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Dynamics of microbial contamination of protein during ruminal *in situ* incubation of feedstuffs*

K. Krawielitzki[1], Th. Schmidt[1], J. Voigt[2], J. Kowalczyk[3] and M. Gabel[1,1]

[1]University of Rostock, Institute for Farm Animal Sciences and Technology
18051 Rostock, Germany
[2]Research Institute for the Biology of Farm Animals,
Department of Nutritional Physiology "Oskar Kellner"
W.-Stahl-Allee 2, 18196 Dummerstorf, Germany
[3]The Kielanowski Institute of Animal Physiology and Nutrition,
Polish Academy of Sciences
05-110 Jabłonna, Poland

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ABSTRACT

The aim of this study was to determine the dynamics of bacterial contamination of feedstuffs during ruminal *in situ* incubation and to quantify the effects of bacterial contamination on the ruminal *in situ* degradation of crude protein (CP). Two different approaches using ^{15}N as a marker were followed, namely: 1. ^{15}N-labelled rumen bacteria in combination with unlabelled feedstuffs and 2. ^{15}N-labelling of feeds while leaving rumen bacteria unlabelled. Using the nylon bag technique, a total of 31 feedstuffs was incubated in the rumen of 3 steers. Both marker methods gave in principle similar results. The course of bacterial contamination was best described by an exponential function of the general type $A = A_{max} [1 - \exp(-C \cdot t)]$, where A denotes bacterial contamination (% of residue N), A_{max} is the maximum of bacterial contamination for $t \approx \infty$, C is the rate of contamination [$\% \cdot h^{-1}$] and t denotes the incubation time (h). The A_{max} values of most cereals ranged from 32 to 38% of the residue N. For roughages and straw, A_{max} reaches 45 to 100%. A_{max}, but not C, is significantly positively influenced by the NDF content in feedstuffs (P=0.049). The CP content of feedstuffs negatively affects C (P=0.006). The proportion of bacterial CP of total CP within the *in situ* bag residues was used to correct the apparent CP degradation rate. This correction was numerically lowest for protein concentrates and grains (0 to 3%) and highest for roughages (up to 50%). In conclusion, correction of ruminal protein degradation for bacterial contamination is necessary, especially for roughages. For cereal grains and protein concentrates it seems to be irrelevant.

KEY WORDS: rumen, microbial contamination, crude protein degradation, nylon-bag method, ^{15}N marker

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[14] Corresponding author: e-mail: martin.gabel@uni-rostock.de

INTRODUCTION

Data obtained by the nylon bag technique (NBT) often do not agree with the three factorial model function of Ørskov and McDonald (1979) used for description of ruminal crude protein (CP) degradation (Robinson and Tamminga, 1984). A great source of error with this method is the microbial contamination of feed particles within the artificial fibre bags, which results in an underestimation of CP degradation (Mathers and Aitchison, 1981; Südekum, 2005). Only few studies for quantifying this microbial contamination have been carried out and most *in situ* estimates of ruminal CP degradation simply neglect this microbial contamination.

Bauchop (1979) observed the contamination of feed particles microscopically. Other authors tried to extract or separate the microbes by physico-chemical treatments using salts in combination with chilling (Dehority and Grubb, 1980; Craig et al., 1987), ultrasonic (Puelo et al., 1967; Sharpe and Kilsby, 1970) or mechanical treatments such as stomaching (Legay-Carmier and Bauchart, 1989; Weisbjerg and Hvelplund, 2005). Ould-Bah (1988) combined these three techniques to remove the bacterial mass from bag residues and obtained the best results. All methods gave highly variable results in dependence on treatment time, showing that the separation of bacteria was incomplete.

An alternative way to take into account microbial contamination is the use of marker methods. Mehrez and Ørskov (1977) used diaminopimelic acid (DAPA), Mathers and Aitchison (1981) ^{35}S, and Varvikko and Lindberg (1985) ^{15}N and DAPA as microbial markers.

The aim of this study was to determine the dynamics of microbial contamination of feedstuff residues in artificial fibre bags during *in situ* incubations and to quantify the error associated with this microbial contamination in estimating the ruminal protein degradability of different feeds.

MATERIAL AND METHODS

The study was carried out with three adult German Holstein steers equipped with ruminal cannulae (inner diameter 100 mm, Bar Diamond, Inc., USA). The animals were feed twice per day at 6.30 and 18.30 with a diet consisting of (on air-dry matter basis) 60% of roughage (artificially dried grass) and 40% concentrates. The concentrate portion contained, %: winter barley 60, winter wheat 25, oat 7, soyabean meal 2, rapeseed-cake 2, molasses 3, and mineral feed 1. A detailed listing of feedstuffs used in the NBT study is given in Table 1.

Table 1. Nutrient contents of feedstuffs and feed mixtures investigated by NBT, % of dry matter

Feedstuff	DM	CA	CP	NDF	ADF	ADL	Starch
Unlabelled							
oats	95.0	2.8	11.4	27.7	13.2	2.9	46.8
winter wheat grain	94.4	1.8	14.1	14.1	3.2	0.6	64.9
winter barley grain	93.1	2.0	12.5	18.4	5.9	0.9	60.0
summer barley grain	93.8	2.0	12.1	17.0	3.5	0.4	57.4
winter triticale grain	94.0	2.0	12.5	14.7	3.3	0.8	65.3
maize grain	94.0	1.4	10.6	13.1	3.9	0.7	71.4
wheat bran	93.2	6.1	16.9	42.2	13.1	3.9	14.0
brewer's grain	96.6	3.0	21.7	58.8	24.8	5.2	1.9
bean (*Vicia faba*)	93.2	3.8	26.4	16.7	13.4	3.7	40.1
field pea	93.5	3.1	23.8	24.9	9.2	0.1	45.1
soyabean meal	95.8	7.0	49.5	15.6	11.2	1.2	4.6
rapeseed meal	95.8	8.5	37.1	27.7	18.4	8.0	2.8
Grass (Perennial ryegrass)							
immature	95.0	9.2	24.6	35.9	20.3	1.9	n.d.
med-maturity	94.9	8.7	17.5	49.8	28.4	1.4	n.d.
mature	93.7	6.0	9.8	58.2	32.9	2.1	n.d.
Maize (whole crop)							
immature	94.1	5.9	9.0	53.6	25.8	3.6	8.3
med-maturity	93.6	5.1	8.4	43.0	23.4	2.3	17.9
mature	92.7	2.9	8.3	40.0	17.8	1.6	39.7
Lucerne hay	96.0	11.2	18.6	43.8	32.3	7.2	n.d.
Oat straw	95.4	5.0	5.6	81.7	54.6	10.0	n.d.
Winter wheat straw	93.6	6.0	6.2	74.2	48.6	7.7	n.d.
Summer barley straw	94.1	4.6	4.3	79.6	48.4	5.3	n.d.
Pea straw	95.3	8.5	5.3	67.3	58.1	10.7	n.d.
Feed mixtures							
soyabean meal : winter wheat straw							
20 : 80	98.0	6.0	18.4	43.5	40.2	8.1	n.d.
40 : 60	97.7	6.2	23.2	48.2	31.2	4.0	n.d.
60 : 40	98.4	6.2	30.8	38.0	25.3	3.4	n.d.
80 : 20	93.8	6.1	39.5	29.1	18.3	2.0	n.d.
wheat grain : winter wheat straw							
20 : 80	91.5	5.2	8.2	61.1	41.3	6.5	17.2
40 : 60	91.9	4.0	9.2	48.2	27.3	4.1	27.7
60 : 40	92.0	3.5	10.4	40.7	21.1	3.2	41.5
80 : 20	92.6	2.5	11.7	20.8	19.7	1.9	51.7
^{15}N-labelled				*^{15}N (at-%)*			
rye grain	90.6	1.9	15.7	1.71			
summer wheat grain	91.3	1.9	15.0	33.86			
summer barley grain	92.4	1.8	11.6	4.64			
bean (*Vicia faba*)	91.5	3.4	29.1	0.85			
perennial ryegrass	93.7	8.0	10.1	3.02			
summer barley straw	93.4	4.6	4.2	4.58			

The application of NBT was done according to the recommendations of Madsen and Hvelplund (1994). Polyester bags (100 × 225 mm; Ankom Technology Corp., USA) with a pore size of 50±15 μm were used for the incubations. In each bag, 5 g of dry matter of milled (2 mm screen for concentrate, 3 mm screen for roughage) sample were weighed. Eighteen bags were prepared in parallel for all feedstuffs and every incubation time. The bags were divided evenly among the three animals. After incubation the bags were washed in a washing machine. More details about the experimental animals and NBT are presented by Schmidt et al. (2005).

^{15}N was used as a marker to estimate microbial N in residues of nylon bags. Two different ^{15}N marker methods were tested: 1. ^{15}N-labelled rumen bacteria in combination with unlabelled feedstuffs (Beckers et al., 1995) and 2. ^{15}N labelled feedstuffs and unlabelled rumen microbes. The samples using method 1 were incubated for 2, 4, 8, 12, 24 and 48 h, and those using method 2, for 4, 8, 12, 24 and 48 h. Roughages were also incubated for 72 h.

The determination of the ^{15}N level in residue and isolated ruminal bacteria was carried out by isotope ratio mass spectrometry using an elemental analyser (EA 1108, Fisons Instr., Rodano, Italy) coupled on-line to an isotope ratio mass-spectrometer (delta S, Finnigan MAT, Bremen, Germany).

Calculations

The apparent disappearance rate (aDR) of N or CP (N×6.25) at different incubation times was calculated as follows:

$$\text{aDR}\,[\%] = \frac{\text{Initial N}\,[\text{mg}] - \text{Residue N}\,[\text{mg}]}{\text{Initial N}\,[\text{mg}]} \cdot 100 \qquad (1)$$

The true disappearance rate (tDR) of N was computed considering the estimated bacterial N contamination of incubation residues:

$$\text{tDR}\,[\%] = \frac{\text{Initial N}\,[\text{mg}] - \text{Residue N}\,[\text{mg}] + \text{Bacterial N}\,[\text{mg}]}{\text{Initial N}\,[\text{mg}]} \cdot 100 \qquad (2)$$

where bacterial N for method 1 was calculated as:

$$N_{bact}.\,[\text{mg}] = \text{Residue N}\ [\text{mg}] \cdot \frac{^{15}\text{N' in Residue N}\,[\text{atom\%}]}{^{15}\text{N' in Bacterial N}\,[\text{atom\%}]} \qquad (3)$$

(^{15}N' [atom %] = $^{15}N_{excess}$ [atom %])

For trials using ^{15}N labelled feeds (method 2) the following equations are used:

$$\text{tDR}\,[\%] = \frac{\text{Initial } ^{15}\text{N}\,[\text{mg}] - \text{Residue } ^{15}\text{N}\,[\text{mg}]}{\text{Initial } ^{15}\text{N}\,[\text{mg}]} \cdot 100 \qquad (4)$$

$$N_{bact.}[mg] = \text{Residue N}[mg] \cdot \frac{(^{15}N' \text{ in Initial N} - {}^{15}N' \text{ in Residue N})[atom\%]}{^{15}N' \text{ in Initial N}[atom\%]} \quad (5)$$

The dynamic of bacterial N contamination A (% of total N) was described by the following exponential equation:

$$A_{contam.}[\%] = f(t) = A_{max.}[\%][1 - \exp(-C \cdot t)] \quad (6)$$

Ruminally undegraded CP (respectively N) in the bag residues ($N_{undegraded}$) was estimated as follows:

$$N_{undegraded}[\%] = 100 - N_{degraded}[\%] = f(t) = 100 - \{a + b[1 - \exp(-C \cdot t)]\} \quad (7)$$

For $t \approx \infty$ not degraded N will then become the potentially undegradable N or CP:

$$\{N_{undegraded}\}_{t \to \infty} = N_{undegradable}$$

$$N_{undegradable}[\%] = 100 - (a + b)[\%], \text{ where: } \{N_{degraded}\}_{t \to \infty} = a + b \quad (8)$$

In combination with the data of bacterial contamination ($A_{contam.}$), the proportion of undegradable protein of bacterial source ($N_{undegradable\ bact.}$) in this not degraded residue protein was calculated by use of equation 9:

$$N_{undegradable\ bact.}[\%] = \frac{N_{undegradable}[\%] \cdot A_{contam.}[\%]}{100} \quad (9)$$

Descriptive statistics and linear regression analysis were carried out by SPSS (version 13.0, SPSS Inc., Chicago/Illinois, USA). CADEMO (Rasch et al., 1987) was used for calculation of the exponential equations.

RESULTS

Method 1 (^{15}N-laballed rumen bacteria)

The course of the N-amounts [mg] for total N (N total) and bacterial N ($N_{bact.}$) in dependence of the incubation time is shown for oat straw in Figure 1.

The relative contamination (% of total residue N) of the feedstuff residues in the nylon bags increased continuously in an exponential manner. This is illustrated in Figure 2 for winter barley grain and barley straw.

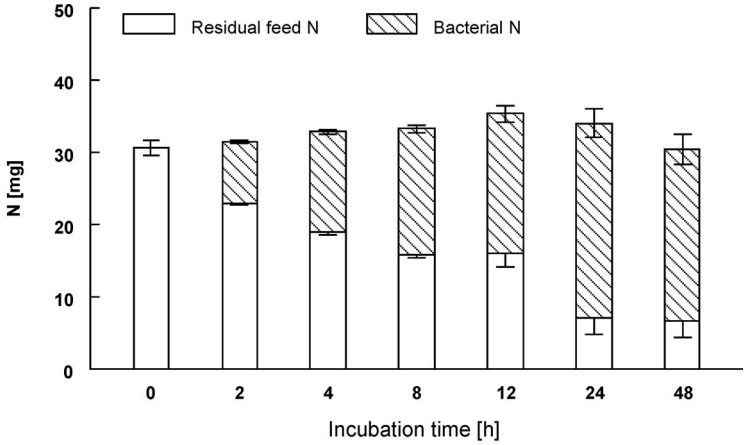

Figure 1. Course of feed N and bacterial N in incubation residues of oat straw in dependence of incubation time ($x \pm s$, $n = 36…54$)

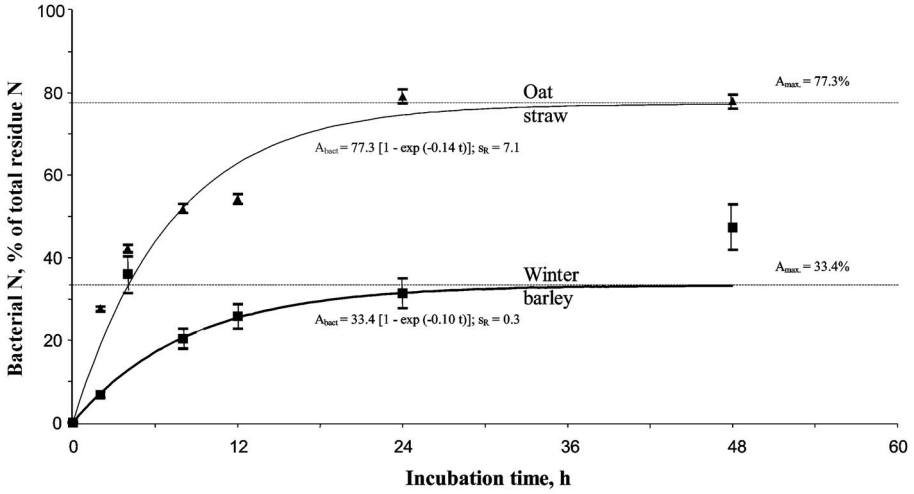

Figure 2. Exponential course of bacterial contamination of winter barley and oat straw during incubation

The course of both curves shows a steep ascent at the beginning of the incubation, reaching relatively high contamination values within 2-4 h. There follows a period of slow increase (4-8 h) and then approximates to a maximum, showing that a saturation point or a steady state for microbial contamination of feed particles is reached. This is the typical course of a growth function. From a number of different growth functions (Logistic-, Sigmoid-, Exponential-, Gompertz-, Bertalanfy-function) the best fitted one with the smallest residue

variance (s_R) was chosen using the CADEMO program (Rasch et al., 1987). This was the exponential function (equation 6), which also by heuristic model choice would be the function with the logically best understanding of the parameters for the assessed growing process.

Table 2 summarises the calculated parameter of bacterial contamination for all feedstuffs and feed mixtures during ruminal digestion. For calculation of these exponential functions, the values which were distinctly outliers of a continuous course were ignored. This concerns especially some 24 and the 48 h data, where marginal amounts of residues in combination with very small differences in ^{15}N excess values between residue and bacterial N causes a high uncertainty in the estimation of bacterial contamination.

The meaning of the parameter $A_{max.}$ of the exponential function (equation 6) is the final value of bacterial contamination of feed residues at saturation state in % of total N in the residue. This value extrapolated for $t = \infty$ is nearly equal to the data of bacterial contamination estimated analytically for 24 or 48 h. For most cereals, this value is about 32-38%, except for oats and triticale (48%) and brewer's grain (12.5%). The exponent C of equation 6 represents the rate of bacterial contamination and 100·C denotes the increase of this process in $\%\cdot h^{-1}$. The rate varies for cereals between 5 and $15\%\cdot h^{-1}$, showing very similar dynamics of bacterial contamination for all cereal products. Contamination starts immediately at the onset of ruminal incubation, because the calculated lag was always zero within the limits of error (maximum lag was approximately 0.15 h for winter wheat). In contrast to cereal grains, the by-products showed some lag before contamination started (0.6 and 0.5 h). For protein concentrates, the evidence for exponential bacterial contamination of feed particles was difficult to assess, because CP degradation occurred at a much higher rate (9.5- $10.8\%\cdot h^{-1}$) than the slow process of bacterial contamination, which ranged from only $2.1\%\cdot h^{-1}$ for *Vicia faba* to $3.1\%\cdot h^{-1}$ for soyabean meal. For field peas and rape seed, the calculation was not possible.

Table 2 demonstrates that bacterial contamination takes place very rapidly (up to $20\%\cdot h^{-1}$). The proportion of bacterial N of total N had reached about 100% within 24-48 h for all whole crop maize samples (not shown). Thus, after prolonged incubation periods, all the N of the residues was of bacterial origin and the microbes within the bags were attached to undegraded N-free residues, which consist mainly of cell wall material.

The grass and straw samples behaved similarly, but the saturation point was reached later. The study on the feed mixtures (soyabean meal with winter wheat straw and winter wheat grain with winter wheat straw) showed that the saturation value for contamination rises with the proportion of straw in the mixtures. Also, an increase in the velocity of this process was observed with an increasing straw proportion within the feed mixture. For the wheat grain-wheat

Table 2. Bacterial contamination calculated by use of the exponential Function $Y = f(t) = A_{max.} (1 - \exp(-C \cdot t))$

Feedstuff	Contamination A_{max}, %	Rate of contamination C, $\% \cdot h^{-1}$	Residual variance
Oats	47.7	15.0	6.1
Winter wheat	31.8	10.2	3.9
Winter barley	33.4	12.0	0.3
Summer barley	47.7	5.7	5.1
Winter triticale	47.1	11.9	7.9
Grain maize	38.6	5.2	1.5
Wheat grain	32.1	7.4	2.6
Brewer's grain	12.5	11.4	1.4
Bean (*Vicia faba*)	60.2	2.1	2.7
Field pea	Exponential function not calculable		
Soyabean meal	1.3	3.1	2.1
Rapeseed meal	Exponential function not calculable		
Grass (*Perennial ryegrass*)			
immature	45.5	8.6	3.6
med-maturity	62.2	19.0	9.8
mature	84.9	8.4	16.3
Maize (whole crop)			
immature	91.5	20.4	15.0
med-maturity	104	20.1	11.7
mature	106	18.4	8.8
Lucerne hay	47.9	5.1	4.1
Oat straw	77.3	13.8	7.1
Wheat straw	66.7	24.4	14.9
Summer barley straw	102.0	9.9	8.9
Pea straw	83.8	24.0	5.7
Soyabean meal : winter wheat straw			
20 : 80	53.0	6.3	2.6
40 : 60	48.5	5.6	5.3
60 : 40	(96.7)	2.8	4.4
80 : 20	53.9	3.0	5.2
Winter wheat grain : winter wheat straw			
20 : 80	58.7	19.0	9.0
40 : 60	54.6	23.0	7.9
60 : 40	49.5	12.0	4.7
80 : 20	47.5	10.0	4.7

Y - bacterial contamination at time t

A_{max} - maximal bacterial contamination at time $t \approx \infty$ (% of total N)

C - speed of bacterial contamination ($\% \cdot h^{-1}$)

Table 3. Residues of nitrogen and potential ruminal degradability of CP

Feedstuff	Total residue N	Bacterial residue N	Potential N degradability
	% of initial N		
Oats	6.2	3.0	97
Winter wheat	3.5	1.1	98
Winter barley	5.8	1.9	96
Summer barley	3.5	1.7	98
Winter triticale	4.6	2.2	98
Grain maize			
Wheat grain	7.8	2.5	95
Brewer's grain	9.7	1.2	92
Bean (*Vicia faba*)	-1.1	0.0	101
Field pea	-3.0	0.0	103
Soyabean meal	1.7	0.0	98
Rapeseed meal	0.7	(-2.0)	99
Grass (*Perennial ryegrass*)			
immature	3.5	1.6	98
med-maturity	2.9	1.8	99
mature	(-7.3)	(-6.2)	101
Maize (whole crop)			
immature	13.6	12.4	99
med-maturity	7.8	8.1	100
mature	6.0*	6.4	100
Lucerne hay	13.3	6.4	93
Oat straw	28.0*	13.3	85
Wheat straw	20.0*	13.3	93
Summer barley straw	36.0*	36.7	101
Pea straw	60.0*	50.3	90
Soyabean meal : winter wheat straw			
20 : 80	19.4	10.3	91
40 : 60	9.8	4.8	95
60 : 40	4.2	4.1	100
80 : 20	1.2	0.6	99
Winter wheat grain : winter wheat straw			
20 : 80	(42.8)	(25.1)	82
40 : 60	29.0	15.8	87
60 : 40	21.2	10.5	89
80 : 20	12.1	5.7	94

* calculation of apparent degradability rate (aDR) was not possible. Values were recalculated from true degradability (tDR) and bacterial contamination

straw combination, this course is very exactly fulfilled, whereas the soyabean meal-wheat straw mixtures had some irregularities for $A_{max.}$, especially for the 60:40 mixture, which results from the extremely high values for 48 and 72 h incubation. The latter values are probably overestimated.

Potential ruminal degradation for t ≈ ∞ was estimated by equation (7). The results of this estimation are summarized in Table 3.

This table shows that the N of the bacterial source ranged greatly between 0 and 50% of the residual N within the bags after ruminal incubation. For cereal grains and protein concentrates, this correction for bacterial contamination is negligible, but for maize (whole crop) and especially for straw and straw mixtures, neglecting to account for bacterial contamination is the cause of important systematic errors in estimation of ruminal CP degradation (underestimation). For all types of straw, calculation of ruminal degradation of CP by the model of Ørskov and McDonald (1979) was only possible after correction for bacterial contamination.

Method 2 (^{15}N labelled feedstuffs)

As expected, bacterial contamination also follows an exponential function of the type $Y = A_{max}. [1 – exp (- C·t)]$. In Table 4 the results of bacterial contamination of these estimations are compared with those from studies using method 1.

Table 4. Kinetic of bacterial contamination of ^{15}N-labelled and unlabelled feedstuffs calculated by use of the exponential function $Y = f(t) = A_{max.}[1 – exp (-C·t)]$

Feedstuff	Contamination A_{max}, %	Rate of bacterial contamination C, $\%·h^{-1}$	Residual variance S_R
Rye grain			
^{15}N-labelled	30.8	12	0.8
unlabelled		n. d.	
Summer wheat grain			
^{15}N-labelled	48.0	5	2.4
unlabelled	31.8	10	3.9
Summer barley grain			
^{15}N-labelled	47.2	6	2.9
unlabelled	47.7	6	5.1 (t_{lag} = 6.5h)
Bean (*Vicia faba*)			
^{15}N-labelled	60.8	2.1	6.6 (t_{lag} = 3.3h)
unlabelled	55.1	1.3	1.7
Grass *(Perennial ryegrass)*			
^{15}N-labelled	69.4	9	2.7
unlabelled	84.9	8	16.3
Summer barley straw			
^{15}N-labelled	62.8	18	1.9
unlabelled	102.5	10	8.9

Y - bacterial contamination at time t; A_{max} - maximal bacterial contamination at time t ≈ ∞ (% of total N); C - speed of bacterial contamination ($\%·h^{-1}$)

The origin of ^{15}N labelled and unlabelled samples of barley grain and straw was the same; for the other comparative investigations, the cultivars were not exactly the same and thus permit only limited comparison.

The levels of microbial contamination ($A_{max.}$[%]) were roughly in the same range as for unlabelled feed residues except for barley straw, where the value for unlabelled straw differs from those of the other straw types and appears to be overestimated.

Contamination was lowest for cereal grains and bean (*Vicia faba*) and highest for barley straw. The contamination data were within the limits of error. As for *Vicia faba,* in both experimental series (with ^{15}N labelled and unlabelled beans), the course of bacterial contamination could be calculated only if a discrete lag (t_{lag}) was accepted before microbial contamination of the feed particles started.

DISCUSSION

Neglecting bacterial contamination caused a relevant underestimation of CP degradation for feeds with low CP contents but potentially high CP degradation (fresh and conserved forage) but was negligible for feedstuffs with a high CP content and low degradation rates (cereal grains and other concentrates) (Varvikko and Lindberg, 1985; Katzy et al., 1993). At longer incubation times (>12 h) the residual feed N of some of the feedstuffs approximated zero, demonstrating that the residual N was solely of bacterial origin. Table 3 demonstrates that for all feeds, CP degradation corrected for bacterial contamination amounts to ≥ 95%, showing that feed CP is (almost) completely degradable in the rumen if time is not a limiting factor.

This leads to the question, which feed component is responsible for the assimilation of rumen bacteria? The most probable component seems to be the fibre fraction of forages, which is not fully degraded even after lengthy incubations. Furthermore, the protein content is to be considered. The following significant relation was found between A_{max} and the content of these 2 components: $A_{max}(\%)=50.6\pm14.6+0.48\pm0.23\cdot NDF-0.77\pm0.44\cdot CP$; n=29; R=0.57; P=0.006. The positive influence of NDF was significant (P=0.047), in contrast to the negative influence of CP (P=0.094). In this and the following regressions, the possible intercorrelation between CP and NDF was not taken into account. The calculation of single regressions delivered comparable results.

Contamination with bacterial N increased in order: soyabean meal < cereal grains < grass < maize whole crop < straw, nearly identically as already found by Varvikko and Lindberg (1985). Results from both methods coincided in the following sequence for speed of contamination: protein concentrates < cereals < by-products < green feeds < straw.

This is in contrast to the results of Alexandrov (1998), who found that the speed of contamination of wheat straw was much slower than that of other feeds (fish meal, sunflower meal, lucerne hay, maize), even though his data of bacterial contamination (% of residual N) correspond well with our results. Estimation of bacterial contamination for pure straw samples is problematical, due to the only marginal feed N contents in bag residues after incubation. Extrapolation of C values for feed mixtures with increasing proportions of straw does, however, seem to substantiate our results.

Also the rate or half-life of contamination depends on the feed composition. For cereals and their by-products, the rates of contamination varied between 5 and 15%·h^{-1}, which are low in comparison with the rate of ruminal CP degradation of cereals (25-48%·h^{-1}), which was estimated on the basis of ^{15}N labelling of bacteria (Schmidt, 2005, unpublished). The CP in residues of such feedstuffs decreases faster than bacterial contamination can grow. On the other hand, for roughages and straw, the access of ruminal bacteria to feed proteins seems to be (partly) hampered by fibre substances. These may be polyphenolic compounds like tannins and lignin (McAllister et al., 1994), which reduce the speed of protein degradation and therefore the rate of contamination is somewhat higher (9-24%·h^{-1}). As can be seen in Table 2, the rate of contamination seems to be unaffected by stage of maturity of grass or maize. The following regression equation confirms this. The influence of fibre on C is not significant (P=0.086). In contrast to $A_{max,}$ a negative influence of the CP content on C existed (P=0.002):

$$C\ (\%\cdot h^{-1}) = 13.3\pm3.3 + 0.09\pm0.05\cdot NDF - 0.35\pm0.10\cdot CP;\ n=29;\ R=0.70;\ P<0.0001.$$

For some feeds, especially for bean and barley, bacterial contamination of nutrient particles does not start immediately with the incubation, but shows a certain delay (lag time t_{lag}). McAllister et al. (1994) described the attachment of particle substrate as the initial step in the digestion process. Before starting this process, microbes often had to penetrate or skirt resistant barriers of incrustation substances on the surface of feed particles. The degree to which microbes overcome these physical barriers is reflected in the contamination lag time that characterizes the ruminal degradation of some feeds.

For the majority of feeds, both ^{15}N labelling methods (rumen bacteria or feeds) gave similar results for the degree of contamination and the rate of contamination (Table 4). These observations confirm earlier results of *in situ* experiments with ^{15}N labelled feed samples carried out by Varvikko and Lindberg (1985). The latter method is less convenient to handle than ^{15}N labelling of rumen bacteria because of the difficulties in producing uniformly labelled feeds. If rapidly degraded N fractions of feeds are labelled differently with ^{15}N compared with average N, the ^{15}N dilution method will result in distorted values for bacterial contamination of

feed particles. Therefore, ^{15}N labelling of rumen bacteria appears superior over the labelling of feedstuffs.

Protein degradation and contamination of feed particles during incubation are both temporal processes. This leads to the problem of the efficiency and dynamics of bacterial contamination. In other words, the production of bacterial N and attachment of bacteria to feed residues per unit of initial feed N in the nylon bag depends on the incubation time. This bacterial attachment capacity was calculated using equation (9), where $N_{undegraded}$ (%) was estimated using equation (7). At the beginning of the incubation, bacterial contamination ($A_{contam.}$) is very small and therefore the part of bacterial N as a percentage of initial N is also low. Bacterial contamination then rises exponentially with time, while in contrast, the residue feed N ($N_{undegraded}$), and therefore also the adhesion sites for bacteria, decreases rapidly. As the product of these two opposing processes, the part of bacterial N in % of initial N increases, reaching a maximum at about 12 h after the start of incubation. The amount of bacterial N increases slowly after this time, while the majority of feed residue N is disappearing. This decrease is so dominant, that the proportion of bacterial N to initial N also goes into decline (Figure 3).

In Table 5, the changes in bacterial N expressed as part of the initial N are presented for all studied feedstuffs.

Some remarkable distinctions exist between the different feedstuffs. Whereas they seem to be marginal between cereals, by-products and protein concentrates (≤3.1%) except for maize grain, for roughages this value in the time interval of 8-

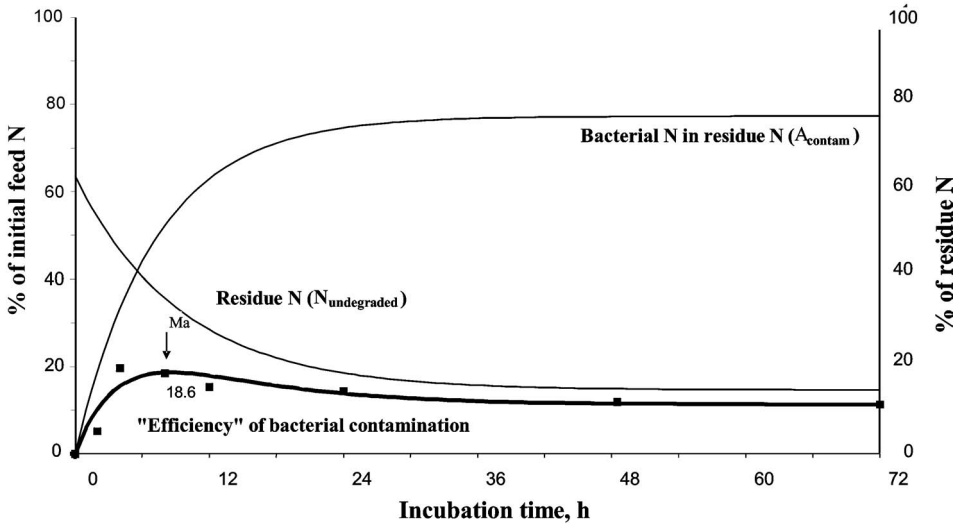

Figure 3. Production of bacterial N during incubation of oat straw

12 h is relatively high (amounting to 7-24% for grass and up to 40-70% for maize and straw), showing the high efficiency of bacterial contamination of these feeds during incubation.

Tables 5. Efficiency and dynamic of bacterial contamination

Feedstuff	Bacterial N in % of initial sample N, h						
	2	4	8	12	24	48	72
Oats	2.3	3.0	3.0	2.9	2.9	2.9	n. d.
Winter wheat	2.7	3.1	2.1	1.4	1.0	1.1	n. d.
Winter barley	3.3	3.6	2.7	2.0	1.8	1.9	n. d.
Summer barley	1.7	1.5	0.9	0.9	1.2	1.5	n. d.
Winter triticale	3.3	3.5	2.5	2.0	2.0	2.2	n. d.
Grain maize	3.1	5.8	10.5	13.9	(22.3)	12.1	n. d.
Wheat grain	1.8	2.3	1.9	1.9	2.4	2.5	n. d.
Brewer's grain	1.7	2.2	2.1	1.0	2.0	(2.4)	n. d.
Bean (*Vicia faba*)	0	1.1	1.6	1.8	1.5	0.4	n. d.
Field pea	Exponential function not calculable						
Soyabean meal	3.7	6.1	7.9	7.6	3.3	0.0	n. d.
Rapeseed meal	Exponential function not calculable						
Grass (*Perennial ryegrass*)							
Immature	3.6	5.7	6.7	6.0	3.3	1.8	1.6
med-maturity	7.0	11.3	15.1	15.9	13.4	8.3	5.3
Mature	7.9	14.3	23.4	29.0	34.0	27.7	19.6
Maize (whole crop)							
Immature	18.9	30.0	39.6	41.1	34.7	23.1	17.6
med-maturity	22.9	36.4	48.1	49.9	41.5	25.8	17.4
Mature	21.6	35.7	49.9	54.4	49.3	28.9	10.3
Lucerne hay	3.3	5.8	8.7	10.0	9.8	7.4	6.6
Oat straw	10.2	15.5	18.5	17.9	13.8	11.6	11.3
Wheat straw	16.4	26.0	38.4	44.2	47.9	42.2	37.6
Summer barley straw	14.8	28.3	53.0	71.3	90.1	67.3	48.0
Pea straw	23.4	39.4	55.3	63.3	57.1	44.7	42.0
Soyabean meal : winter wheat straw							
20 : 80	4.5	7.8	12.0	14.0	14.6	12.1	11.0
40 : 60	3.5	5.8	7.4	8.1	6.5	4.9	4.7
60 : 40	3.6	5.9	8.0	8.3	6.0	3.6	3.6
80 : 20	2.3	3.8	5.0	5.1	3.3	1.0	0.7
Winter wheat grain : winter wheat straw							
20 : 80	8.7	13.6	19.6	25.2	25.1	24.9	25.1
40 : 60	8.1	10.7	13.5	14.8	15.8	15.8	15.8
60 : 40	3.4	4.2	5.4	6.5	8.0	8.5	8.4
80 : 20	3.1	3.6	4.5	4.8	5.4	4.9	4.4

The "efficiency of bacterial contamination" showed a strong dependence on the fibre contents of feedstuffs ($P<0.0001$), but not on the CP content ($P=0.19$), as shown in the following equation for bacterial N (BN) in bags after 24 h incubation:

$$BN_{[\%\text{ of initial sample N}]} = -4.9 \pm 10.7 + 0.69 \pm 0.17 \cdot NDF - 0.44 \pm 0.19 \cdot CP;$$
$$n=29;\ R=0.72;\ P<0.0001.$$

Also, the time to reaching the peak value seems to rise with increasing fibre contents of feedstuffs.

CONCLUSIONS

The time course of bacterial contamination of incubation residues follows an exponential model for nearly all studied feedstuffs.

The final value of bacterial contamination of feed residues at the saturation state in % of total N in the residue (A_{max}) is positively influenced by the NDF content in feedstuffs. In contrast, the speed of the contamination ($\%\cdot h^{-1}$) is independent of the NDF content. However, the CP content of feedstuffs affects the speed negatively.

Contamination of feed particles by bacteria, expressed as g $N\cdot g^{-1}$ initial feed N, reaches a maximum 8-24 h after the start of incubation. The maximum values ranged from only 1-3% for cereal grains to 65-80% for straw. Correction of ruminal protein degradation for bacterial contamination is necessary for roughages in particular. For cereal grains and protein concentrates it seems to be irrelevant.

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