Characterization of proteins in feeds according to the CNCPS and comparison to *in situ* parameters

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ABSTRACT: Several methods were used for the evaluation of feed protein for rumen degradability and intestinal digestibility of rumen undegraded protein (RUP). The aim of this work was to explore the possibility of using the Cornell method of predicting the fraction RUP. An *in situ* method was adopted for estimation of degradability parameters (a, b, c) and effective degradable crude protein (EDCP), and five nitrogen fractions (A, B_1, B_2, B_3, and C) were determined according to the Cornell Net Carbohydrate and Protein System (CNCPS). Fifty-one feedstuffs – 13 cereals grains, 5 legume seeds, 3 oilseeds, 11 oilseed by-products, 4 distillers dried grains with solubles (DDGS), and 15 silages (maize, lucerne, grass, and grass-clover) – were used in this work. The examined feedstuffs varied widely in nutrient composition. Fraction B_1 (soluble true protein) in forages was small (2.5–5.7% of total N), but it varied to a large extent in concentrates (DDGS 0.9–1.2, legumes 46.5–63.7, oilseeds meal 17.1–51.8% of total N). Fraction B_2 represented a large proportion of the total protein in oil seed meals (44.3–82.6% of total N) and in DDGS (55.8–77.8% of total N), too. Fraction B_3 was relatively small (less than 10% of total N) in all feedstuffs and declined with increasing acid detergent insoluble nitrogen (ADIN) concentration. The concentration of ADIN fraction in feeds affected ruminal degradability. Lucerne silage, with a high content of ADIN (30.9% of total N), had a low effective crude protein (CP) degradability (57%). Correlation between EDCP and fraction A was $r = 0.76$. A weaker correlation ($r = 0.67$) was found between *in situ* parameter "b" and fractions B_2 and B_3 ($r = 0.59$), respectively. The results show that much more samples of all feed types should be analyzed to obtain results allowing a more exact prediction of CP degradability and RUP.

Keywords: feedstuffs; nitrogen fractions; Cornell Net Carbohydrate and Protein System; *in situ* method; effective crude protein degradability

INTRODUCTION

Estimates of potential degradability and rates of degradation in the rumen are prerequisites in feed/ration evaluation systems that model rumen dynamics. Currently, *in situ* and *in vivo* methods are the main reference methods used in European systems (Hvelplund et al., 2009). However, these methods require too many resources to be appropriate for running calibrations; hence, wet chemistry laboratory methods would be a prerequisite for a robust feed analysis programme. Therefore, the aim of this work on laboratory methods was to improve and develop methods for determination of the intrinsic value of feeds.

Existing protein models (PDI – Vérité and Peyrand, 1989; DCE/OEB system – Tamminga et al., 1994; AAT/PBV – Madsen et al., 1995; NRC, 2001) for ruminants are used to predict the protein value of feeds and assess animal requirements for rumen degraded and intestinally digested and absorbed protein. These models help predicting

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animal performance and nutrient excretion and can be used in diet formulation under a range of management and feeding situations (Fox et al., 2004). These systems estimate rumen degradable protein and separate requirements into the needs of microorganisms and the needs of the animal. Utilizable protein is defined as the true protein absorbed by the intestine, supplied by microbial crude protein and undegraded intake protein.

Nutritive value of crude protein (CP) in feedstuffs for ruminants is described by the rate and extent of degradation in the rumen and intestinal digestibility of rumen undegraded protein (RUP). The amount of CP that will be degraded in the rumen (RDP) is affected by many factors, including proportional content of proteins and non-protein nitrogen (NPN), physical and chemical properties of proteins, and rumen retention time. To characterize feed protein it is essential to obtain accurate and precise values of RUP and RDP. The current models of feed evaluation are based on protein fractionation. In Europe, the *in situ* technique of Ørskov and McDonald (1979), and modifications of this procedure have been well accepted for estimating rumen protein degradability (Hvelplund et al., 2009). *In situ* techniques (PDI, DCE/OEB, NRC, AAT/PBV) and solubility in buffers and detergent solutions (Cornell Net Carbohydrate and Protein System – CNCPS) are used to fractionate protein in feeds.

The aim of this study was to determine rumen *in situ* degradability characteristics of CP and intestinal digestibility of RUP, and to fractionate CP according to the CNCPS for samples of various concentrate feedstuffs and silages, and to determine whether the CNCPS can be used to estimate *in situ* values for degradation.

MATERIAL AND METHODS

Fifty-one feed samples: 13 cereals grains, 5 legume seeds, 3 oilseeds, 11 oilseed by-products, 4 distillers dried grains with solubles (DDGS), and 15 silages (maize, lucerne, grass, and grass-clover) were used in this study. Silages were freeze-dried. The feeds are commonly used in cattle rations in practice. Twenty samples of feed were from Denmark, five samples from Germany, six samples from the Czech Republic, and twenty samples from Slovakia.

Samples were milled on hammer mill with a 3 mm screen for *in situ* incubations and on a 1 mm screen for chemical and solubility analysis. Nitrogen (Kjeldahl method), ash, and dry matter contents were determined according to the Weende analysis (Commission Regulation, 2009), and neutral detergent fibre (NDF), acid detergent fibre (ADF), and lignin contents were determined by Van Soest procedures (Lutonská and Pichl, 1983). Starch content was determined by the enzymatic method according to Salomonsson et al. (1984). Nitrogen fractionation was based on solubility in borate-phosphate buffer and in detergent solutions (Figure 1). The choice of the method for NPN determination depends on the desired objectives. The most common precipitants are tungstic acid and trichloracetic acid (Licitra et al., 1996). Tungstic acid was used in this experiment.

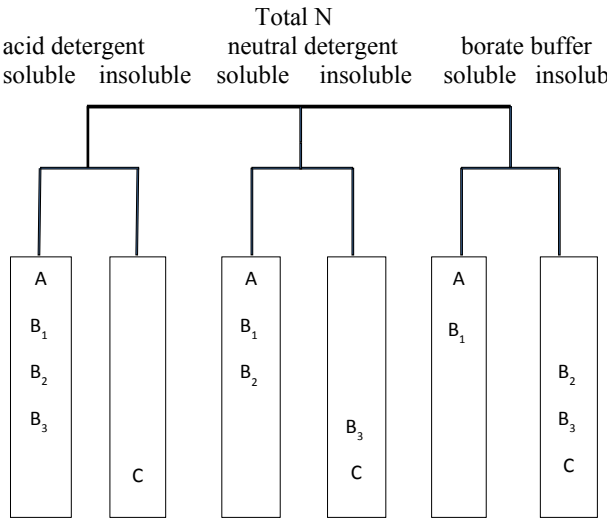

Figure 1. Nitrogen fractionation based on solubility in detergent solutions and borate-phosphate buffer (Tham et al., 2008)

fraction A – soluble in buffer and tungstic acid, fraction B_1 – soluble in buffer and precipitated by tungstic acid, fraction B_2 – insoluble in buffer but soluble in neutral detergent, fraction B_3 – soluble in acid detergent but insoluble in neutral detergent, fraction C – insoluble in acid detergent

Effective protein degradation (ECPD) and degradation parameters (a, b, c) were determined by the *in situ* method (Harazim and Pavelek, 1999). Three rumen fistulated cows (fed twice a day, diet 70% forage and 30% concentrate on dry matter basis) were used. Samples were incubated for 3, 6, 9, 16, 24, 48, 72, and 96 h (with a minimum of three bags per animal, incubation, and feed). Pore size of bag material was 47 µm. Particle losses were determined by washing the bags filled with feeds in domestic washing machine in the cycle

3 × 5 min without spinning. Protein degradation parameters and ECPD were calculated using equations of Ørskov and McDonald (1979) assuming a rumen outflow rate of 0.06/h.

Results were analyzed by One-Way and Two-Way Analysis of Variance with unequal subclass numbers of observations per cells (Grofík and Fľak, 1990) with Bonferroni multiple comparison test, and by using Student's *t*-test. The Pearson's correlation between CNCPS and *in situ* characteristics was measured. The statistical package Statistix (Version 8.0, 2001) was used for statistical analysis.

RESULTS AND DISCUSSION

Chemical composition. The examined feedstuffs varied widely in nutrient composition (Tables 1 and 2). The content of cell walls (NDF) and their lignification were partially responsible for the degree of CP availability in feedstuffs. Large variability in NDF content was found in some groups of feeds (triticale, sunflower meal, maize and lucerne silages). Oil seed feeds, lucerne silages, and grass clover silage were also high in lignin content.

Protein degradation and solubility. Proteins were fractionated using the CNCPS system into three major fractions (Pichard and Van Soest, 1977): NPN (A), true protein (B), and unavailable nitrogen (C) true protein. Then, based on the inherent rates of ruminal degradation, fraction B was fractionated into subfractions B_1, B_2, and B_3 (Van Soest et al., 1981; Sniffen et al., 1992).

The proportion of total N that was buffer soluble N (SN) was the lowest in maize grain and distillers grain, followed by palm kernel cake, rapeseed meal, and sunflower cake (20–28%), and the highest in blue lupine, pea, and field beans (60–73%); in oil seeds, SN was about 50% of total N (Table 3). According to Lanzas et al. (2007), fraction A represents approximately 50% of the soluble protein in concentrates. A very high concentration of buffer soluble N and fraction A was found in lucerne silage as a result of intensive protein hydrolysis during ensiling. Like the percentage of buffer soluble CP, NPN was very high in forages, ranging 81–95% in lucerne silages and 84–97% in maize silages. High proportions of NPN in soluble CP in fermented forages and low (5%) proportion in soybean meal have also been commented by Van Amburgh et al. (2012). Feeds like DDGS and heat processed cereal grains were very low in both NPN and buffer soluble N (Chrenková et al., 2010b, 2011a, 2012b).

Soluble true protein (B_1) in forages represented a small fraction of total N; however, B_1 varied widely in concentrates. Except for oats and peanut meal, most feedstuffs contained less than 10% of total N in the form of soluble true protein (Krishnamoorthy et al., 1982). These samples of DDGS contained very low amounts of B_1 (0.9–1.2% of total N) (Chrenková et al., 2010b). In wheat, barley, and maize, B_1 constituted 17.5, 13.0, and 2.4% of total N, respectively (Chrenková et al., 2012b); this proportion decreased due to flaking process in wheat (3.9%) and barley (1.0%). Changes in B_1 were not observed with flaking of maize. Oilseed meals (rapeseed meal, sunflower meal, soya bean meal) were significantly different ($P < 0.01$) in fraction B_1 (Table 4); fraction B_1 was only 8.7% of total N in soybean meal.

The B_2 fraction corresponds to the protein fraction of intermediate degradability and is estimated as buffer insoluble protein minus the protein insoluble in neutral detergent. As a proportion of

Table 1. Chemical composition (mean ± SD) of examined samples of cereals and legumes

Feeds	*n*	Content of nutrients (g/kg DM)				
		CP	starch	NDF	ADF	lignin
Maize	4	84.2 ± 4.0	733.3 ± 8.7	139.3 ± 29.8	37.4 ± 4.4	14.8 ± 3.1
Triticale	3	112.6 ± 0.5	675.3 ± 6.3	127.5 ± 42.4	38.7 ± 0.1	20.5 ± 1.7
Rye	3	129.8 ± 13.3	592.0 ± 23.9	155.9 ± 11.9	36.3 ± 2.7	9.3 ± 0.7
Wheat	3	137.0 ± 5.9	671.5 ± 17.3	143.2 ± 40.3	36.9 ± 6.97	9.3 ± 0.6
Pea	2	237.1 ± 35.8	447.2 ± 100.6	191.8 ± 9.4	78.6 ± 7.4	16.8 ± 7.5
Field beans	1	289.8	329.5	223.6	110.2	2.3
Field pea	1	204.3	485.6	179.9	135.5	26.4
Lupine	1	325.9	39.4	303.6	256.2	40.7

CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre

Table 2. Chemical composition (mean ± SD) of examined samples of oilseed feeds, DDGS, and silages

Feeds	*n*	Content of nutrients (g/kg DM)			
		CP	NDF	ADF	lignin
Linseed	1	235.6	253.0	166.6	75.2
Rapeseed	2	194.1 ± 7.3	329.6 ± 14.4	249.5 ± 3.7	*
Linseed cake	1	367.4	288.3	185.4	69.0
Rapeseed cake	1	306.2	201.5	223.6	93.6
Sunflower cake	1	317.4	277.8	229.5	111.4
Linseed meal	1	400.1	242.2	152.6	65.2
Rapeseed meal	3	366.6	254.8	244.9	103.7
Sunflower meal	4	375.8 ± 69.4	355.2 ± 109.6	279.7 ± 89.4	97.1 ± 34.5
DDGS	4	306.5 ± 15.0	324.0 ± 37.7	156.5 ± 5.1	54.4 ± 11.7
Maize silage	6	69.4 ± 18.6	450.5 ± 83.5	225.1 ± 33.0	31.7 ± 9.3
Lucerne silage	7	154.4 ± 37.9	474.2 ± 116.6	396.8 ± 102.9	98.5 ± 49.6
Grass silage	1	139.7	628.1	298.3	57.8
Grass clover silage	1	228.1	397.6	279.5	71.5

DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, DDGS = distillers dried grains with solubles
*not analyzed

Table 3. Crude protein solubility (mean of analyzed samples) for concentrate feeds and silages

Feed	*n*	CP (g/kg DM)	Soluble N (% of total CP)		
			SN	A	B_1
Maize	2	100	15.9	13.6	2.4
Triticale	2	113	37.1	11.0	26.6
Pea	2	237	70.5	11.3	59.2
Field bean	2	300	59.8	12.9	46.5
Lupine (blue)	1	326	73.2	9.5	63.7
Linseed	1	236	55.8	8.1	47.7
Linseed cake	2	376	47.3	9.9	36.8
Linseed meal	1	176.	59.2	9.5	49.7
Rapeseed	2	194	45.5	10.6	34.9
Rapeseed meal	3	366	27.0	9.9	17.1
Soybean meal	4	512	11.7	2.8	8.8
Sunflower cake	1	317	28.4	8.8	19.2
Sunflower meal	4	353.2	41.2	10.3	51.8
Palmkernel cake	1	176	20.4	10.4	9.9
Distillers grain	2	318	15.6	14.5	1.5
DDGS	3	293	7.7	7.0	0.5
Maize silage	3	62	25.9	18.1	6.5
Lucerne silage	3	186	64.5	56.6	7.9

CP = crude protein, DM = dry matter, DDGS = distillers dried grains with solubles, SN = buffer soluble N, A = tangstic acid soluble N (non-protein nitrogen), B_1 = soluble true protein

total N, the B_2 fraction varied over a wide range, from 23% for oilseeds to 86% for soybean meal. The results in Table 4 also show the significant difference among crude protein feeds in fraction B_2. The fraction B_2 represented the largest proportion of the total protein in oilseed meals. Lower proportions were found in silages (23–59%) (Chrenková and Čerešňáková, unpublished). The fraction B_2 is significantly affected by the amount of neutral detergent insoluble nitrogen (NDIN). More than 12.5 g/kg DM of NDIN was determined in DDGS from maize, wheat, and triticale (Chrenková et al., 2010b). Heat processing significantly affected concentration of NDIN as also was found for cereal grains, where the concentration of NDIN increased with heat processing. Heat processing increased the B_2 fraction by about 20%, from (% of total N) 56.4 to 77.9 for wheat, from 57.2 to 75.5 for barley, and from 56.2 to 78.2 for maize. Fraction B_3 was less than 10% of total N (Chrenková et al., 2012a).

Concentrations of B_2 were significantly higher ($P < 0.05$) in lucerne silages (LS) than in maize silages (MS) but, like a proportion of total N (Table 5), the difference was non-significant (32.1 and 39.9%, respectively). Fraction C (unavailable protein) varied considerably in both LS and MS (6.2–30.9% of total N for LS and 6.8–42.6% of total N for MS) but means for these silages were similar (17.4 and 12.3%, respectively). Incorrect technology of silaging occurred leading to heat-

ing of the ensiled mass and thermal damage of proteins. This caused increase in the fraction C. At higher temperatures the Maillard reaction between proteins (between the free-NH_2 groups of lysine) and carbohydrates, especially in maize silage, takes place. In lucerne silage, also complexes between tannins and proteins (Reed, 1995; Halalsheh et al., 2007) are formed. *In situ* effective CP degradability (EDCP) of LS and MS was about 75% in both types of silage (Chrenková et al., 2011b). Very weak correlations were found in maize silages between selected parameters of quality (between RUP and NDIN, B_2 and C fractions, buffer insoluble N). The correlations between those parameters were significant in lucerne silages. The variability (v) of some observed parameters was high in both groups of silages (Table 5).

The NDIN forms a small fraction of total N in protein supplements; however, by-products such as brewers grains, beet pulp, and DDGS contain a large amount of NDIN and ADIN (Krishnamoorthy et al., 1982). According to Chrenková et al. (2012b), ADIN (% of total N) was about three times higher in DDGS than in wheat and triticale, 22.2 vs 6.2% and 27.9 vs 9.2%, respectively. Fraction B_3 was relatively low in all feedstuffs and declined with increasing ADIN concentration in feeds. The NDIN fraction forms an essential part of RUP. Accumulation of ADIN also occurs in response to spontaneous heating during ensiling (Rotz and Muck, 1994) and with targeted processing of feeds (such as flaking, toasting, and extruding). This decreases rumen protein degradation and increases the N of dietary origin flowing to the intestine. For lucerne silage with high ADIN (30.9% of total N) EDCP was very low (57%) and the degradation rate was only 0.057/h; however, effective CP degradation of lucerne silage with 9.6% ADIN of total N was 88.9% with a high degradation CP rate (0.112/h). Harvest and storage reduced protein degradation and truly CP digestion of baled hay as a consequence of spontaneous heating during storage (Coblentz and Hoffman, 2010). Protein degradation rate was reduced from 0.171 to 0.075/h, and increased RUP during harvest and storage (balled) of alfalfa hay (Broderick et al., 1992).

Correlations between in situ degradation and protein solubility. Buffer soluble N, CNCPS NPN (fraction A) and soluble true protein (fraction B_1) were compared with the protein degradation characteristics estimated *in situ* for selected concentrate feeds and silages (Chrenková et al., 2011b). Feeds with high proportions of buffer soluble N also had high *in situ* CP degradability, except for rapeseed meal, sunflower cake, soybean meal, and triticale. Correlation between the NPN fraction and the *in situ* "a" fraction was high for silages (r = 0.93 for silages and r = 0.70 for all samples). Correlation between buffer soluble N and EDCP was modest across all tested feeds (r = 0.63) (Chrenková et al., 2010a). The *in situ* fraction "a" and buffer soluble fraction A were negatively related to RUP (Lanzas et al., 2007).

When comparing *in situ* results with CNCPS data, differences were observed but some strong correlations were found to CNCPS fractions. Correlation between EDCP and fraction A was r = 0.76, and between *in situ* parameter "a" and fraction A the correlation was r = 0.84; however, a weaker correlation of r = 0.67 was found between *in situ* parameter "b" and fraction B_2 for DDGS (Chrenková et al., 2011c). Significant correlation was between subfraction B_2 and *in situ* potential degradable fraction "b" (r = 0.90), and between effective CP degradation (r = –0.64) for extracted oil seeds. The relationship between *in situ* parameter "b" and protein subfraction B_2 was: "b" = 24.54 + 0.852 × B_2, (R^2 = 0.802).

A higher proportion of ADIN in maize DDGS samples was correlated with a lower degradation rate (0.0156/h) and its ECPD was only 45.9% (Chrenková et al., 2010b). The ADIN and the pa-

Table 4. Comparison of crude protein content and protein fractions (% of CP) between protein concentrates

Feeds	CP (g/kg DM)	Protein fraction (% of CP content)					
		A	B_1	B_2	B_3	C	ISCP
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Rapeseed meal (n = 3)	$366.2^{a} \pm 3.84$	6.6 ± 1.67	$19.2^{a} \pm 1.03$	$60.4^{a} \pm 0.68$	$2.5^{a} \pm 0.19$	$11.6^{a} \pm 1.59$	$74.5^{a} \pm 0.87$
Sunflower meal (n = 4)	$353.2^{a} \pm 24.59$	$8.9^{a} \pm 0.72$	$38.4^{b} \pm 4.61$	$44.3^{b} \pm 4.46$	$1.6^{b} \pm 0.53$	$6.9^{b} \pm 0.52$	$52.7^{b} \pm 4.65$
Soya bean meal (n = 4)	$512.5^{b} \pm 6.05$	$3.2^{b} \pm 0.71$	$8.7^{c} \pm 1.57$	$82.6^{c} \pm 1.54$	$1.4^{bc} \pm 0.39$	$6.52^{bc} \pm 1.32$	$88.1^{ac} \pm 1.93$

CP = crude protein, DM = dry matter, ISCP = buffer insoluble protein

means with different superscript in the same column are significantly different at $P < 0.05$

Table 5. Characteristics of protein and NDF assessed by the *in situ* or CNCPS methods

Item	Method	Feedstuffs				*P*-value
		maize silage (n = 6)		lucerne silage (n = 7)		
		$\bar{x}$	v%	$\bar{x}$	v%	
CP (g/kg DM)		69.4	15.6	154.4	22.5	0.01
NDF (g/kg DM)		457.0	13.9	458.7	24.8	0.98
ADF (g/kg DM)		237.8	13.6	389.4	24.6	0.004
NDIN (g/kg DM)	CNCPS	16.9	46.6	24.9	71.0	0.34
Total N (%)	CNCPS	24.0	47.6	16.5	79.9	0.30
C (g/kg DM)	CNCPS	12.2	79.8	18.3	57.3	0.30
Total N (%)	CNCPS	17.4	77.5	12.3	68.8	0.43
B_2 (g/kg DM)	CNCPS	27.2	28.3	48.0	32.7	0.01
Total N (%)	CNCPS	39.9	37.7	32.1	39.7	0.33
EDCP (%)	*in situ*	75.6	6.7	76.3	12.1	0.87
cEDCP (%/h)	*in situ*	0.042	50.1	0.063	43.0	0.16

DM = dry matter, CP = crude protein, NDF = neutral detergent fibre, ADF = acid detergent fibre, NDIN = neutral detergent insoluble nitrogen, C, B_2 = nitrogen fractions, EDCP = effective degradable crude protein, cEDCP = effective crude protein degradation rate, CNCPS = Cornell Net Carbohydrate and Protein System, v = variability

rameters of CP degradability (*in situ* "b" fraction, rate of degradation, effective CP degradation) were significantly affected in cereal grains by flaking at 90°C. The fraction "b" was increased and degradability rate "c" and effective CP degradability was reduced. The weakest effect was observed with maize, which normally has little ADIN but also a low degradation rate and EDCP. This was attributed to the structure of zein, a major corn protein.

Fraction C (ADIN) is assumed to be unavailable. Nitrogen in this fraction is associated with lignin and Maillard products that originate during heating processes. The ADIN fraction is insoluble and assumed to be undegraded in the rumen, indigestible in the small intestine and, thus, cannot provide amino acids postruminally (Krisnamoorthy et al., 1982; Waters et al., 1992; Licitra et al., 1996; Lanzas et al., 2007).

CONCLUSION

Estimates of potential protein degradability and rates of degradation in the rumen are prerequisites in feed/ration evaluation systems modelling rumen dynamics. The aim of the present paper was to compare solubility methods to *in situ* degradation. There was found a good correlation between *in situ* degradability characteristics of "a", "b" EDCP and fractions A, B_2, B_3 according to CNCPS with the same feed. To derive equations to predict the RDP and/or RUP values it is necessary to analyze a much larger number of feed samples.

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