



Evaluation of ruminal crude protein degradation of common feeds used in temperate climates

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ABSTRACT

The objective of this study was to evaluate the nutritional value of different feeds using chemical analysis and procedures to determine the nitrogen fractions expressed in g/kg of crude protein (CP) using a Cornell system. The experiment includes samples of common feeds used for ruminants in the Czech Republic. Fraction A is most commonly available from silages (average 468.2 g/kg CP) and least available in soybean (83.9 g/kg CP). In contrast, rapidly degradable protein (fraction B1) showed the lowest values in silages (average 31.5 g/kg CP) and the highest values in rapeseed cakes (average 195.7 g/kg CP) and lupines (average 308.7 g/kg CP). The intermediately degradable protein fraction B2 had a high value in almost all of the samples and especially in lupines (average B2 fraction 384.7 g/kg CP). The remaining fractions (B3 and C) represent slowly degraded proteins and unavailable proteins and represented a very small part of CP (average 18.9 and 34.9 g/kg CP, respectively). A strong relationship was found between fraction A and soluble protein (SOLP) and fraction B1 and SOLP. Other strong correlations were found between fraction B2 and CP, B2 and insoluble protein (IP), B3 and IP and B3 and neutral detergent insoluble nitrogen (NDIN).

Key words: Cornell system, Nitrogen fractions, Ruminal degradability, Soluble protein.

INTRODUCTION

System for evaluation of the fractions of nitrogenous substances is the Cornell Net Carbohydrate and Protein System (CNCPS) (Lanzas *et al.*, 2008), which uses the chemical fractionation procedure described by Licitra *et al.* (1996). Crude protein (CP) is subdivided into 5 fractions (A, B1, B2, B3 and C) according to their degradability and passage rate in the gastrointestinal tract (Van Soest, 1994). Fraction A consists of non-protein nitrogen (NPN) (Bovera *et al.*, 2003) that can be separated using trichloroacetic acid (TCA) according to the method described by Licitra *et al.* (1996). Fraction B1 is expressed by estimating the true soluble protein (SOLP) in borate phosphate buffer at pH from 6.7 - 6.8. Fraction B2 is classified as neutral detergent soluble protein and is estimated as the difference between buffer insoluble protein (IP) and neutral detergent insoluble nitrogen (NDIN). Fraction B3 is insoluble in neutral detergent but soluble in acidic detergent; thus, acid detergent insoluble nitrogen (ADIN) is subtracted from NDIN (Licitra *et al.*, 1996). The amount of soluble fibre-bound CP (fraction B3) is calculated as the difference between the NDIN and ADIN. Fraction C is referred to as ADIN (Bovera *et al.*, 2003) and is measured by estimating the nitrogen in the acid detergent fibre (ADF) residue (Parashuramulu *et al.*, 2013).

Protein is considered as basic feed component (Katsande *et al.*, 2015) and knowledge of the kinetics of ruminal degradation of feed proteins is fundamental to formulating diets for adequate amounts of rumen degradable protein for rumen microorganisms and for the host animal (Kumar and Ravi, 2015). The CNCPS uses feed carbohydrate and protein degradation and passage rates to predict the extent of ruminal fermentation, microbial

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protein production, post-ruminal absorption and the total supply of metabolized energy and protein for the animal (Fox *et al.*, 2004; Lanzas *et al.*, 2008). To improve the feed efficiency in ruminants, carbohydrate and protein availability in the rumen should be precisely balanced. Nutritional models aid in the process of farm decision making by predicting the animal performance and nutrient excretion and assessing diet adequacy under a range of management and feeding situations (Fox *et al.*, 2004). Decreasing negative environmental nitrogen pollution due to farming is necessary. Therefore, it is important to optimally formulate feed rations to meet but not exceed the nitrogen requirements of rumen microbes and the amino acid requirements of the ruminants (Schwab *et al.*, 2003).

For this purpose, the study was aimed to determine the nutritional value of various feeds using chemical analysis and procedures to determine the nitrogen fractions using a CNCPS model to estimate A, B1, B2, B3,

C, NPN, SOLP, ADIN and NDIN. The benefit of this determination is more accurate characterization of the nutritional value of the feeds, detailed laboratory procedures and a tendency to apply this CNCPS into routine practice.

MATERIALS AND METHODS

Various feed samples ($n = 15$) were selected for the experiments, including lupine seeds, concentrates and roughages. List of the evaluated samples is provided in Table 1. Estimated samples (not including samples number 14 and 15) were originated from the common agricultural conditions. Mountain pasture forages (harvested at the Krkonoše Mountains National Park, Czech Republic, locality of Zadní Rennerovky, altitude from 1170 - 1320 m above sea level) was evaluated as two mitres of the same sward harvested during one vegetation season. The fresh material was dried at 50°C. The dried material was subsequently milled and passed through a 1 mm sieve for laboratory analysis.

The dry matter (DM), ash, CP, ether extract (EE), neutral detergent fibre (NDF), ADF and acid detergent lignin (ADL) were analyzed in the samples. Ash-free concentrations of NDF, ADF and ADL were determined according to the methods described by Van Soest *et al.* (1991). Crude protein was analyzed according to the Kjeldahl method (nitrogen $\times 6.25$). Ether extract was determined using Soxtec extraction with petroleum ether and ash was determined after 4.5 h of combustion at 550°C (AOAC, 2005). The total heating value (gross energy; GE) was measured using a calorimeter (IKA C 5000 control, IKA-Werke GmbH and Co. KG, Staufen, Germany).

The methods (Licitra *et al.*, 1996) used to determine the fractions of CP in ruminant feed of as follow: (1) non-protein nitrogen was measured using TCA; (2) determination of soluble nitrogen and protein; (3) determination of nitrogen insoluble in acid detergent using the Fibertec apparatus and (4) determination of nitrogen insoluble in neutral detergent

using the Fibertec apparatus. These procedures required the Kjeltex 2400 analyzer (apparatus for determining the residual protein and nitrogen according to Kjeldahl) and the Fibertec, which is an apparatus used to determine individual fractions of structural carbohydrates (ADF, NDF, ADL). Nitrogen fractions were calculated according to Kelzer *et al.* (2010). Fraction A is the same as NPN; fraction B1 is calculated as the difference of NPN from SOLP; fraction B2 is CP without SOLP and NDIN; fraction B3 is calculated by subtracting ADIN from NDIN and fraction C is the same as ADIN.

The data were statistically analyzed using the SAS 9.3 (SAS, 2003) GLM procedure (PROC GLM) and the PROC CORR procedure to evaluate the correlation coefficients between the observed variables. Statistically significant differences were evaluated using Scheffe's method (SAS, 2003). Statistical significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

The chemical composition and energy values of lupine seeds, concentrates and roughages are presented in Table 2. As expected, the CP of pasture forages decreased and the fibre and lignin contents increased as the plants matured towards the first mitre (sample 14) and second mitre (sample 15) (Table 2). Other feeds showed typical CP and fibre contents, chemical compositions and GE. The chemical compositions of the feeds were affected by many factors, such as the soil type, fertilization, climate and processing to by-products (Van Soest, 1994; Valderrama and Anrique, 2011). The CP of the samples ranged from 69.5 to 531.3 g/kg DM. The CP values of the Amiga and Prima lupines (293.3 and 386.3 g/kg DM, respectively) were not significantly different from the results reported by Straková *et al.* (2006) (329.6 and 305.7 g/kg DM, respectively). Variations in the NDF, ADF and ADL values are shown in Table 2. Forage exhibited increasing lignification with rising plant maturity (ADL = 43.9 and 64.9 g/kg DM for the first and second mitres, respectively). This trend has been cited in several publications (Rinne and Nykänen, 2000; Homolka *et al.*, 2012). The GE of the estimated samples had an average of 19.7 MJ/kg DM.

The nitrogen fractions of the estimated samples are provided in Table 3. Fraction A is instantaneously degraded in the rumen. In this study, fraction A varied from 83.9 to 612.1 g/kg CP. The smallest value was calculated for the soybean seed. The low NPN content in the soybean seed may occur because soybeans possess anti-nutritional substances associated with tannins and a trypsin inhibitor that blocks digestive enzymes and reduces protein digestibility (Mahmood *et al.*, 2007). Therefore, the soybean NPN is low (84.0 g/kg CP), whereas the intermediately soluble nitrogen (B2 fraction, 356.4 g/kg CP) and slowly soluble nitrogen (fraction B3, 422.2 g/kg CP) were considerably higher. The average fraction A content for lupines was 252.9 g/kg CP. This value is close to the fraction A value found by Cazzato *et al.* (2012) for lupines. Alzueta *et al.* (2001) determined that the fraction A content in

Table 1: List of forage samples.

Sample No.	Feed
1	White lupine variety AMIGA
2	White lupine variety PRIMA
3	Clover silage
4	Corn silage
5	Rye grass silage
6	Grass silage
7	Alfalfa silage
8	Barley meal
9	Wheat meal
10	Rapeseed cake
11	Extruded rapeseed cake
12	Non-extruded rapeseed cake
13	Soybean seed
14	Mountain pasture forage of first miter
15	Mountain pasture forage of second miter

legumes *Vicia sativa* L., vetch forage conventionally used as the animal feeds (Swain *et al.*, 2016), ranged from 211.0 to 329.0 g/kg CP. The fraction A for our results of grain meals, rapeseed feeds, pasture forages and silages achieved mean values of 144.9, 206.8, 296.3 and 468.2 g/kg CP, respectively. These findings are in agreement with several authors (Choi *et al.*, 2002; Chrenková *et al.*, 2014; Polat *et al.*, 2014). Fraction A exhibited a strong correlation ($P < 0.001$) only with SOLP ($r = 0.758$). Fraction B1 represents a portion of the SOLP. Thus, fraction A (NPN) and B1 are SOLP. The lupine fraction B1 (average 308.7 g/kg CP) and B2 (average

384.7 g/kg CP) corresponded to the values cited by Cazzato *et al.* (2012) (B1 = 214.0 g/kg CP and B2 = 346.0 g/kg CP). The same author noted that data on the nitrogen fractions determined according to the CNCPS system of lupine grains were lacking. Therefore, the determination of protein quality is useful for determining the nutritive value and the degradability properties of seeds. The authors further confirmed in their study that the lupine CP contents were likely to be largely degraded in the rumen (Cazzato *et al.*, 2012). The fraction B1 of rapeseed feed (349.0 g/kg CP), rapeseed meal (171.0 g/kg CP) and

Table 2: Chemical composition (g/kg DM) and gross energy value (MJ/kg DM) of the estimated samples.

Sample	DM	CP	EE	OM	ADF	NDF	ADL	GE
1	907.5	293.3	97.9	957.4	320.4	550.6	60.0	20.6
2	891.5	386.3	95.6	956.1	237.4	495.6	54.9	20.8
3	933.5	179.8	17.5	905.6	380.3	423.1	52.9	18.3
4	931.3	69.5	28.1	955.2	259.9	434.4	28.0	18.2
5	943.5	192.8	26.4	807.8	257.8	364.2	21.1	18.7
6	898.7	161.3	24.4	926.1	409.7	459.4	103.7	19.3
7	903.4	187.8	17.3	876.7	79.9	468.6	94.0	19.3
8	885.2	115.1	26.0	970.1	77.4	195.4	12.6	18.4
9	962.9	144.1	128.3	703.2	74.8	147.1	8.2	16.8
10	910.0	314.6	109.9	914.4	166.3	188.5	70.1	21.3
11	933.4	313.2	141.4	934.5	214.9	197.7	79.4	21.9
12	938.4	319.1	133.2	935.0	234.6	188.3	87.4	21.6
13	926.7	531.3	26.4	923.6	79.9	151.2	6.3	19.8
14	891.7	237.5	18.7	940.0	308.6	724.4	43.9	20.2
15	897.8	132.0	22.6	936.5	381.2	676.2	64.9	19.6

ADF = acid detergent fibre, ADL = acid detergent lignin, CP = crude protein, DM = dry matter, EE = ether extract, GE = gross energy, NDF = neutral detergent fibre, OM = organic matter

The values are arithmetic means ($n = 2$) based on dry matter.

Table 3: Determination of the nitrogen fractions (g/kg CP) of the estimated samples.

Sample	A	B1	B2	B3	C
1	223.3 ^{b,c}	301.8 ^b	414.0 ^d	21.8 ^{h,i,j}	39.1 ^g
2	282.5 ^a	315.5 ^a	355.4 ^c	16.0 ^{i,j}	30.6 ^g
3	384.9 ^{b,c}	66.5 ^d	183.2 ^{g,h,i}	224.4 ^d	141.0 ^c
4	327.3 ^e	17.6 ^d	456.2 ^{h,i}	125.2 ^{g,h}	73.8 ^h
5	594.7 ^a	68.9 ^d	215.4 ^{f,g,h}	20.8 ^{i,j}	100.2 ^f
6	422.0 ^{b,c}	1.0 ^d	177.9 ⁱ	7.0 ^j	391.6 ^a
7	612.1 ^a	3.6 ^d	192.6 ^{f,g,h,i}	60.4 ^{f,g}	131.2 ^{c,d}
8	156.3 ^e	115.0 ^d	396.3 ^f	287.8 ^e	44.6 ^h
9	133.4 ^e	127.7 ^{c,d}	607.5 ^e	80.9 ^{f,g}	50.5 ^h
10	193.3 ^c	118.3 ^c	534.3 ^b	42.5 ^f	111.6 ^b
11	243.3 ^b	244.0 ^b	432.3 ^c	13.8 ^{i,j}	66.4 ^{e,f}
12	183.7 ^c	224.9 ^b	346.8 ^d	174.9 ^c	69.7 ^{d,e}
13	83.9 ^d	74.5 ^c	356.4 ^a	422.2 ^a	62.9 ^b
14	296.9 ^{b,c}	25.7 ^d	356.9 ^e	268.6 ^b	51.9 ^g
15	295.6 ^d	44.3 ^d	337.1 ^{f,g}	226.8 ^e	96.2 ^g

A = non-protein nitrogen, B1 = rapidly degradable protein, B2 = intermediately degradable protein, B3 = slowly degradable protein, C = bound (indigestible) protein, CP = crude protein

The values are arithmetic means ($n = 2$) on a dry matter basis

a,b,c,d,e,f,g,h,i,j Means with different superscript differ significantly ($P < 0.05$).

soybean meal (88.0 g/kg CP) reported by Chrenková *et al.* (2014) roughly corresponded to the rapeseed feed (average 195.7 g/kg CP) and soybean seed (74.5 g/kg CP) B1 found in this study. The study of Mikolayunas-Sandrock *et al.* (2009) showed nitrogen fractions for soybean meal that corresponded to different values for fraction B1 (90.3 g/kg CP) and especially fraction B2 (708.8 g/kg CP). Our nitrogen fraction calculations provided results of 74.5 g/kg CP for the soybean seed fraction B1 and 356.4 g/kg CP for fraction B2. This difference may be due to the impact of feed processing as discussed by earlier Hvelplund and Weisbjerg (2000), Givens and Rulquin (2004). Fraction B1 has a very strong correlation ($P < 0.0001$) with SOLP ($r = 0.839$). Moreover, a strong correlation was confirmed ($P < 0.01$) between B1 and the CP ($r = 0.665$), EE ($r = 0.700$) and GE ($r = 0.653$) (Table 4). Fraction B2 ranged from 177.9 to 607.5 g/kg CP. The higher range of the B2 fraction was found in

concentrated feeds (average 445.6 g/kg CP) and lupines (average 384.7 g/kg CP). These high values correspond to the values reported for soybeans (567.0 g/kg CP) by Bertipaglia *et al.* (2008). Alzueta *et al.* (2001) reported a B2 fraction value of 337.0 g/kg CP for the common vetch (*Fabaceae*) and Cazzato *et al.* (2012) showed a B2 fraction value of 346.0 g/kg CP for lupines; both of these results corresponded with the results of our study. A very strong correlation ($P < 0.0001$) was confirmed between fraction B2 and CP ($r = 0.891$; Fig 1) and B3 and IP ($r = 0.836$; Fig 2).

Lower correlation coefficients with a significance level $P < 0.01$ were found between fraction B2 and B1 ($r = 0.682$). The correlations ($P < 0.05$) between B2 and EE and B2 and GE achieved values of 0.595 and 0.621, respectively. Fraction B3 (Table 3) varied from 7.0 to 422.2 g/kg CP and accounted for an average of 184.4 g/kg CP for grain meals,

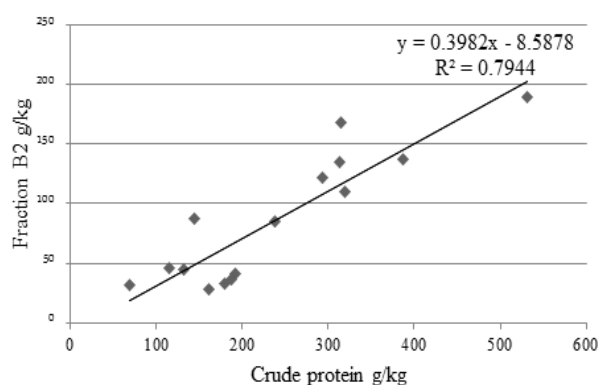


Fig 1: The trendline of the nitrogen fraction B2 with crude protein (g/kg).

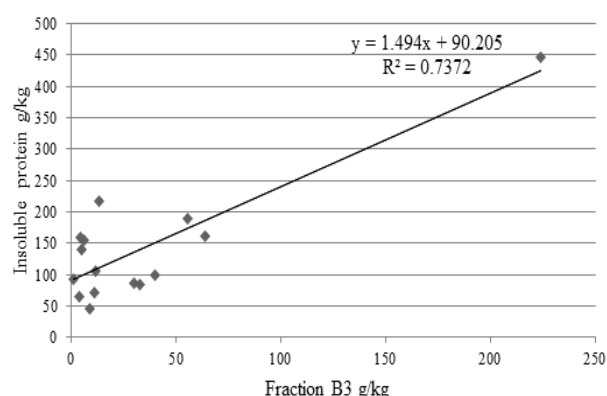


Fig 2: The trendline of the nitrogen fraction B3 with insoluble protein (g/kg).

Table 4: Correlation coefficients of selected variables (chemical analysis of essential nutrients and the fractions of CP).

Items	CP	EE	NDF	ADL	OM	GE	NPN ¹	IP	SOLP	NDIN	B1
EE	0.349										
NDF	-0.285	-0.487									
ADF	-0.240	-0.222	0.669**								
ADL	0.013	0.181	0.263								
OM	0.208	-0.174	0.309	0.317							
GE	0.634**	0.469	0.021	0.572*	0.559*						
NPN ¹	0.283	-0.045	0.292	0.442	-0.020	0.373					
IP	0.890***	0.175	-0.389	-0.189	0.156	0.411	-0.085				
SOLP	0.612**	0.450	0.061	0.356	0.178	0.655**	0.758***	0.185			
ADIN ²	0.243	-0.124	-0.113	0.537*	0.057	0.215	0.257	0.275	0.046		
NDIN	0.642**	-0.252	-0.259	-0.232	0.136	0.107	-0.151	0.870***	-0.124		
B1	0.665**	0.700**	-0.155	0.155	0.279	0.653**	0.281	0.344	0.839***	-0.056	
B2	0.891***	0.595*	-0.413	-0.083	0.129	0.621**	0.014	0.836***	0.471	0.457	0.682**

ADF = acid detergent fibre, ADIN = acid detergent insoluble nitrogen, ADL = acid detergent lignin, B1 = rapidly degradable protein, B2 = intermediately degradable protein, CP = crude protein, EE = ether extract, GE = gross energy, IP = insoluble protein, NDF = neutral detergent fibre, NDIN = neutral detergent insoluble nitrogen, NPN = non-protein nitrogen, OM = organic matter, SOLP = soluble protein

¹The results of NPN determinations are identified with nitrogen fraction A

²The results of ADIN determinations are identified with nitrogen fraction C

*statistical significance $P < 0.05$, **statistical significance $P < 0.01$, ***statistical significance $P < 0.001$.

77.1 g/kg CP for rapeseed feeds, 247.7 g/kg CP for pasture forages and 87.6 g/kg CP for silages. The highest value (422.2 g/kg CP) was obtained for soybeans. The results of Cazzato *et al.* (2012) illustrated that the lupine CP was largely degraded in the rumen. Our results with lupines (mean B1, B2 and B3 values of 308.7, 384.7 and 18.9 g/kg CP, respectively) showed the same trend, with the results (214.0, 346.0 and 175.0 g/kg CP, respectively) of Cazzato *et al.* (2012). These differences between our results and the results of Cazzato *et al.* (2012) may be due to the different NDF (523.1 versus 215.0 g/kg DM) and ADF values (278.9 versus 121.0 g/kg DM). A strong relationship ($P < 0.001$) was found between fraction B3 and IP ($r = 0.859$) and B3 and NDIN ($r = 0.969$) and between B3 and CP ($r = 0.924$) ($P < 0.01$). The fraction C of our grain meals was 44.6 g/kg CP (barley meal) and 50.5 g/kg CP (wheat meal). These C values almost corresponded with the values of Choi *et al.* (2002) (14.0 g/kg CP for barley), Gupta *et al.* (2011) (26.0 g/kg CP for barley grain), and Polat *et al.* (2014) (19.0 g/kg CP for wheat and 31.0 g/kg CP for barley). Generally, the highest C values were found for silages (Table 3). Fraction C, which is referred to as ADIN, was measured by estimating the nitrogen in the ADF residue and had a strong correlation ($P < 0.05$) with ADL ($r = 0.537$). Significant differences ($P < 0.05$) were observed in the individual nitrogen fractions (A, B1, B2, B3 and C) among the evaluated feeds.

In conclusion, this study highlights the importance of the Cornell system, which targets the nutrition and digestion of ruminants to account for the physiological dynamics of the digestive tract and the animal nutrient requirements. The fractionation of CP provides a broader view of the digestibility of the received feeds in the ruminant digestive tract. These procedures allow proper balancing of feed nutrients, their ratios and their concentrations for the proper functioning of the rumen. Especially during the period of calving and during the transit period is important to regulate the supply of nutrients, including proteins. Information on protein degradability helps to reduce the potential for protein transfer, which can arise during this period with the supply of more contrived and better feed. The results are a valuable source of feed database information that is important for formulating livestock rations.

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