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Determination of proteins in different feeds using the *in sacco* method in comparison to the CNCPS method

Stanovenie bielkovín v rôznych krmivách metódou *in sacco* v porovnaní s metódou CNCPS

Zuzana MLYNEKOVÁ (✉), Zuzana FORMELOVÁ, Mária CHRENKOVÁ, Matúš RAJSKÝ, Mária POLAČIKOVÁ

National Agricultural and Food Centre, Research Institute for Animal Production – Nitra, Hlohovecká 2, SK- 95141 Lužianky, Slovakia

✉ Corresponding author: zuzana.mlynekova@nppc.sk

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ABSTRACT

The aim of this study was to determine the correlations between the output parameters of the *in sacco* method and the CNCPS method (Cornell Net Carbohydrate and Protein System). Both methods were tested on 106 samples from the feeds classified into following categories: extracted meal and cakes, cereals, legumes, maize and alfalfa silage, other silages, DDGS (dried distiller's grains with solubles), oilseeds and various hays. The effective degradability of crude protein (EDg CP) by the *in sacco* method varied depending on the type of feed from 44.97% (hays) to 82.59% (alfalfa silage). The lowest degradation parameter of rapidly degraded fraction (a=24.3%) and the highest potentially degraded fraction (b=70.52%) were found in oilseeds. Five nitrogen fractions (A, B_1, B_2, B_3, C) were determined according to the CNCPS method. Fraction A (NPN – non-protein nitrogen) was different in examined feed with the highest in silages (44.95% of the total CP). Fraction B_2 represented in tested feeds the highest part of the total CP, (except for silages, legumes, and oilseeds). Fraction B_3 ranged from 1.96% (legumes) to 19.25% (hays) of the total CP. The correlation between EDg CP and soluble fractions was low (r=0.5464) in concentrate feeds, the correlation between EDg and soluble fractions was (r=0.6323) also low in forages.

Keywords: CNCPS method, crude protein degradability, *in sacco* method

ABSTRAKT

Cieľom tohto príspevku bolo stanoviť korelačné vzťahy medzi výstupnými parametrami metódy *in sacco* a metódy CNCPS (Cornell Net Carbohydrate and Protein System). Metódy *in sacco* a CNCPS boli testované na rôznych krmivách (n=106). Krmivá boli rozdelené do jednotlivých kategórií: extrahované šroty a výlisky, obilniny, strukoviny, kukuričná a lucernová siláž, ostatné siláže, DDGS (sušené liehovarnícke výpalky s rozpustnými zložkami), olejniny a rôzne seno. Efektívna degradovateľnosť dusíkatých látok (EDg N-látok) metódou *in sacco* sa pohybovala v závislosti od typu krmiva od 44,97 % (seno) do 82,59 % (lucernová siláž). Najnižší parameter rozpustnej a degradovateľnej frakcie (a) a najvyššia nerozpustná a degradovateľná frakcia (b) bola zistená v olejnatých semenách (70,52 %). Päť dusíkových frakcií (A, B_1, B_2, B_3, C) bolo stanovených podľa metódy CNCPS. Frakcia A (NPN - nebielkovinový dusík) bola v skúmanom krmive odlišná, najvyššia v silážach (44,94 % z celkových dusíkatých látok). Frakcia B_2 predstavovala v testovaných krmivách najvyšší podiel z celkových dusíkatých látok, okrem siláží. Frakcia B_3 sa pohybovala od 1,96 % (strukoviny) do 19,25 % (seno) z celkových dusíkatých látok. Korelácia pre jadrové krmivá medzi EDg N-látok a rozpustnými frakciami bola slabá (r=0,5464). Podobne to bolo aj u objemových krmív, kde korelácia medzi EDg N-látok a rozpustnými frakciami bola r=0,6323.

Kľúčové slová: CNCPS metóda, degradovateľnosť N-látok, *in sacco* metóda

INTRODUCTION

Controlling the protein fractions in forages is one way to improve the efficiency of nitrogen use and decrease nitrogen excretion to the environment on ruminant farms (Haugen et al., 2006). From the point of view of current nutritional assessment systems, in addition to the energy value, very important indicators of nutritional value of feed are the degradability of feed nutrients in the foreguts, the content of undegraded nutrients and their digestibility in the small intestine. In general, with increasing performance the proportion of degraded crude protein in the rumen must be decreased and the digestibility of undegraded proteins in the small intestine must be increased.

Whereas numerous proteins and NPN (non-protein nitrogen) compounds contribute to CP (crude protein), it is understood that the nutritive value of CP in a feedstuff for ruminants is best described by its rate and extent of degradation in the rumen and the composition of the RDP (rumen degraded protein) and RUP (rumen undegraded protein) fractions that result. Many factors affect the amount of CP that will be degraded in the rumen. These include the proportional content of proteins and NPN, the physical and chemical properties of proteins, the rumen retention time of the protein, microbial proteolytic activity and ruminal pH (Schwab et al., 2003).

The *in sacco* method according to Ørskov and McDonald (1979), known also as nylon bag technique, has been used for more than 40 years. Modifications of the *in sacco* method have been well accepted (Michalet-Doreau and Ould-Bah, 1992; Hvelplund et al., 2009) to determine the degradability of CP and other important nutrients (neutral detergent fibre, acid detergent fibre and starch) significantly associated with the nitrogen metabolism (Čerešňáková et al., 2005; Tham et al., 2008; Chrenková et al., 2010; Chrenková et al., 2012a). The *in sacco* method is the most widely used research approach for measuring the ruminal protein degradation. This method has been adapted for use in several countries. The greatest advantage of the *in sacco* method is that it allows exposure of feedstuffs to the digestive conditions thought to be similar to those existing *in vivo*. Coupled with the use of equations that predict rates of passage of undigested feed, a dynamic system is in place for predicting the RDP and RUP content of feedstuffs (Schwab et al., 2003). The nylon bag technique is now accepted as one of the basic methods required by the protein evaluation methods proposed by NRC (2001). The primary shortcoming of the *in sacco* method is that it is labour intensive and requires the use of cannulated animals, both of which makes it the costliest method for obtaining the RDP and RUP values for a feedstuff. A few commercial laboratories maintain ruminal cannulated animals and provide *in sacco*-derived estimates of ruminal protein degradation. Another shortcoming of this method is the disappearance of soluble proteins from the bag. Soluble proteins vary in the ruminal degradation rate and, therefore, cannot be assumed to be degraded completely in the rumen (NRC, 2001).

The Cornell Net Carbohydrate and Protein System (CNCPS) is one of the schemes developed for the fractionation of protein in feeds. The CNCPS method is a mathematical model to evaluate cattle ration and animal performance based on principles of rumen fermentation, feed digestion, feed passage and physiological status of the animal (Fox et al., 2004). The Cornell Net Carbohydrate and Protein System (CNCPS) is a widely applied and internationally recognized analysis (Licitra et al., 1996). The method enables dividing the crude protein (CP) into five fractions according to the type of N compound and degradability. The nomenclature of the CNCPS has changed over the years and according to the system concept described in 2008 (Tylutki et al., 2008), the CP fractions are divided into A, B_1, B_2, B_3 and C, where especially B fractions are interesting as A is non-protein nitrogen and C is cell wall-bound N.

The aim of this study was to determine the rumen *in sacco* degradability characteristics of CP, to fractionate CP according to CNCPS for different feeds and to determine, whether CNCPS can be used to estimate the *in sacco* values of degradation by examining the interrelationships between the output parameters.

MATERIALS AND METHODS

One hundred and six samples collected from cereals, legumes, maize and alfalfa silages, other silages, extracted meal and cakes, hays, oilseed and DDGS (dried distiller's grains with solubles) were used in this study. Effective crude protein degradability and degradation parameters (a, b) were determined using the *in sacco* method (Chrenková et al., 2012b). The study was carried out at the National Agricultural and Food Centre - Research Institute for Animal Production Nitra, Slovak Republic. In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of Slovak Republic, (permission code: SK U 03021).

In sacco experiments were carried out on four non-lactating heifers of Holstein-Friesian cattle with large rumen cannulas (diameter of 10 cm). The animals were fed a diet consisting of 70% forage and 30% concentrate on a dry matter basis at the maintenance level twice a day. The ration consisted of maize silage (12 kg/day), alfalfa hay (4 kg/day), wheat meal (0.5 kg/day), barley meal (0.5 kg/day) and vitamin-mineral premix (0.150 kg/day). Access to water was *ad libitum*. Milled samples were weighed (approx. 2.50 g dry matter) into the bags (9 x 15 cm) made of Uhelon 120T with pore size of 48 µm. At least three separate bags per sample (12 bags in total) were used. The bags were incubated during 3, 6, 9, 16, 24 and 48 hours for concentrate feeds and during 72 and 96 hours for forage feeds. The 0 h time bags were only washed in a washing machine in the cycle 3 x 5 min without spinning to determine washing losses. The other bags were washed in a washing machine without spinning (cycle 3x5 min) and subsequently were dried at 50 °C for 24 hours and the residues were weighed. Protein degradability parameters and EDg CP (effective degradability of crude protein) were calculated using equations of Orskov and McDonald (1979):

$$p = a + b\ (1 - e^{-ct}),\ \text{where}$$

p - represents degradability of CP at time t; (a + b) is their potential degradability; c - is the rate of their degradability. The effective rumen degradability of CP was calculated as: ED = a + (b x c) / (c + k), assuming the rumen outflow rates (k) of 0.06 h^{-1}.

CNCPC method is based on the solubility of nitrogen in borate-phosphate buffer and in detergent solutions (Figure 1). In this experiment, the method according to Licitra et al. (1996) was used. Samples were milled using a hammer mill with a 1 mm screen for chemical and solubility analysis. Samples were fractionated into three major protein fractions: non-protein nitrogen (A), true protein (B) and unavailable protein (C). Fraction B was subsequently fractionated into sub-fractions B_1, B_2 and B_3. Protein fractionation is based on the solubility in a borate-phosphate buffer and in detergent solutions. Tungstic acid was used as a precipitant (Licitra et al., 1996).

Contents of crude protein, neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined according to AOAC (1995) and Van Soest et al. (1991).

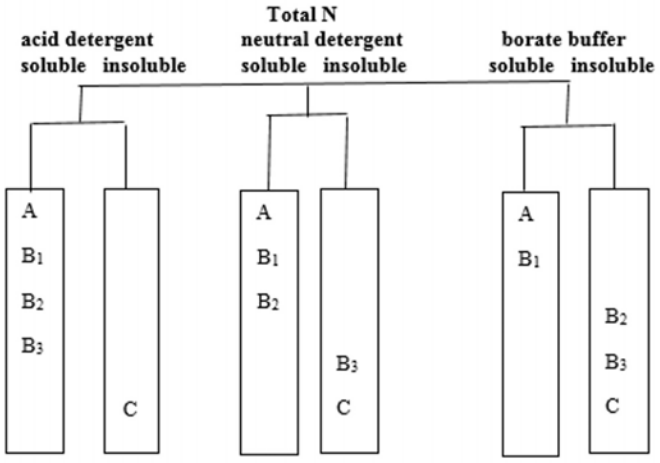

Figure 1. Nitrogen fractionation based on the solubility in detergent solutions and borate-phosphate buffer (Tham et al., 2008). fraction A - soluble in a buffer and tungstic acid, fraction B_1 - soluble in a buffer and precipitated by tungstic acid, fraction, B_2 - insoluble in a buffer but soluble in a neutral detergent, fraction B_3 - soluble in an acid detergent but insoluble in a neutral detergent, fraction C - insoluble in an acid detergent.

The Pearson's correlation between CNCPS and *in sacco* characteristics was measured. For statistical analysis, an XL STAT software (2014) was used. The significant differences were declared at $P \leq 0.01$ and $P \leq 0.05$ using T-test.

RESULTS AND DISCUSSION

In Europe, current systems for assessing feed quality and predicting nutrient needs in animals are based on energy and crude protein degraded and fermented in the rumen and actually digested in the small intestine. Determining the degradability of crude protein by the *in sacco* method is very time consuming and requires fistulated animals, which are also not commonly available. However, it is still used as a reference method and is more biologically reliable than *in vitro* methods (Thomas, 2004). Crude protein in feeds has different qualities (solubility and availability to enzymatic digestion), which determine the degree of their degradation in the rumen and the amount of crude protein available in the small intestine and their subsequent digestibility. Using chemical analytical methods, it is possible to obtain new quality parameters for fractions of crude protein in feed. CNCPS method is based on the solubility in a buffer, acid and neutral detergent. This method requires an analytical laboratory with good basic and specific (nitrogen determination) equipment and, unlike the *in sacco* method, it does not require cannulated animals.

An effective CP degradability of different groups of feeds is shown in Table 1 (106 different samples). Oilseeds revealed the lowest rapidly degraded fraction (a=24.3% and the highest potentially degraded fraction (b=70.52%). Alfalfa and maize silages were characterized by a high value of the "a" fraction.

González and Andrés (2003) found that the effective degradability of crude protein from various legumes ranged from 69.3 to 80.7%, which was similar to our values (73.58% - 85.2%). Higher degradation parameters, as well as the effective degradability of CP, were found also for cereals in comparison to Polat et al. (2014).

Hays had the lowest effective degradability of crude protein; the highest value was observed in alfalfa and other silages (Table 1). DDGS was also characterized by relatively low CP effective degradability. DDGS is a high-value protein-energy feed produced during the fermentation of grains into alcohol; these are a good source of non-degradable protein.

Table 1. Average values of effective degradability of crude protein and its parameters in different groups of feeds determined by the *in sacco* method

Samples (n)	a (%)	b (%)	EDg CP (%) 0.06.h^{-1}
Cereals (23)	36.55	62.52	65.41
Legumes (14)	46.06	52.29	77.23
Maize silages (8)	69.88	14.11	75.4
Alfalfa silage (7)	72.81	18.49	82.59
Other silages (7)	58.49	33.24	82.55
Extracted meal and cakes (32)	30.27	64.01	61.12
Oilseed (4)	24.30	70.52	74.73
Hays (4)	34.50	53.05	44.97
DDGS (7)	41.64	58.45	52.66

a - rapidly degraded fraction, b - potentially degraded fraction, c - rate of degradation, EDg CP - effective degradability of crude protein
DDGS - dried distiller's grains with solubles

From the viewpoint of cattle nutrition, it is desirable that the degradability of nutrients could be low and that most of the crude protein pass into the small intestine, where it is available to the animal itself and not only to rumen microorganisms.

Using the CNCPS method

Crude protein is divided into five fractions with different properties. Fractions A and B_1 are soluble in a buffer and B_1 is determined as the trifluoroacetic acid (TCA) precipitable fraction (Van Soest et al., 1981). Nonprotein N (ammonia, peptides, amino acids) is rapidly converted to ammonia in the rumen. Fraction B is subdivided to estimate rates of ruminal degradation. Fraction B_1 is rapidly degraded in the rumen (Van Soest et al., 1981). In harvested forages, fraction B_1 is a small fraction of the total soluble protein (approximately 5%), while concentrate feeds can contain twice as much.

Fraction B_2 is mostly fermented in the rumen and partially escapes to the lower gut. The fate of fraction B_2 depends on the relative rates of digestion and passage. Fraction B_2 is typified by the glutelin protein found in small grains (Van Soest et al., 1981). The B_2 fraction is

a fraction of proteins insoluble in a buffer but soluble in ND (neutral detergent) and AD (acid detergent) reagents. The rate of degradation of this fraction in the rumen is lower. This fraction, the largest of protein feeds, is of great importance in feed evaluation for ruminants.

Fraction B_3 is slowly degraded in the rumen because it is associated with the cell wall (Van Soest et al., 1981; Krishnamoorthy et al., 1983). This protein fraction is soluble in the AD reagent and is less prevalent.

Fraction C is the protein that is insoluble in the acid detergent (acid detergent insoluble protein, ADIP) fraction. Fraction C contains protein associated with lignin, tannin-protein complexes and Maillard products that are highly resistant to microbial and mammalian enzymes (Krishnamoorthy et al., 1982, 1983). Fraction C cannot be degraded by ruminal bacteria and, thus, does not provide amino acids post-ruminally (Krishnamoorthy et al., 1982).

The same 106 feed samples grouped into nine feed categories were tested using the CNCPS method. The results are presented in Table 2. The proportion of total N soluble in a buffer (soluble protein SOLP, Table 2) was lowest in the DDGS, extracted hemp meal and treated cereals, followed by meadow hay and rapeseed extracted meal, cereal grain, while highest in blue lupine, peas and feed beans. In oilseeds, SOLP was about 50% of the total CP. According to Lanzas et al. (2007), fraction A represents approximately 50% of the soluble protein in concentrate feeds. Very high SOLP were also present in alfalfa silage, due to the intense protein hydrolysis during silage processing.

NPN (fraction A - non-protein nitrogen) was very high in fermented feeds (silages). On the contrary, the feeds, such as DDGS and heat-treated feeds, had a NPN fraction and a buffer-soluble fraction very low (9.63%), which is confirmed by the results of Chrenková et al. (2012a). Polat et al. (2014) determined 9.15% of NPN from the total CP in maize grain, 20.40% - in wheat grain and 9.39% - in barley.

Fraction B_1 was represented with less than 10% of the total soluble nitrogen in the treated feeds and in DDGS. Fraction B_1 was decreased in the treated feeds.

The fraction B_2 is degraded relatively quickly in the rumen and is soluble in the NDF reagent. This fraction is the largest proportion of protein feeds and is of great importance in feed evaluation. Similar results in all investigated samples were also reported by Thers et al. (2021). Heat treatment of feed increased the B_2 value in wheat by almost 20% and in soybeans by 40% of the total CP (25.42% vs. 65.43% of the total CP). The monitored silages in this experiment were characterized by a lower B_2-value than other examined feed samples; the lowest B_2-value was measured in alfalfa silage (11.3% of the total CP) and the highest - in pumpkin cakes (83.77% of the total CP). The treated extracted hemp meal had a high B_2 value - above 70%.

As a part of the evaluation of the efficiency of the thermal process, the change in the representation of insoluble B_2 and B_3 fractions was determined. In particular, B_3 fraction shows the lowest rate of degradation and, therefore, when this fraction increases, the degradability of crude protein decreases. Heat-treated feed properties depend not only on the used technology (micronization, flocculation, toasting, extrusion...), temperature but also vary with the humidity. The highest B_3 value was observed in the analysed hay samples (19.25% of the total CP).

Regardless of a treatment method, all kinds of treatments decreased soluble crude protein fraction (SOLP) and increased the intermediately degradable protein (B_2) and slowly degradable protein (B_3) fraction. Treated varieties had greater B_2 and B_3, and lower A and B_1 fractions compared to untreated varieties (Kafilzadeh et al., 2013).

An important parameter is also the proportion of C fraction; its disproportionate increase causes a decrease in the intestinal digestibility. In this experiment it was found that the C parameter (ADIP) was different in maize and alfalfa silages (Table 2). High values of the C fraction are due to the incorrect silage technology, which led to heating of the silage and thermal damage to proteins. At higher temperatures, a Maillard reaction occurred between proteins (between the free NH_2 groups of lysine) and carbohydrates, especially in maize silage. In alfalfa silage, there are also complexes between tannins and proteins (Halalsheh et al., 2007).

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Table 2. Content of CP and protein fraction (CNCPS - Cornell Net Carbohydrate and Protein System) in different feed types

Samples (n)	CP	% CP						
	(g·kg-1 DM)	A	B_1	B_2	B_3	C	Insoluble P	Soluble P
	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$	$\bar{x} \pm s_x$
Cereals (23)	110.55±3.75	5.87 ± 1.07	13.26±1.84	58.16±2.65	6.79±0.82	15.61±1.91	80.56±3.13	19.30±2.21
Legumes (14)	281.58±14,93	7.75± 0.73	45.47±4.31	35.48±4.45	1.96±0.74	9.51±1.26	46.95±4.88	53.20±4.57
Maize silage (8)	68.69±2.99	36.52± 3.34	7.84±1.73	33.66±5.39	12.35±2.36	19.94±3.96	65.66±10.54	44.36±4.67
Alfalfa silage (7)	174.14±16.45	48.41±9.69	5.16±2.45	24.03±5.20	5.38±1.70	14.01±3.18	43.42±9.97	53.58±9.62
Other silages (7)	181.79±19.84	49.91± 1.69	1.92±0.29	28.21±1.67	10.75±1.62	9.06±0.97	48.02±2.75	51.83±1.97
Extracted meal and cakes (32)	351.50±12.92	8.37± 1.07	18.59±3.11	60.21±3.02	3.74±0.37	9.81±0.81	73.76±3.58	25.99±3.51
Oilseed (4)	204.44±10.59	9.90± 0.63	38.14±4.58	32.75±2.38	3.44±0.55	15.74±3.54	51.93±4.00	48.04±4.35
Hays (4)	160.05±32.01	22.16±9.23	9.28±0.45	38.49±1.73	19.25±7.44	10.83±0.74	68.57±9.00	31.44±8,99
DDGS (7)	310.20±5.97	12.50± 1.33	2.63±1.44	60.19±4.10	4.58±0.83	16.76±1.05	81.54±4.33	15.13±2.45

A - fraction of non-protein nitrogen - NPN
B_1 - fraction of true soluble proteins that are soluble in a buffer and are precipitated with TCA
B_2 - fraction of proteins insoluble in a buffer but soluble in ND and AD reagents
B_3 - protein fraction soluble in the AD
C - fraction of proteins which are insoluble even in the acid-detergent agent, may be represented by lignin-bound proteins, tannins and bound in Maillard reaction
Insoluble P - Insoluble protein
Soluble P- Soluble protein (SOLP)
DDGS - dried distiller's grains with solubles

Table 3. Correlation between the *in sacco* method and the CNCPS method on the basis of dividing the N- fraction selected parameters

Correlation *in sacco*: CNCPS	r	R^2	Equation	*P*-Value
All feeds (106 samples)				
EDg CP: A	0.42	0.176	y = -15.53509+0.46922x	0.0001
a: A	0.60	0.360	y = -4.47615+0.48930x	0.0001
b: B_2	0.55	0.306	y = 25.09338+0.43536x	0.0001
Cereals (23)				
EDg CP:SOLP	0.76	0.571	y = -11.05014+0.46397x	0.0001
Legumes (14)				
b: B_2	0.85	0.715	y = -18.73751+1.03676x	0.0001
Maize silage (8)				
EDg CP: A	0.88	0.770	y = 93.03194+1.71810x	0.0042
Alfalfa silage (7)				
EDg CP: A	0.71	0.507	y = -279.22389+3.96696x	0.0728
Other silages (7)				
b: B_3	0.92	0.839	y = 1.61114+0.27497x	0.0037
Oilseeds (4)				
EDg CP: A	0.99	0.957	y = -134.98432+1.93890x	0.0215
b: B_3	0.97	0.943	y = -34.08508+0.53460x	0.0292
b: A	0.60	0.3662	y = -16.91687+0.38207x	0.3948
Hay (4)				
a: A	0.97	0.936	y = -1.36533+0.68189x	0.0326
EDg CP: A	0.94	0.882	y = -24.05078+1.02759x	0.0609
Edg CP: B_3	0.97	0.932	y = -17.92120+0.70059x	0.0345
DDGS (7)				
EDg CP: A	0.85	0.725	y = -11.48266+0.45539x	0.0150
Concentrate feeds (58)				
EDg CP: SOLP	0.55	0.299	y = -20.26423+0.79927x	0.0001
Forage feeds (26)				
EDg CP: SOLP	0.63	0.399	y = -8.69812+0.74498x	0.0005

r = correlation coefficient, R^2: coefficient of determination, SOLP (A+B_1) (soluble protein)

The lowest value of the C fraction from total N was recorded in untreated lupine (0.27% of the total CP), which, however, had a high value of the B_1 fraction. C fraction is non-degradable in the rumen and, according to Sniffen et al. (1992), is indigestible in the small intestine.

Correlation between the in sacco method and the CNCPS method

Currently, there is a pressure to limit experiments on live animals, therefore, new laboratory methods are being search. In this study, we determined whether the CNCPS method can be used to estimate *in sacco* values for degradation and to find correlation between these two methods.

Usually, feeds with a high value of SOLP (soluble protein, A fraction + B_1 fraction) also have a high EDg CP, except soybeans and linseed cakes.

When the parameter "b" and B_2 fraction were compared, a slight correlation between the methods and all feeds was found (Table 3). Similarly, slight correlation but high statistical significance was determined between the parameter "a" (*in sacco* method) and fraction A (CNCPS) for all feeds. Very similar results (r=0.70) are described by Chrenková et al. (2014).

Other correlation coefficients were determined in different feed samples. A higher correlation coefficient between EDg CP and SOLP (cereals) and “b” and B_2 was determined for cereals and legumes (Table 3).

The DDGS had a higher correlation coefficient between the compared methods for EDg CP and NPN. DDGS had the low EDg CP and high content of insoluble CP as a result of the heat treatment (Table 3). Chrenková et al. (2011) found a weaker correlation (r=0.67) between the *in sacco* parameter “b” and fraction B_2 for DDGS.

Concentrate and forage feeds were also compared but very low correlation coefficient between EDg CP and soluble fractions was determined.

CONCLUSIONS

Estimates of potential protein degradability and rates of degradation in the rumen are prerequisites in feed evaluation systems modelling rumen dynamics. Therefore, it is necessary to focus on methods for determining the extent and rate of degradation of N fraction maintaining good accuracy and repeatability of results. In this article, the laboratory method based on nitrogen solubility was compared to the *in sacco* method for determining the CP effective degradability. The data obtained so far provide a more detailed picture of the quality of proteins in feed. Because significant correlation between the *in sacco* and CNCPS degradability was determined, we can conclude that both methods are suitable giving similar results of protein characteristics. A correlation between the *in sacco* degradability characteristics of “a”, “b”, EDg CP and fractions A, B_2, B_3 according to CNCPS was found with the same feed.

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