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Organic Matter and Crude Protein Digestibility Predicted from Nitrogen and Fibre Fractionation of Festulolium Hybrids

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ABSTRACT

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The objectives of the experiment were to study nitrogen fractions of the Cornell Net Carbohydrate and Protein System (CNCPS) and digestibility of organic matter (OMD) and crude protein (CPD) along with the possibilities for predicting OMD and CPD based on chemical composition and CNCPS nitrogen fractions. Thirty samples of festulolium hybrids (fescue hybrids and ryegrass hybrids) commonly used and certified in the Czech Republic were analyzed for chemical composition, brutto energy, and nitrogen fractions, these fractions being A (non-protein nitrogen), B1 (rapidly degradable protein), B2 (intermediately degradable protein), B3 (slowly degradable protein), and C (unavailable protein). OMD and CPD were determined by near-infrared reflectance spectroscopy. Nitrogen fractions showed similar mean values for both analyzed groups of festulolium hybrids. Mean rapidly, intermediately, and slowly soluble fractions (B1, B2, and B3, respectively) were 29.2, 30.7, and 33.7 g/kg of dry matter (DM) for fescue hybrids and 21.7, 33.5, and 31.9 g/kg of DM for ryegrass hybrids. There was a trend for fraction B2 to decrease with advancing maturity in relation to neutral detergent insoluble protein value. The mean content of bound fraction C was 10.4 g/kg of DM for fescue hybrids and 7.3 g/kg of DM for ryegrass hybrids. Three groups of hybrids were evaluated statistically: fescue (I), ryegrass (II), and combined fescue/ryegrass (III). Better regression equations were found for fescue/ryegrass hybrids. High degrees of CPD determination were identified with predictors of individual nitrogen fractions (R^2 = 0.975; P > 0.0001). OMD was described in regressions only using nitrogen fractions A and B1 (R^2 = 0.500; P > 0.0001).

Keywords: nitrogen fractions; Cornell system; nutritive value; ruminant

Ryegrass (*Lolium* L.) and fescue (*Festuca* L.) species are agronomically important grasses in temperate regions worldwide (Kubota et al. 2015). Intergeneric hybrids between ryegrass and fescue species, termed "festuloliums," are also very intriguing from a strictly cytogenetic point of view (Kopecky et al. 2008) and reflect the agronomic benefits of both grass genera (Yamada et al. 2005; Kubota et al. 2015). The superior agronomic characteristics of festulolium hybrids combine the yield performance of ryegrasses (*Lolium multiflorum* and *Lolium perenne*) and tolerance against abiotic stress of fescues (*Festuca pratensis*, *Festuca arundinacea*, and *Festuca arundinacea* var. *glaucescens*) (Kopecky et al. 2006).

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Some hybrids of *Festuca* and *Lolium* are commonly used for feeding ruminants (Jancik et al. 2008). It is important to quantify all factors influencing the optimal formulation of rumen feed rations in order to meet but not exceed the nitrogen requirements of rumen microbes and the amino acid requirements of the ruminants (Schwab et al. 2003). Information on the metabolism of common protein feedstuffs is essential for models used in optimizing dairy cow diets in terms of cost and efficient use of nutrients. Dietary protein provides peptides, amino acids, and ammonia for rumen microbial N synthesis as well as undegradable feed protein to the animal in excess of the microbial N yield. Rates of protein degradation and passage determine the relative proportions of substances either metabolized in the rumen or escaping ruminal degradation (Ahvenjarvi et al. 1999). Potential degradability and rates of degradation in the rumen are important and used in several nutritional systems (Chrenkova et al. 2014). The Cornell Net Carbohydrate and Protein System (CNCPS) is one such a system, and it uses feed carbohydrate and protein degradation and passage rates to predict the extent of ruminal fermentation, microbial 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). According to Purwin et al. (2012), particular nitrogen fractions have different impacts on the efficiency of microbial protein synthesis due to their differential degradability. Validation of the accuracy and precision of the CNCPS in predicting animal responses to variations in factors influencing feed utilization require information on feed carbohydrate and protein fraction composition, in addition to accurate information on animal requirements (Fox et al. 1992).

This method involving soluble and insoluble nitrogen fractions has been developed to meet the need for uniform procedures for partitioning feed nitrogen into the individual fractions A, B, and C (Pichard and Van Soest 1977), where fraction B itself is subdivided into three fractions (B1, B2, and B3) that are believed to have different rates of ruminal degradation (Alzueta et al. 2001). Nitrogen fractions are classified according to their solubility into fractions A (non-protein nitrogen), B1 (rapidly degradable protein), B2 (intermediately degradable protein), B3 (slowly degradable protein), and C (unavailable protein) (Liu et al. 2015, 2017).

Due to the variability in the nitrogen fractions' rates of degradability and resulting influence on animal performance, knowledge of forage digestibility is important in formulating effective rations for ruminants. The present study was directed at predicting nitrogen and organic matter digestibility (OMD) using basic chemical analyses and individual nitrogen fractions.

MATERIAL AND METHODS

Hybrids and sampling. Thirty different festulolium hybrids were chosen for this study. The hybrids were divided by the predominance of each grass species' representation into fescue hybrids and ryegrass hybrids, all of which are commonly used and certified in the Czech Republic. Each festulolium hybrid was sown in a separate field during 2011 and first cuttings were taken in May 2012 (in botanical phase beginning of earing). The experimental fields (in three repetitions) were on fertile ground at Hladké Životice, Czech Republic (49°67'N, 17°94'E, 254 m a.s.l.). These fields all were placed at the same locality and were fertilized with 60 kg N/ha. Chemical treatment against dicotyledonous plants was used in the first year of crop growth. Long-term (2011–2012) annual rainfall for this locality is 738 mm and average annual temperature 8.2°C.

Laboratory analyses. The chemical composition as dry matter (DM), ash, ether extract (EE), crude fibre (CF), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), brutto energy (BE), and fractions of crude protein (CP) for the hybrid grass samples were analyzed in the accredited laboratories of the Institute of Animal Science, v.v.i., Prague, Czech Republic. The fresh-cut grass samples were dried at 55°C as described by Harazim et al. (1999). The dried material was then milled to pass through a 1 mm sieve for laboratory analyses. Ash and CF were determined in accordance with methods prescribed by AOAC (2005). Ash-free NDF, ADF, and ADL were determined according to the methods described by Van Soest et al. (1991), and BE was measured using a calorimeter IKA C 5000 control (IKA-Werke GmbH and Co. KG, Germany). The contents of EE and CP were commercially determined by near-infrared reflectance spectroscopy at the Crop Research Institute v.v.i., Jevíčko

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Research Station, Czech Republic. Nitrogen-free extract (NFE) was calculated as DM – (CP + CF + EE + Ash).

The digestibilities of OM and CP were also commercially determined at the Jevíčko Research Station. The quality of forage dry matter was evaluated using a FOSS NIRSystems 6500 instrument (Foss NIRSystems, USA) fitted with a transport sample module, in reflectance from 1100 to 2500 nm, bandwidth 2 nm, measured in small ring cups.

Nitrogen fractionation analyses. The chemical analyses of individual nitrogen fractions were described by Licitra et al. (1996). The calculations (units of individual fractions in g/kg of DM) were according to Lanzas et al. (2008), as follows:

Fraction A = NPN × (SOLP/1000) × (CP/1000)

Fraction B1 = (SOLP/1000) × (CP/1000) – A

Fraction B2 = CP – A – B1 – B3 – C

Fraction B3 = (NDIP – ADIP) × (CP/1000)

Fraction C = ADIP × (CP/1000)

where:
A = non-protein nitrogen (g/kg of DM)
ADIP = acid detergent insoluble protein (g/kg of CP)
B1 = rapidly degradable protein (g/kg of DM)
B2 = intermediately degradable protein (g/kg of DM)
B3 = slowly degradable protein (g/kg of DM)
C = unavailable protein (g/kg of DM)
CP = crude protein (g/kg of DM)
NDIP = neutral detergent insoluble protein (g/kg of CP)
NPN = non-protein nitrogen (g/kg of SOLP)
SOLP = soluble protein (g/kg of CP).

Laboratory methods were carried out in accordance with Licitra et al. (1996), which involved partitioning into laboratory procedures as depicted in Figure 1. CP was precipitated as insoluble protein (IP), borate-phosphate buffer IP, NDIP, and ADIP (Valdes et al. 2006). NDIP and ADIP were analyzed using a Fibertec 2010 system (Tecator Comp., Sweden). The residues of crude protein, which is important for the calculation of individual nitrogen fractions, were analyzed according to the Kjeldahl method.

Statistical analyses. For the statistical analyses, data were statistically evaluated as group I (fescue hybrids), group II (ryegrass hybrids), and group III (fescue/ryegrass hybrids together). Data were ana-

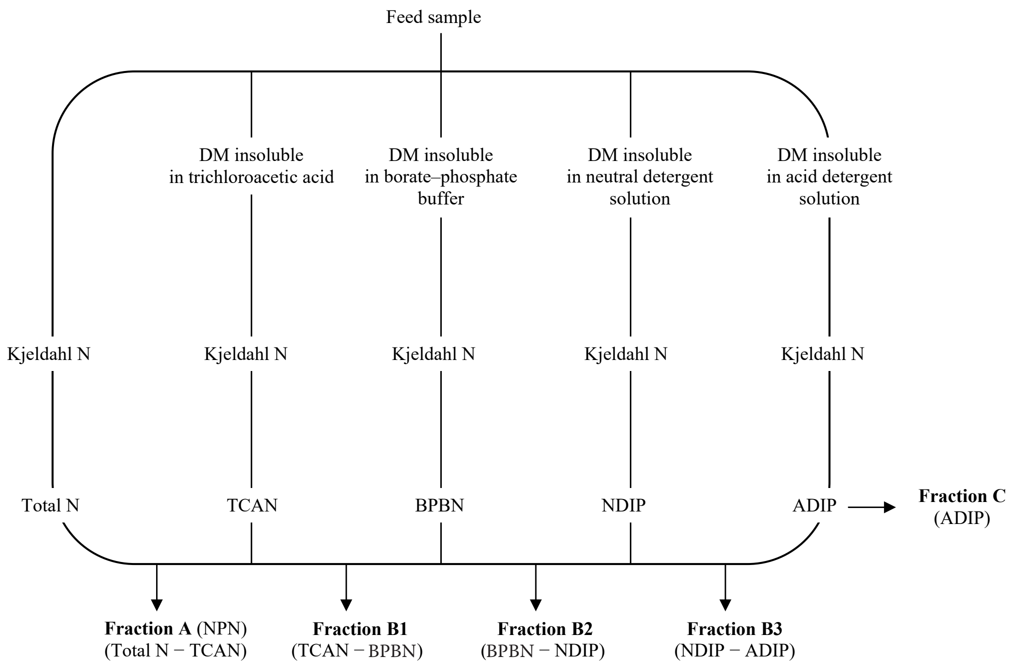

Figure 1. Fractionation according to Valdes et al. (2006) of crude protein (N × 6.25) in feedstuffs
ADIP = acid detergent insoluble protein, BPBN = borate-phosphate buffer insoluble nitrogen, DM = dry matter, N = nitrogen, NDIP = neutral detergent insoluble protein, NPN = non-protein nitrogen, TCAN = nitrogen in trichloroacetic acid precipitated matter

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lyzed using the PROC GLM procedure in the SAS software (Statistical Analysis System, Version 9.3, 2003), and the PROC CORR was used to compute correlation coefficients ($P < 0.05$) between the observed variables. The PROC REG procedure was used to determine the prediction ability of the estimated models. Statistical significance for regression models was declared at $P < 0.0001$.

RESULTS AND DISCUSSION

The chemical compositions of festulolium hybrids (fescue hybrids and ryegrass hybrids) are shown in Table 1. Mean original laboratory DM values for fescue hybrids were 231.8 g/kg and for ryegrass 231.0 g/kg. Mean values in fescue hybrids (g/kg of DM) were 141.1 for CP, 26.8 for EE, and 76.1 for ash. Mean values in ryegrass hybrids were 140.2 for CP, 28.9 for EE, and 72.8 for ash. BE values for fescue and ryegrass hybrids were 18.1 and 18.3 MJ/kg of DM, respectively. Mean fibre fractions (Table 1) of ADF, NDF, and ADL, respectively, were 546.2, 324.9, and 31.1 g/kg of DM for fescue hybrids and 515.7, 314.8, and 49.1 g/kg of DM for ryegrass hybrids. Lignification, representing a very important parameter affecting forage quality (Wang et al. 2017; Loucka et al. 2018) and expressed as CF contents ($r = -0.728$) and NDF ($r = -0.684$), generally corresponded ($P < 0.01$) with the degree of CP availability in forage hybrids. Fibrous feeds generally are characterized by a high lignocellulose content, low crude protein content, poor palatability, and low nutrient digestibility (Elghandour et al. 2016).

Nitrogen fraction contents are shown in Table 2. Nitrogen fraction values were similar for both groups of hybrids. Mean non-protein nitrogen was 37.2 g/kg of DM for fescue hybrids and 43.5 g/kg of DM for ryegrass hybrids. For both grass types, fraction A constituted the largest part among all fractions. Fraction A is a part of CP that is very rapidly available. It is soluble in buffer but does not precipitate as a protein in trichloroacetic acid (Lanzas et al. 2008). It can be said that this nitrogen fraction is immediately available in the rumen. Generally, with maturity, fraction A decreased, likely due to increased CP bonding to cell wall constituents and being expressed in the not tabulated NDIP value. This is in accordance with the findings of Abbasi et al. (2012). Degradable fractions B1, B2, and B3 had mean values of 29.2, 30.7, and 33.7 g/kg of DM for fescue hybrids and 21.7, 33.5, and 31.9 g/kg of DM for ryegrass hybrids, respectively. Similarly as did Ehsani (2007) and Abbasi et al. (2012), we observed a negative but not statistically significant relationship between NDF and fraction B1. There was a trend for fraction B2 to decrease with advancing maturity in relation to NDIP value, but this was significant only for

Table 1. Chemical composition (g/kg of DM) and brutto energy value (MJ/kg of DM) of analysed samples ($n = 30$)

	Fescue hybrids ($n = 14$)				Ryegrass hybrids ($n = 16$)			
	mean	SD	min	max	mean	SD	min	max
Original DM	231.8	9.6	213.4	249.6	231.0	19.5	209.5	275.8
Chemical composition (g/kg DM) **and brutto energy** (MJ/kg DM)								
CP	141.1	11.0	113.5	156.2	140.2	16.1	111.9	163.9
EE	26.8	3.3	18.8	32.6	28.9	3.7	23.0	36.6
NFE	488.5	9.1	475.1	505.8	515.0	9.4	498.1	534.7
Ash	76.1	3.8	69.7	80.9	72.8	6.9	63.4	88.8
CF	267.5	11.9	240.6	290.4	243.1	16.4	218.4	270.3
ADF	546.2	12.5	520.3	567.0	515.7	16.9	491.6	549.5
NDF	324.9	25.9	290.8	370.4	314.8	19.5	280.2	346.1
ADL	31.1	6.1	23.1	40.9	49.1	16.6	30.7	81.6
BE	18.1	0.1	18.0	18.3	18.3	0.2	18.0	18.6

ADF = acid detergent fibre, ADL = acid detergent lignin, CF = crude fibre, CP = crude protein, BE = brutto energy, DM = dry matter, EE = ether extract, NDF = neutral detergent fibre, NFE = nitrogen-free extract, max = maximum, min = minimum, SD = standard deviation

values are arithmetic means ($n = 2$) based on DM

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Table 2. Determination of nitrogen fractions (g/kg of DM) in analysed samples (n = 30)

Nitrogen fraction	Fescue hybrids (n = 14)				Ryegrass hybrids (n = 16)			
	mean	SD	min	max	mean	SD	min	max
A	37.2	7.6	21.5	52.1	43.5	9.8	24.1	57.7
B1	29.2	10.4	9.1	42.7	21.7	14.4	1.6	38.2
B2	30.7	14.2	7.1	56.8	33.5	15.4	18.3	64.3
B3	33.7	10.3	20.0	55.1	31.9	5.9	23.2	46.4
C	10.4	3.0	7.1	15.1	7.3	1.4	5.0	9.9

A = non-protein nitrogen, B1 = rapidly degradable protein, B2 = intermediately degradable protein, B3 = slowly degradable protein, C = unavailable protein, CP = crude protein, DM = dry matter, max = maximum, min = minimum, SD = standard deviation

fescue hybrids (r = –0.709; P < 0.01). Differential changes in NDF concentration of plant while approaching maturity may explain the differences in proportions of B3 (Alzueta el al. 2001; Abbasi et al. 2012), and this corresponds to our finding of a significant relationship between NDIP and B3 for both groups of hybrids (r = 0.954; P < 0.0001). The insoluble fraction C constitutes the smallest part of CP. It is unavailable to the animal (Lanzas et al. 2008). The mean fraction C content in fescue hybrids was 10.4 g/kg of DM and for ryegrass hybrids 7.3 g/kg of DM. According to several authors (Ehsani 2007; Abbasi et al. 2012), fraction C increases due to increasing lignification as indicated by ADL (r = –0.584; P < 0.001). The content of our fraction C, however, was not significantly related to NDIP value.

The results obtained show that group III (fescue/ryegrass hybrids together) provides a better base for evaluating the regression equations than do the groups I and II separately. It can be assumed that the results of regression equations for groups 1 and 2 separately were not significant due to their low numbers of data points (for fescue hybrids n = 14 and for ryegrass hybrids n = 16). Some authors (Yamada et al. 2005; Kopecky et al. 2006; Kubota et al. 2015) describe fescue and ryegrass hybrids as a single group due to their similar nutrient compositions. Group III thus provides a dataset sufficiently large (n = 30) for more robust regression equations.

The best combinations of regression procedure for the OMD prediction equations are shown in Table 3. Expectedly, CF and NDF were the best predictors for OMD when simple linear regression was used (P > 0.0001). Moreover, the best multiple regressions for OMD (P > 0.0001) were found for combinations of CP with NDF and EE with NDF. The only nitrogen fractions, which can be used to

Table 3. Regression equations for determination of organic matter digestibility (OMD) of group III (n = 30) based on chemical composition and nitrogen fractions (units are in g/kg of DM)

OMD (y) Equation	R^2	RMSE	Probability
Simple linear regression			
y = 760.151 + 1.161 A	0.500	11.32	> 0.0001
y = 1092.576 – 0.537 NDF	0.536	10.90	> 0.0001
y = 985.740 – 0.699 CF	0.703	8.72	> 0.0001
Multiple regression			
y = 761.341 – 1.183 B1 + 1.151 SOLP	0.500	11.52	> 0.0001
y = 712.238 + 3.021 EE + 0.280 ADL	0.538	11.08	> 0.0001
y = 997.388 + 0.329 CP – 0.445 NDF	0.602	10.28	> 0.0001
y = 960.892 + 1.537 EE – 0.370 NDF	0.609	10.19	> 0.0001

A = non-protein nitrogen, ADL = acid detergent lignin, B1 = rapidly degradable protein, CF = crude fibre, CP = crude protein, EE = ether extract, NDF = neutral detergent fibre, SOLP = soluble protein, R^2 = coefficient of determination, RMSE = residual mean square error

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Table 4. Regression equations for determination of crude protein digestibility (CPD) of group III (n = 30) based on chemical composition and nitrogen fractions (units are in g/kg of DM)

CPD (y) Equation	R^2	RMSE	Probability
Simple linear regression			
y = 37.562 + 0.961 A	0.513	9.134	> 0.0001
y = −52.652 + 0.923 CP	0.969	2.308	> 0.0001
Multiple regression			
y = 147.944 – 0.360 CF + 0.316 SOLP	0.511	9.313	> 0.0001
y = 181.918 – 0.472 CF + 1.757 C	0.530	9.140	> 0.0001
y = 216.909 – 0.494 CF – 0.342 ADL	0.552	8.919	> 0.0001
y = 145.645 – 0.365 CF + 0.582 NDIP	0.554	8.896	> 0.0001
y = 22.869 + 0.862 A + 0.564 B3	0.641	7.988	> 0.0001
y = 732.738 – 0.559 NDF – 0.714 NFE	0.773	6.355	> 0.0001
y = 518.413 – 0.605 CF – 0.572 NFE	0.842	5.302	> 0.0001
y = −51.441 + 0.817 A + 1.067 B1 + 1.042 B2 + 1.040 C	0.917	3.994	> 0.0001
y = −52.473 + 0.913 IP + 0.932 SOLP	0.969	2.337	> 0.0001
y = −56.631 + 0.895 A + 0.992 B1 + 0.960 B2 + 0.927 B3 + 1.170 C	0.975	2.236	> 0.0001

A = non-protein nitrogen, ADL = acid detergent lignin, B1 = rapidly degradable protein, B2 = intermediately degradable protein, B3 = slowly degradable protein, C = unavailable protein, CF = crude fibre, CP = crude protein, IP = insoluble protein, NDF = neutral detergent fibre, NDIP = neutral detergent insoluble fibre, NFE = nitrogen-free extract, SOLP = soluble protein, R^2 = coefficient of determination, RMSE = residual mean square error

describe OMD (P > 0.0001), are fractions A and B1 (when SOLP is added to the equation).

Predictions of crude protein digestibility (CPD) have been reported numerous times, examples of which include Villamide and Fraga (1998) and Valiente et al. (2004).

Expectedly, the close relationship between CPD and CP and their fractions was confirmed (P > 0.0001) (Table 4). CPD depends upon the type of feed protein and is associated with modification of protein body structure, amino acid composition, and starch associated molecules (Xia et al. 2012). The best simple linear regression was found for CP. Due to the relatively easy and precise determination of the CP value, this regression equation seems to be usable for accurate calculation of CPD. Also, a statistically significant (P > 0.0001) simple linear regression for CPD was determined using fraction A, but its R^2 was just 0.513. Among the multiple regression equations we found interactions affirmed by R^2 greater than or equal to 0.773 (P > 0.0001) for NFE with CF and NFE with NDF. Strong coefficients of determination (R^2 more than 0.917; P > 0.0001) were found for combinations of individual nitrogen fractions (Table 4).

CONCLUSION

This study confirms the importance of nitrogen fraction determinations. The equations reported here with a high degree of determination can be used to describe the digestibility of crude protein. The digestibility of organic matter and crude protein can be derived not only from CF fractions, but also from CP fractions.

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