were all greater than those obtained in vitro (Table 5); statistical analyses revealed no agreement (concordance coefficient <0.01) between the 2 procedures. The magnitude of the difference between the 2 methods was less for SE (10%) than for the treated SBM products (25%). Stern et al. (1997) also found a difference of 22% between values of intestinal digestion in vitro and those estimated with the mobile bag technique for SE and LS. Woods et al. (2003) reported higher values for in situ compared with in vitro intestinal digestion of CP in SBM. The higher digestion values for the in situ method could be explained by the fact that an artificially designed technique to study enzymatic digestion is unlikely to represent the environment or the function of the intestine exactly (Stern et al., 1997). Furthermore, particle losses from the mobile bags during intestinal transit, or loss of material during ma-

chine washing of the bags could be additional factors explaining the higher values for the in situ method.

Given the fact that the mobile bags were recovered from feces, another possible explanation for the discrepancy between in situ and in vitro AA disappearance is that hindgut bacteria could have contributed to proteolysis in the in situ procedure. However, using the mobile bag technique, Prestløkken and Rise (2003) could find no influence of the site of recovery (ileum or feces) on intestinal disappearance of AA from untreated or LS-treated SBM. Given the lack of agreement between the results in vitro and in situ, and the discrepancy with published data for in vitro intestinal digestion for EP, it seems that the determination of in vitro intestinal disappearance of AA cannot be used as a reliable replacement for in situ measurements.

Availability of AA

This is the first study with dairy cattle to comprehensively evaluate the kinetics of rumen degradation and intestinal availability of AA in SBM products subjected to multiple methods of rumen protection. Such information is needed for the development of diet formulation models to optimize the AA nutrition of dairy cattle. Calculations of AA availability were based on the in situ procedure, and these results are presented in Table

Table 6. Effects of different methods of treatment of soybean meal on essential and nonessential AA availability¹ (% of original feed)

Item	Treatment ²				SEM, n = 4	Contrasts		
	SE	EP	LS	HS		SE vs. others	EP vs. LS, HS	LS vs. HS
Essential AA, %								
His	32.7	69.3	62.0	62.8	5.74	0.001	0.243	0.901
Ile	38.0	62.1	65.1	51.9	3.80	<0.001	0.163	0.003
Leu	36.7	62.0	64.1	54.8	4.15	<0.001	0.373	0.024
Lys	37.7	65.6	65.2	63.9	4.68	<0.001	0.789	0.801
Met	31.3	67.8	71.3	71.9	1.67	<0.001	0.092	0.809
Phe	36.1	62.0	64.3	54.2	4.25	<0.001	0.339	0.020
Thr	40.4	67.2	65.3	55.5	4.45	0.011	0.186	0.166
Val	38.0	61.7	68.1	50.2	3.56	<0.001	0.373	0.002
Nonessential AA, %								
Ala	35.9	62.0	63.5	49.7	4.62	<0.001	0.052	0.008
Arg	25.2	61.3	63.9	69.9	1.35	<0.001	0.008	0.012
Cys	31.9	60.8	65.8	71.4	1.90	<0.001	0.006	0.055
Glx ³	33.2	67.6	59.8	61.2	5.34	<0.001	0.146	0.807
Gly	37.9	66.3	62.8	64.4	5.12	0.001	0.571	0.792
Pro	31.8	67.4	70.2	75.0	3.09	0.002	0.187	0.275
Ser	37.3	66.9	64.7	55.5	4.15	0.001	0.129	0.124
Tyr	37.4	62.2	64.1	60.1	6.66	0.003	0.980	0.575

¹(100 – % effective ruminal degradation) × % intestinal disappearance in situ ÷ 100.

²Treatments: SE = solvent-extracted; EP = expeller; LS = lignosulfonate; HS = heat and soyhulls.

³Glx = Glu plus Gln.

6. The estimates of AA availability of EAA and NEAA were, on average, 30% higher ($P < 0.001$) for treated SBM products than for SE; values for individual EAA in SE ranged from 31% for Met to 40% for Thr. In contrast, EAA availability for the treated SBM products ranged from 50 to 71%, depending on the particular EAA and the type of SBM treatment. When EP was compared with LS and HS, there was no significant difference in EAA availability. Only 3 NEAA (e.g., Ala, Arg, and Cys) were significantly lower ($P < 0.053$) for EP compared with LS. When compared with LS, the HS SBM product had lower availability values ($P < 0.05$) for the BCAA and Phe. This difference is explained mainly by the significantly higher rumen degradability of BCAA and Phe for HS (Table 3). The present study shows that the main factor contributing to differences in AA availability among the SBM products was the difference in rumen degradability; differences in intestinal digestion were minimal.

There is a scarcity of in vivo data on the intestinal supply of AA from rumen-protected SBM products; even less has been published on the availability of EAA from such supplements (Ipharraguerre and Clark, 2005). In a study with wethers fed heated SBM contributing 20% of the dietary CP, Demjanec et al. (1995) observed a linear effect of time of roasting on the duodenal flow of nonbacterial AA; there was a significant increase in intestinal disappearance for most EAA but not for Lys and Met. Based on the rumen and mobile bag techniques, Schroeder et al. (1995) also showed that heating

SBM increased the amount of available AA in the small intestine of dairy cows. Ipharraguerre et al. (2005) quantified the flow to the duodenum of AA in the non-ammonia nonmicrobial fraction when expeller SBM or xylose-treated SBM partially replaced SE SBM in the diets of dairy cattle. These authors found that EP and xylose treatment of SBM tended to increase the duodenal flow of total AA, and that xylose treatment increased the flow of some EAA. However, these authors made no attempt to measure the intestinal availability of EAA in the RUP fraction of the SBM products.

CONCLUSIONS

Solvent-extracted SBM exhibited greater effective degradability of CP and AA when compared with treated SBM products, EP, LS, and HS SBM. This was due to a greater fraction of soluble protein and a faster rate of rumen degradation of protein in SE SBM. Treatment of SBM protected the CP and EAA from ruminal degradation, thereby increasing RUP from 42 to 68%. Estimates of effective degradability of BCAA and Phe differed significantly among the treated SBM products, but for the remaining EAA, rumen degradability was similar for all products. Irrespective of the SBM product, the pattern of EAA degradation, after 16 h of ruminal incubation, was characterized by a rapid rate of degradation of Lys and His and by a slower degradation of Met and BCAA compared with that of the remaining EAA. In situ intestinal disappearance of AA ranged

from 97 to 100% and all values were greater than those obtained *in vitro*. Because the magnitude of the difference between the 2 methods was greater (25%) with treated SBM products than with SE (10%), further development of the *in vitro* method should be considered before it can be used to replace the *in situ* procedure. There were no differences in EAA availability between EP SBM and LS- or HS-treated SBM. However, when the latter 2 chemical methods were compared, EAA availability was lower with HS than with LS. The differences were due mainly to lower values of available BCAA and Phe. The results of this *in situ* study suggest that the EP and LS SBM treatments could be used as an effective means of enhancing the supply of EAA to the small intestine of dairy cattle. The use of soyhulls to protect SBM was not as efficacious as the other treatment methods for some EAA.

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APPENDIX

Table A1. Effects of different methods of soybean meal treatment on the kinetics of ruminal degradation¹ of nonessential AA

Item ²	Treatment ³				SEM, n = 4	Contrasts		
	SE	EP	LS	HS		SE vs. others	EP vs. LS, HS	LS vs. HS
ED, ⁴ %								
Ala	60.9	37.6	36.2	38.8	4.82	0.003	0.973	0.689
Glx ⁵	66.8	32.2	39.0	38.4	4.16	<0.001	0.100	0.897
Gly	61.4	32.8	36.7	31.6	4.22	<0.001	0.777	0.387
Ser	62.3	32.5	35.7	44.4	3.21	<0.001	0.035	0.052
Tyr	62.3	37.0	35.5	38.7	5.60	0.001	0.987	0.581
L, h								
Ala	6.2	7.3	8.8	12.1	4.68	0.317	0.317	0.390
Glx	4.1	1.6	10.1	8.0	3.38	0.456	0.044	0.588
Gly	3.1	2.7	8.5	4.6	3.54	0.589	0.314	0.407
Ser	2.8	2.3	8.7	11.5	3.04	0.204	0.056	0.514
Tyr	6.4	7.5	8.9	12.3	4.80	0.238	0.232	0.274
a, %								
Ala	10.9	10.3	5.8	6.9	1.98	0.156	0.085	0.641
Glx	11.6	7.0	6.6	7.9	1.68	0.046	0.900	0.557
Gly	10.7	8.7	6.7	4.6	1.97	0.093	0.166	0.424
Ser	10.9	8.0	5.0	7.9	1.34	0.013	0.205	0.079
Tyr	10.2	9.8	5.8	6.2	1.93	0.131	0.050	0.851
b, %								
Ala	85.7	86.7	91.2	90.0	2.01	0.118	0.085	0.621
Glx	84.9	89.8	90.4	89.1	1.81	0.046	0.978	0.589
Gly	86.1	87.0	90.3	92.5	2.26	0.117	0.073	0.414
Ser	85.1	88.7	92.0	89.1	1.54	0.006	0.168	0.092
Tyr	86.8	87.2	91.2	90.8	1.93	0.131	0.050	0.851
c, h ⁻¹								
Ala	0.12	0.04	0.04	0.05	0.008	<0.001	0.460	0.479
Glx	0.18	0.03	0.04	0.04	0.029	0.003	0.670	0.946
Gly	0.12	0.03	0.04	0.03	0.007	<0.001	0.376	0.453
Ser	0.12	0.03	0.04	0.06	0.006	<0.001	0.004	0.034
Tyr	0.11	0.04	0.04	0.05	0.009	<0.001	0.464	0.407

¹Rumen degradation estimated using the in sacco technique.²Model parameters of rumen degradability (p) according to Ørskov and McDonald (1979) and Denham et al. (1989): If lag time L = 0 : p = a + b(1 - e^{-ct}); if lag time L ≠ 0 : if t ≤ L : p = a, and if t > L : p = a + b[1 - e^{-c(t-L)}].³Treatments: SE = solvent-extracted; EP = expeller; LS = lignosulfonate; HS = heat and soyhulls.⁴ED = effective degradability, a + [bc/(c + k)]e^{-kL} (Denham et al., 1989); estimated with a rate of passage (k) = 0.08 h⁻¹.⁵Glx = Glu plus Gln.