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Rumen degradability and ileal digestibility of proteins and amino acids of feedstuffs for cows

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Abstract

Knowledge of the profile of amino acids of the rumen-undegradable protein can help in the formulation of diets to provide amino acids that complement microbial protein as well as supply amino acids, which are most limiting for milk production. Three non-lactating cows fitted with rumen cannulas were used to determine the effect of *in situ* rumen degradation on crude protein and amino acid profile of rumen-undegraded protein of feedstuffs. The obtained values of rumen degradability of crude protein with significant difference ($P < 0.001$) between feeds ranged from 20.3 to 76.3% (mean 62.0 ± 17.9%) and values of total amino acids ranged from 30.9% in corn gluten meal to 83.8% in corn gluten feed (mean 67.5 ± 16.4%). An *in vitro* modified 3-step method was used to determine intestinal digestibility. Intestinal digestibility of undegraded protein varied from 54.5 ± 1.4% in raw soybean to 95.2 ± 1.0% in corn gluten feed. The absorbable amino acid profile of rumen-undegraded protein for each feedstuff was compared with profiles of the original feedstuff and the rumen-exposed undegraded protein. Absorbable lysine (9.3 ± 1.1 g/kg of crude protein) was higher in products of soybean and sunflower cake. Corn gluten feed and meal supplied more absorbable methionine (3.6 ± 0.6 g/kg of crude protein). This study showed that the digestibility factor of crude protein and amino acid based on *in situ* and *in vitro* methods for thermal treatment of protein feeds can be used in models to optimize the amino acid nutrition of dairy cows and expand knowledge about rumen degradability and ileal digestibility of amino acids in feedstuffs.

Rumen degradation, undegradable, intestinal, thermal treatment, milk protein

A limiting factor of protein nutrition in cows is lower utilization of nitrogen (N). Overall average efficiency of utilization of N (N g feed/g N intake) in dairy cows with high production is approximately 25% (Huhtanen and Hristov 2009). The total milk yield and milk protein production in high-producing dairy cows is limited due to inadequate intake of certain amino acids. The most limiting amino acids for synthesis of milk protein were reported to be lysine (Lys) and methionine (Met) (Rulquin et al. 2001). More recent studies confirm the content of histidine and leucine amino acids as potentially limiting for milk protein synthesis. The amino acid profile of the intestinal contents in dairy cows depends on the amino acid (AA) content of microbial protein as well as rumen undegradable protein (RUP) and their intestinal digestibility (Rulqiun et al. 2001). The amino acid profile and intestinal digestibility of individual AA of RUP vary widely among and within feedstuffs (Prestlokken and Rise 2003; Mjoun et al. 2010). Using the small intestinal digestibility of RUP as a constant factor will lead to errors in prediction of nutrient needs. Therefore, intestinal digestibility of RUP has become an important variable in recent protein evaluation systems for ruminants (Hvelplund and Nørgaard 2003). Current feed evaluation and formulation is generally based on mean table values for the content and digestibility of AA of the individual feedstuffs. New approaches for analysis of digestible AA based on rapid *in vitro* and *in situ* analysis technique appear to result in more correct estimates of the actual nutritional needs.

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The main objectives of this study were to determine the rumen degradation and intestinal digestibility of crude protein, AA composition of RUP, and intestinal digestibility of the AA in RUP from several feedstuffs with high crude protein concentration.

Materials and Methods

Feedstuffs

Different protein concentrates routinely used in Slovakia were examined: raw soybean (RS), soybean meal (SM), Hyterso (toasted soybean - TS), Soypass (soybean meal treated with xylose - SP), corn gluten meal (CGM), corn gluten feed (CGF), rapeseed cake (RC), sunflower cake (SC), brewers grain dehydrated (BGD), malt culms (MC).

Animals and *in situ* rumen incubation

Indicators of rumen degradation of crude protein (CP) were determined, using *in situ* methods (Orskov and McDonald 1979), where 5 g of sample of each feed was weighed into bags (10 × 20 cm, 53 ± 10 µm pore size R1020 ANKOM technology, Macedon, NY) and heat-sealed. Samples of each feedstuff were incubated in duplicate, in the rumen of each cow for 0, 3, 6, 9, 12, and 24 h. The *in situ* procedure was conducted using 3 non-lactating dairy cows (600 kg of body weight) fitted with rumen cannulas. Animals were fed a lactation diet *ad libitum* (CP 15.0%, neutral detergent fibre 36.8%, and acid detergent fibre 25.3% of dry matter) composed of 53% forage and 47% of concentrate. After ruminal degradation, bags were rinsed with cold water to remove particulate matter. The intestinal digestibility of RUP of selected protein feeds was performed by modified three-step method (MTSP) (Gargallo et al. 2006). Approximately 1.0 g of the pooled rumen-exposed residue after 12 h rumen incubation were weighed into nylon bags (5 × 10 cm, 50 µm pore size, R 510 ANKOM Technology), heat-sealed and placed in a Daisy^{II} incubator (ANKOM, Fairport, NY, USA). Samples were incubated in 2 l of pre-warmed 0.1 N HCl solution adjusted to pH 1.9 and containing 1 g/l of pepsin (P-7000, Sigma, St. Louis, MO, USA). They were rotated constantly at 39 °C for 1 h. After pepsin digestion, samples were rinsed in cold tap water until the runoff was clear before they were incubated in 2 l of pre-warmed pancreatin (Sigma P-7545, St. Louis, MO, USA) solution (0.5 M KH_2PO_4 buffer standardized at pH 7.8 and containing 50 ppm of thymol and 3 g/l of pancreatin) and rotated constantly at 39 °C for 24 h. After incubation, samples were rinsed in cold tap water until the runoff was clear and oven-dried at 55 °C for 48 h.

Chemical analysis

Samples of feeds and bag residues after ruminal degradation and ileal digestion were analyzed for AA using the AAA 400 analyzer of amino acids (INGOS, Czech Republic). The methods incorporated an ion-exchange column, multiple sequential sodium-based eluents, and sodium hydroxide, for column regeneration. Absorbance was measured following post-column colour development by ninhydrin reagent at 131 °C. All individual AA were calculated on the basis of quantification by acid-stable hydrolysis (6 N HCl at 110 °C for 24 h) and the cystine and methionine contents were also quantified by means of performic acid hydrolyzed AA analysis. Feed samples were analyzed for dry matter (DM), crude protein (CP), crude fibre (CF), and ether extract (EE) according to conventional methods (Committee Regulation ES No.152/2009). Dry matter was determined by weight upon drying the sample at 105 °C under prescribed conditions. The CP content was determined according to the Kjeldahl methods (N × 6.25) using a 2300 Kjeltec Analyser Unit (Foss Tecator AB, Hoganas, Sweden). Fat (as etheric extract) was determined by the Det-gras device (JP SELECTA, Spain). Crude fibre was determined by the Dosi-Fibre Analyzer device (JP SELECTA, Spain).

The results were processed by mathematical-statistical methods using the statistical program GraphPad Prism4. We evaluated results of rumen degradation of CP and individual AA, their ileal digestibility and their differences for each feed as variable using Tukey-HSD test at significance levels of $P \leq 0.01$ and $P \leq 0.05$. Each indicator was presented by its mean (x), standard deviation (SD) and standard error of means (SEM).

Results

Chemical analysis of nutrients and calculated nutritional value of feeds tested in the experiment is shown in Table 1. The DM content ranged from 864 to 953.9 g/kg. The CP content of selected feeds varied from 205.7 to 748.4 g/kg of DM with different AA profile among feedstuffs. As expected, there was a large range in EE content among all feedstuffs (6.61–214.2 g/kg of DM). Concentration of total of CP with mean 878.4 ± 76.6 g AA /kg of CP and concentration of essential AA (EAA) g/kg CP with mean 417.9 ± 55.1 g/kg of CP (Table 1) does not differ among feeds. The fraction of EAA in total AA was higher in soybean meal (489.8 g/kg of CP) but lower in malt culms (325.2 g/kg of CP).

Table 1. Chemical analysis of nutrients of experimental feeds in g/kg of dry matter.

Feedstuffs	DM	CP	EE	CF	PDI	Total AA g/kg CP	EAA g/kg CP
Raw soybean	920.2	319.4	188.3	163.5	82.2	926.3	459.1
Soybean meal	878.5	520.0	12.4	49.9	242.5	957.7	489.8
Hyterso	900.0	386.0	214.2	113.0	222.0	877.1	439.4
Soypass	864.9	501.3	47.2	51.1	307.4	867.7	433.2
Sunflower cake	953.9	352.0	79.4	187.7	146.2	944.3	459.8
Rapeseed cake	919.5	376.2	74.2	133.4	132.5	835.5	417.8
Malt culms	928.5	285.3	11.5	90.6	114.2	710.2	325.2
Brewers grains	941.3	278.3	73.3	198.8	192.3	860.5	354.8
Corn gluten feed	885.7	205.7	16.1	81.3	129.5	838.7	350.8
Corn gluten meal	921.7	748.7	6.61	9.1	529.5	966.3	499.0

DM – dry matter, CP – crude protein, EE – ether extract, CF – crude fibre, PDI – protein digestible in intestine, AA – amino acid, EAA – essential amino acid

Results of rumen degradability of proteins (RDP) obtained by the *in-situ* method (Table 2) showed a considerable effect of the time of feed incubation in the rumen environment and effect of the type of feed and the way of its treatment. Thermal treatment of soybean-based feed has a significant effect on degradability of CP compared to RS (76.0%), reaching 46.5% with Soypass (SP). Rapeseed cake and, particularly, shelled sunflower cake showed a mean degradability of CP equal to 60.5% and 53.1%, respectively. By-products such as BGD, MC and CGF with a proportion of CP at the level of 20–30% showed rumen degradability of 74.5–76.3%. Corn gluten meal with the proportion of CP at the level of 748 g/kg of DM showed very high resistance to the action of rumen microflora with degradability at the level of 20.3%. Intestinal digestibility of CP (IDP) of RUP (Table 2) was estimated by the modified method described by Gargallo et al. (2006). The mean

Table 2. Protein digestibility of experimental feeds.

Feedstuffs	RDP (%)	IDP % of CP	IADP % of CP	TDP % of CP
Raw soybean	76.0 ± 8.8	54.5 ± 1.4	13.1 ± 4.2	89.1 ± 4.6
Soybean meal	71.0 ± 1.8	90.1 ± 0.6	26.1 ± 1.4	97.1 ± 0.4
Hyterso	66.8 ± 5.6	80.9 ± 0.4	26.9 ± 5.3	93.7 ± 0.3
Soypass	46.5 ± 1.7	62.8 ± 0.1	33.4 ± 1.6	80.2 ± 0.1
Sunflower cake	53.1 ± 0.8	90.2 ± 0.1	42.3 ± 0.6	95.4 ± 0.1
Rapeseed cake	60.5 ± 3.1	81.3 ± 1.4	32.1 ± 1.9	92.6 ± 1.2
Malt culms	76.3 ± 2.4	74.0 ± 4.0	17.5 ± 0.1	93.8 ± 2.4
Brewers grains	74.5 ± 1.4	70.2 ± 1.8	17.9 ± 0.1	92.4 ± 1.2
Corn gluten feed	74.8 ± 0.1	95.2 ± 1.9	24.0 ± 0.7	98.8 ± 0.4
Corn gluten meal	20.3 ± 0.5	84.7 ± 7.3	67.5 ± 6.4	87.8 ± 8.3
X ± SD	62.0 ± 17.9	78.4 ± 13.0	30.1 ± 15.7	92.1 ± 5.3
SEM	5.662	4.101	4.969	1.685

RDP – rumen degradable protein, IDP – intestinal digestibility of protein, CP – crude protein, IADP – intestinally absorbable dietary protein, TDP – total tract digestibility of crude protein (Mjoun et al. 2010) X – mean, SD – standard deviation, SEM – standard error mean intestinal digestibility of CP was 78.4 ± 13.0%. Rumen degradability of CP as well as intestinal digestibility of CP of RUP in experimental protein feeds was much more variable ($P < 0.01$).

Intestinally absorbable digestible protein - IADP was calculated as multiplying amount of RUP with IDP. After determination of CP of RUP and IDP, the mean of IADP was 30.1 ± 15.7% (Table 2), with more variable values of IADP found between feeds. Values of IADP ranged from 13.1 ± 4.2 to 67.5 ± 6.4% for RS and CGM, respectively.

Total tract digestibility of crude protein (TDP) was calculated as sum of RDP and IADP. The mean total digestibility in tested feeds was 92.1 ± 5.3%. There was a small but significant difference ($P < 0.05$) in the total digestibility of protein of feedstuffs (Table 2). Total tract digestibility of crude protein of Soypass was lower than 85%; it was only one from all selected feeds. The total digestibility of protein varied from 80.2% for Soypass to 98.8% for CGF.

Table 3. Coefficients of ruminal degradability and ileal digestibility of total and essential amino acids.

Feedstuffs	Rumen degradability		Intestinal digestibility	
	Total AA	EAA	Total AA	EAA
Raw soybean	0.789	0.786	0.633	0.657
Soybean meal	0.749	0.753	0.907	0.908
Hyterso	0.680	0.680	0.854	0.881
Soypass	0.526	0.531	0.632	0.629
Sunflower cake	0.616	0.611	0.903	0.905
Rapeseed cake	0.632	0.632	0.821	0.826
Malt culms	0.790	0.784	0.887	0.903
Brewers grains	0.825	0.824	0.762	0.763
Corn gluten feed	0.838	0.834	0.951	0.950
Corn gluten meal	0.309	0.312	0.839	0.833
X ± SD	0.675 ± 0.2	0.675 ± 0.2	0.819 ± 0.1	0.825 ± 0.1
SEM	0.052	0.051	0.035	0.035

X – mean, SD – standard deviation, SEM – standard error mean, AA – amino acid, EAA – essential amino acid

The coefficients of the intestinal digestibility of total AA (Table 3) in protein feeds determined by the MTSP procedure were in relationship to the ileal digestibility of CP. Overall, ruminal degradation of total AA and EAA degradation (Table 3) reflected the overall pattern of CP. Rumen degradation of total AA (Table 3), respective AA (data not presented) varied considerably among and within feedstuffs. Mean coefficients of rumen degradability of total AA (Table 3) (0.675 ± 0.2) were significantly higher ($P < 0.001$) than rumen degradability of CP (0.620 ± 0.2). The mean of intestinal digestibility of CP of RUP was 78.4 ± 13.0% (Table 2), which is similar and without significant difference to the mean of intestinal digestibility of total AA of RUP (81.9 ± 11.7%). Intestinal digestibility of RUP and total AA of RUP in experimental feedstuffs was greater for SM, SC and CGF with mean of 91.8 ± 2.9 % compared to remaining feeds, with the mean of 76.3 ± 7.5%. Effective degradability of Lys was generally very close to degradability of total AA with mean of 84.1.1 ± 12.4% (data not presented), whereas degradability of Met deviated little from the effective degradability of total AA (79.7 ± 12.0%).

The estimated intestinally absorbable amount of each EAA (g/kg of CP) supplied by the RUP fraction of protein feeds is presented in Table 4. Absorbable Lys was greatest

Table 4. Intestinally absorbable essential amino acids supplied by the rumen undegradable protein of feedstuffs.

AA g/kg of CP	RS	SM	TS	SP	SC	RC	MC	BGD	CGF	CGM
Lysine (Lys)	10.5	10.1	9.5	8.5	8.0	6.0	7.6	7.4	5.6	4.2
Histidine (His)	4.1	4.6	2.7	4.3	4.6	3.2	1.9	3.9	4.3	2.1
Arginine (Arg)	9.7	12.5	10.5	9.3	11.7	5.1	4.7	6.8	6.3	5.5
Threonine (Thr)	1.4	5.6	3.9	4.5	6.3	4.9	2.7	5.4	6.5	4.4
Valine (Val)	7.1	8.1	6.7	6.3	8.2	5.6	6.3	7.8	9.6	3.6
Methionine (Met)	1.8	2.2	1.7	2.0	2.5	1.3	1.7	2.0	3.3	3.9
Isoleucine (Ile)	7.7	7.9	6.6	6.2	7.0	4.8	3.4	6.2	6.5	3.2
Leucine (Leu)	12.0	12.7	15.8	10.2	11.2	8.2	8.9	10.8	17.0	12.7
Phenylalanine (Phe)	7.4	8.0	6.4	6.8	7.6	4.4	3.1	7.7	6.9	4.4
Lysine : methionine	5.8	4.7	5.9	4.3	3.2	4.6	4.4	3.8	1.7	1.1
EAA	61.5	71.7	63.7	57.9	62.0	43.5	40.1	57.8	66.0	44.1

AA – amino acid, CP – crude protein, EAA – essential amino acid, RS – raw soybean, SM – soybean meal, TS – Hyterso (toasted soybean), SP – Soypass (soybean meal treated with xylose), CGM – corn gluten meal, CGF – corn gluten feed, RC – rapeseed cake, SC –sunflower cake, BGD – brewers grain dehydrated, MC – malt culms

for soybean products, from 8.5 to 10.5 g/kg of CP (Table 4), but the mean of less absorbable Met was only 1.9 ± 0.2 g/kg of CP. Remaining feeds (SC, RC, MC, BGD, CGF and CGM) had values of absorbable Lys in the range of 4.2–8.0 g/kg of CP. CGF and CGM supplied more absorbable Met (mean 3.6 ± 0.4 g/kg of CP) than products of soybean and of the other analysed feeds. RC and MC supplied less absorbable EAA compared with other feeds.

Discussion

The goal of improved efficiency of transfer of dietary protein into milk protein is to develop models of evaluation of the intestinal digestibility of RUP and AA of RUP. There are various treatments affecting the ruminal degradability and intestinal digestibility of CP from various protein feeds (Stern et al. 2006). Effects of thermal treatment on the rumen degradability of CP and intestinal digestibility of RUP that were observed in this study are consistent with the findings of several studies evaluating protein feeds. Stern et al. (2006) estimated RUP of processed soybean products from 23.2 to 68.3%, and the intestinal digestibility of protein for non-enzymatically browned soybean meal ranged from 57.7% to 83.8%, respectively. Thermal treatment of feeds is a common method that can be performed in a number of ways, including moist heat treatment with a positive relationship between steam pressure and temperature (Van der Poel et al. 2005). Several studies demonstrated that it is possible to move protein digestion of different oilseed feedstuffs from the rumen to the small intestine by heat treatment without decreasing the total digestibility. The values of ruminal degradation of CP for soybean meal (Mjoun et al. 2010) and extruded soybean meal (Borucki Castro et al. 2007) were consistent with similar findings in the literature. Lower ruminal degradation of CP from expeller soybean meal compared to soybean meal was previously reported (Borucki Castro et al. 2007). It was considered as a result of protein solubility reduction and denaturation caused by thermal treatment. Ruminal degradation of AA may be affected by the type of protein in the feedstuffs and their inherent characteristics and AA profiles, and also by different processing methods (Mjoun et al. 2010; Zralý and Písaříková 2009). Ruminal degradation of AA followed a similar pattern to that of CP for different feedstuffs. Increases in RUP often lead to a reduction of RDP and changes in absorbed AA. This intestinal digestibility coefficient is similar to the NRC (2001) assumption of 80%, but the reported estimates are also highly variable ranging from 59.2–95.0%. The wide range of reported estimates of intestinal digestibility of RUP and the number of differing methods used to estimate it illustrate the need for additional study of factors which influence the intestinal digestibility of RUP in ruminant feeds.

In dairy nutrition, Lys is an important nutrient representing 16.0% of the total essential AA contained in milk (NRC 2001). Lys is usually the most sensitive AA to be affected by methods used to protect proteins from ruminal degradation and is often lost at levels 5 ± 15 × higher than other AA. Currently, data reported in the literature on the intestinal digestibility of AA of RUP for individual feedstuffs are insufficient to incorporate AA of RUP digestibility coefficient into nutritional models. Therefore, further research is needed to measure the variation in the intestinal digestibility of AA of RUP within and among feeds. Differences in absorbable AA among feedstuffs resulted mainly from differences in the rumen degradability and degree of digestion in the small intestine. The prevalent factor contributing to differences in availability of AA between our study and the studies by Mjoun et al. (2010); Borucki Castro et al. (2007); and Kleinschmit et al. (2007) was a difference in the rumen degradability and intestinal digestibility. The values of IADP of estimated feeds will help to control the quality of protein feeds as sources of RUP for ruminants. Results of different studies indicate that corn gluten meal (74%) provided the largest amount of IADP, followed by non-enzymatically browned soybean meal (39.4%) and mechanically extracted soybean meal at 41.3% (Stern et al. 2006). The value of IADP or absorbable AA can be used as a guideline to select protein supplements for high producing dairy cows. Such information concerning intestinal digestibility of AA of RUP and absorbable EAA is needed for the development of diet formulation models to optimize the AA nutrition of dairy cows.

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