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Short communication

Development of equations to estimate microbial nitrogen contamination in rumen incubation residues using ^{15}N data and chemical composition of feedstuffs

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ABSTRACT

In situ rumen incubation of feedstuffs is used to obtain estimates for the rate of rumen microbial degradation of nutrients in feedstuffs such as organic matter and protein. These estimated values are used in feed evaluation systems for predicting amounts of nutrients fermented in the rumen. However, with respect to rumen degradation of protein, these rumen degradation estimates may not be accurate due to microbial contamination of in situ nitrogen residues. The objective of this study was to develop equations for predicting microbial contamination of rumen incubation residues. A literature review was carried out in order to build a dataset containing the results of studies in which microbial nitrogen (N) contamination of in situ rumen incubation residues of feedstuffs was measured based on ^{15}N labeled feedstuffs or microbes. These prediction equations may be used in feed evaluation systems to correct in situ incubation results for microbial N contamination and, thereby, in predicting more accurate estimates of rumen protein degradation rate of feedstuffs. The data set contained results of 11 published papers in scientific journals using a ^{15}N labeling method for estimating microbial contamination that had at least an incubation period equal to or larger than 24 h. The dataset contained 22 feedstuffs, of which 10 forages (R; 122 data points) and 12 concentrates (C; 175 data points). From the concentrate dataset, a subset of data was selected containing only low CP concentrate feedstuffs (CP < 300 g/kg DM) to estimate microbial contamination in low protein concentrates (LPC: 9 concentrates; 106 data points). Microbial N-contamination was estimated by the exponential equation of Krawielitzki et al. (2006). This model was further extended by including the effects of feed characteristics such as CP, NDF, and the combination of CP and NDF (CP and NDF expressed in g/kg DM). Coefficient of determination (R^2), root mean squared prediction error (RMSPE), concordance correlation coefficient (CCC), AIC, and BIC were used to assess goodness of fit. The in situ microbial N-contamination (N_{CONT}) of R was best predicted as follows: N_{CONT_R} (%) = (89.0 ± 3.16−0.209 ± 0.0194 × CP) × [1 - e^(-0.117 ± 0.0121 × incubation time (h))] (R^2 = 0.89; CCC = 0.94). For C and LPC the microbial nitrogen contaminationN_{CONT} was best estimated as follows: N_{CONT_C} (%) = (43.8 ± 4.26−0.070 ± 0.0081 × CP + 0.015 ± 0.0063 × NDF) × [1 - e^(-0.068 ± 0.0121 × incubation time (h))] (R^2 = 0.75; CCC = 0.86); N_{CONT_LPC} (%) = (53.0 ± 4.99−0.188 ± 0.0311 × CP + 0.031 ± 0.0084 × NDF) × [1 - e^(-0.072 ± 0.0134 × incubation time (h))] (R^2 = 0.82; CCC = 0.90). These models can be used

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to correct for microbial N-contamination and thereby improve the accuracy in predicting rumen N degradation characteristics of feedstuffs for ruminants.

1. Introduction

Nitrogen disappearance from nylon bags incubated in the rumen (*in situ* rumen incubation technique) as described by Ørskov and McDonald (1979) is a valuable tool to estimate the protein degradability of feedstuffs in the rumen. However, the estimation of *in situ* rumen protein degradability of feedstuffs may be negatively affected by several factors, including microbial contamination (Hristov et al., 2019). It is well documented that adherence of microbial matter to feed particles during the incubation process contaminates feed residues remaining in the bags after incubation. As a result, protein degradability of feedstuffs is underestimated if *in situ* disappearance values are not corrected for microbial contamination (Rodríguez and González, 2006). In the study of Rodríguez and González et al. (2006), values of microbial contamination of in situ incubation residues up to 81 % were measured, which, if not accounted for, resulted in an underestimation of rumen degradation of protein in feedstuffs of up to 13 %. This underestimation of rumen protein degradability may negatively impact the performance of nutritional models that rely on *in situ* evaluation data of feedstuffs such as the NRC 2001 (White et al., 2017) and the DVE/OEB-2011 (Van Duinkerken et al., 2011) system. The extent of microbial contamination is particularly high in forages with low N content and at longer incubation times (Krawielitzki et al., 2006; Rodríguez and González, 2006). Inorganic ^{15}N is a stable isotope that is not found in the diet above natural enrichment and has been used extensively as a relatively inexpensive tracer for labeling microbial protein (Broderick and Merchen, 1992). The use of ^{15}N seems to be an accurate and reliable method of identifying microbial N (Kamoun et al., 2014; Reynal et al., 2005) and is considered a superior marker compared to most internal markers such as diaminopimelic acid and nucleic acids (Broderick and Merchen, 1992). Isotopic ^{15}N can be used to label either the feed protein or the rumen microbial N, but generally, they provide similar corrected degradability values (Kamoun et al., 2014). While correction of the incubation residues for microbial contamination appears to be necessary, it is costly and time-consuming to be implemented as a routine procedure. Therefore attempts have been made to develop equations to predict microbial contamination in ruminal incubation residues as an alternative (Krawielitzki et al., 2006; Machado et al., 2013). Still, the results of these attempts were based on the results of a single experiment with a limited variety in tested feedstuffs. Therefore, the objective of this study was to develop robust equations for predicting microbial contamination that can be used to reliably predict microbial contamination for a wide range of feedstuffs.

2. Material and methods

2.1. Database

A literature study was carried out on available peer-reviewed published studies in scientific journals using as search terms 'microbial contamination', 'in situ incubation', 'rumen', 'cattle or cows', '^{15}N'. Our literature search used PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Google Scholar (http://scholar.google.com/), ScienceDirect, ISI Web of Science (http://apps.webofknowledge.com), CABI (http://www.cabi.org); and investigation of references in papers. Studies that were included in the dataset had used the ^{15}N labeling method for estimating *in situ* microbial N contamination and had at least three measurements at incubation periods greater than 0 h, including a long time incubation period ($\geq$24 h). The data set consisted of 25 feedstuffs, of which 10 roughages (R; 122 data points) and 15 concentrate feedstuffs (C; 175 data points) from 11 published papers in scientific journals that met the criteria for this study (Appendix B). A subset of the C data was further compiled containing low protein concentrate feedstuffs to investigate the microbial contamination in low protein (CP $<$ 300 g/kg DM) concentrate feedstuffs (LPC; 9 concentrates; 106 data points). For this subset of data, rapeseed meal, sunflower meal, full-fat soybean meal, soybean meal, and meat and bone meal were excluded from the C

Table 1
Summary of the data included in the data set.

Dataset	Variable	Mean	SD	Minimum	Maximum
R*	Incubation period (h)	18	20.2	0	72
	Micr. N-contamination (%)	38	27.0	0	95
	CP feedstuffs (g/kg DM)	122	67.5	27	243
	NDF feedstuffs (g/kg DM)	559	142.6	391	817
C*	Incubation period (h)	17	19.0	0	72
	Micr. N-contamination (%)	14	16.0	0	67
	CP feedstuffs (g/kg DM)	281	191.9	80	657
	NDF feedstuffs (g/kg DM)	265	202.8	0	716
LPC*	Incubation period (h)	18	19.6	0	72
	Micr. N-contamination (%)	19	17.8	0	66
	CP feedstuffs (g/kg DM)	138	51.6	80	256
	NDF feedstuffs (g/kg DM)	352	196.2	141	716

*R = roughages, C = concentrate feedstuffs, LPC = low protein concentrate feedstuffs. LPC differs with respect to C in that feedstuffs with protein concentrations higher than 300 g/kg DM such as meat and bone meal, sunflower meal, and soybean meal were excluded from the dataset.

Table 2
Estimated model parameters (values ± SE) and goodness of fit parameters for the Krawielitzki model and extended Krawielitzki models for the roughage dataset (R), the concentrate dataset (C) and the low protein (CP < 300 g/kg DM) concentrate dataset.

Parameter [1]	R		C		LPC	
	Krawielitzki	Extended	Krawielitzki	Extended	Krawielitzki	Extended
N_{max}	65.2 ± 2.93	89.0 ± 3.16	29.3 ± 3.05	43.8 ± 4.26	39.3 ± 3.16	53.0 ± 4.99
k (rate)	0.108 ± 0.0152	0.117 ± 0.0121	0.059 ± 0.0173	0.068 ± 0.0121	0.063 ± 0.0152	0.072 ± 0.0134
b (CP)	–	−0.209 ± 0.0194	–	−0.070 ± 0.0081	–	−0.188 ± 0.0311
c (NDF)	–	–	–	0.015 ± 0.0063	–	0.031 ± 0.0084
AIC	996	915	1366	1261	807	777
R^2	0.770	0.889	0.537	0.749	0.733	0.815
CCC	0.871	0.941	0.694	0.856	0.844	0.897
RMSPE	12.9	9.0	10.8	8.0	9.2	7.6

[1] N_{max} = maximal microbial in situ nitrogen contamination at the time ≈∞ (%), b and c are regression coefficients for, respectively, CP (g/kg DM) and NDF (g/kg DM), k = rate of in situ microbial nitrogen contamination (/h). AIC = Akaike information criterion (smaller is better), R^2 = coefficient of determination, CCC = concordance correlation coefficient, and RMSPE = root mean squared prediction error.

dataset. So the statistical analysis was performed separately for the R, C, and LPC dataset separately. A summary of the data in the R, C, and LPC dataset is provided in Table 1.

2.2. Statistical analysis

The NLMIXED procedure of SAS (SAS, 2003) was used to fit microbial N contamination of the incubation residues at each time point. The model, as described by Krawielitzki et al. (2006), was used with study included as the random effect (St-Pierre, 2001).

$$N_{cont.} = N_{max}[\%]\left[1 - e^{-kt}\right] + study + error$$

Where:
$N_{cont.}$ is the microbial N contamination of the incubation residue (%).
N_{max} represents the maximal microbial N contamination at time ≈∞ (%) (plateau value).
k is the rate of microbial contamination (/h).
t is the incubation period (h).
study is the random study effect.
error is the residual error.

The above model was further extended by allowing the N_{max} or plateau value to vary by the concentration of CP and/or NDF in the feedstuff before incubation. The hypothesis for this extended model was that the plateau value is negatively affected by CP and positively by NDF. The effect of the starch content in the feedstuff on model performance was also investigated but was not included in the final models as it did not result in improved model fits compared to the parameters CP and NDF. The full model containing both correction factors is as follows, and analysis was also performed for sub-models, including each factor solely.

$$N_{cont.}[\%] = [b \times CP + c \times NDF + N_{max}[\%]]\left[1\text{-}e^{-kt}\right] + study + error$$

Where CP = crude protein (g/kg DM) and NDF = neutral detergent fibre (g/kg DM) in the original feedstuff and b and c are regression coefficients for, respectively, CP and NDF.

Prediction errors of models were determined by the root mean squared prediction error (RMSPE), which was calculated according to Conway et al. (1979):

$$RMSPE = \sqrt{\left[\Sigma(observed - predicted)^2/n\right]}$$

In addition, the accuracy and precision of the models were evaluated using the concordance correlation coefficient (CCC) analysis, according to Lin (1989). Concordance correlation coefficients range from –1 to +1, where values closer to +1 indicate a more precise and accurate model.

Since the most parameterized model is not necessarily the best one (Burnham and Anderson, 2004), the Akaike information criterion (AIC) was used to assess if adding explanatory factors to the model is in favor of model performance. Lower AIC values are for a better adjustment (Akaike, 1974). This criterion is expressed as:

$$AIC = -2loglike + 2p$$

where p is the number of parameters, and loglike refers to the logarithmic value of the likelihood function as a function of the

parameter estimates.

Finally, superior models for each dataset were selected based on coefficient of determination (R^2), concordance correlation coefficient (CCC), RMSPE and AIC values.

3. Results and discussion

For the R dataset, the addition of the explanatory parameter CP and for the C and LPC datasets the addition of CP and NDF to the Krawielitzki model improved the goodness of fit characteristics R^2, CCC, RMSPE and AIC. (Table 2). The estimated negative effect of CP on N_{max} and the positive effect of NDF on N_{max} in this study is in agreement with results from the literature. For example, in the study of Machado et al. (2013), the observed contamination levels were negatively related to the CP content of feedstuffs. Furthermore, using DNA as a microbial marker, Paz et al. (2014), reported that microbial contamination of the in situ residues was positively related with NDF concentration of the feedstuffs. Krawielitzki et al. (2006) investigated the dynamics of microbial contamination of feedstuffs during ruminal in situ incubation. Krawielitzki et al. (2006) reported that maximum microbial N-contamination is negatively related to CP content of feedstuffs.

According to Alexandrov (1998) and Krawielitzki et al. (2006), correcting for microbial N-contamination of incubation residues is necessary for roughages but is of less value for protein concentrates and grains. Cellulose is shown as the primary substrate responsible for microbial DM accumulation and its associated microbial N-contamination (Rodríguez and González, 2006). Fiber colonization by rumen microbes, as demonstrated by using different electron microscopic techniques (Akin and Amos, 1975; Dinsdale et al., 1978), can explain the results from studies showing higher microbial N-contamination for feedstuffs with a high NDF content and may also explain the improvement in model prediction in the present study in case NDF was added as an explanatory factor. Using the ^{15}N technique Krawielitzki et al. (2006) reported a N_{max} of 69.4 and 62.8 for perennial ryegrass and summer barley straw, respectively. Using the same technique, Vanegas et al. (2016) reported maximum microbial contamination of 70 % for wheat straw. These values are close to the N_{max} of 65.2 obtained for the R dataset using the original Krawielitzki model (Krawielitzki et al., 2006) in the present study. With respect to protein-rich concentrate feedstuffs, microbial N-contamination exerts probably only a slight influence upon undegraded *in situ* residues. Maximum microbial N-contamination in the incubated residues of protein-rich fish meal and meat meal samples were reported to be <2% determined by continuous intra-ruminal infusion of ^{15}N (González et al., 1998). Vanegas et al. (2016) reported the maximum microbial N-contamination to be <15 % for full-fat soybean (CP = 408 g/kg DM), soybean meal (CP = 551 g/kg DM) and corn gluten feed (CP = 221 g/kg DM) using the ^{15}N labeling method. These are in agreement with N-contamination results of protein-rich feedstuffs included in our dataset (Fig. A1). It shows that microbial protein contamination in concentrate feedstuffs is only relevant for low protein feedstuffs. In the study of Vanegas et al. (2016), the maximum microbial contamination for low protein concentrate feedstuffs wheat bran, corn grain, and sugar beet pulp was reported to be ~30 %. In the study of Krawielitzki et al. (2006), the N_{max} ranged from 31.8% to 47.7% for grains. These results are in agreement with our findings for the C (N_{max}~29.3 %) and LPC (N_{max} = 39.3 %) datasets. Furthermore, results from this study and other studies (González et al., 2006; Rodríguez and González, 2006) show that the microbial N-contamination in concentrate feedstuffs is substantially lower than in roughages.

4. Conclusion

It is concluded from this study that for roughages 89 % and for low protein concentrates 82 % of variation in microbial nitrogen contamination of in situ feed residues can be explained by the extended Krawielitzki models presented in this study. The results of these models can be used in protein evaluation systems to correct for microbial nitrogen contamination of in situ incubation results.

CRediT authorship contribution statement

E. Parand: Methodology, Software, Writing - original draft, Writing - review & editing. **J.W. Spek:** Conceptualization, Methodology, Software, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A

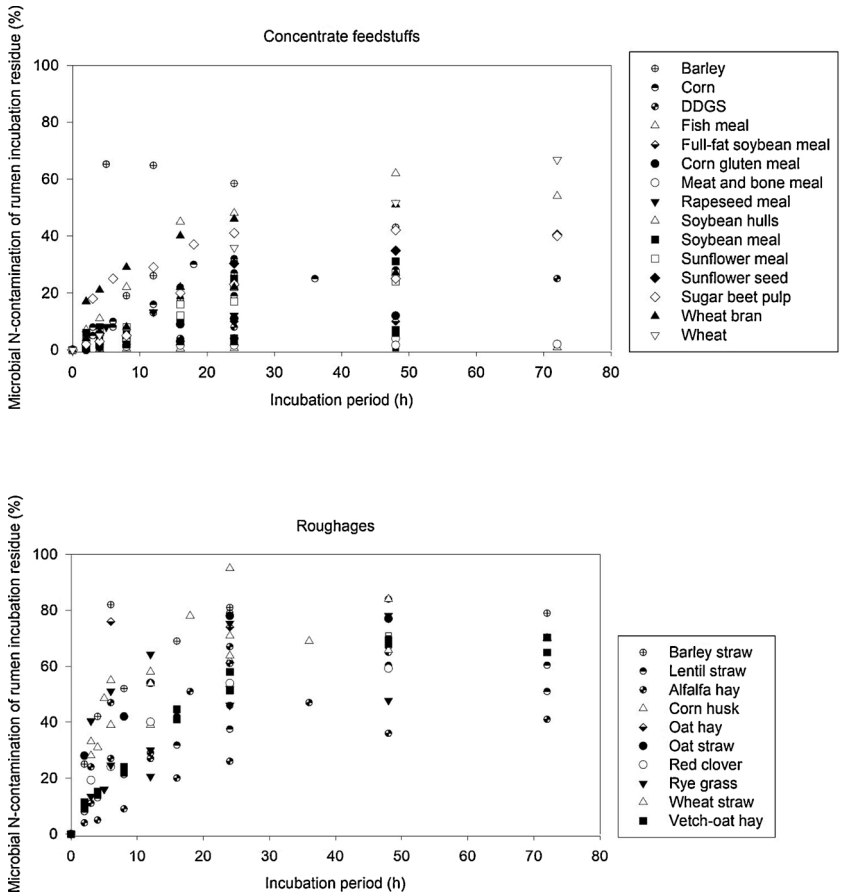

Fig. A1. Overview of observed microbial nitrogen contamination of in situ rumen incubation residues (%) at various incubation periods for concentrate feedstuffs (upper graph) and concentrate feedstuffs (lower graph) based on ^{15}N measurements.

Appendix B

Studies included in the dataset:

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