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Studies on the In Situ Nitrogen Degradability Corrected for Bacterial Contamination of Concentrate Feeds in Steers[1]

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ABSTRACT: Stable ^{15}N was used to evaluate the influence of bacterial contamination on in situ DM and N degradabilities (Dg) of meat and bone meal (MBM), soybean meal (SBM), and wheat bran (WB) in two steers. Bacterial DM and N contamination ranged from 2.4 to 28.6% and 2.1 to 56.8% of residual DM and N, respectively. Effective N degradability increased when bacterial contamination was taken into account ($P < .05$). The difference was low for MBM (2.4%) and for SBM (3.4%) but high for WB (12.2%). Theoretically, using solid-associated bacteria should give the most accurate correction for bacterial contamination; however, results showed that Dg of N based on liquid-associated bacteria were identical for MBM and SBM ($P > .05$) and different for WB ($P < .05$). In a second experiment, five treatments were applied to incubated feeds to remove bacteria fixed to the residues and consequently to determine directly the Dg of DM and N corrected for the bacterial contamination without the need for a marker. These treatments involved chilling for 6 h at 4°C in saline solution alone (T1) or with a commercial detergent (T2), or with sodium dodecyl sulfate (T3) or with methylcellulose (T4), followed by pummeling in a stomacher for 5 min. The last treatment was only machine washing twice (T5). The Dg of DM can be directly determined following the first four treatments, nevertheless their application to MBM and SBM led to higher Dg of N than that corrected for the bacterial contamination determined in the first experiment ($P < .05$). This overestimation was higher than the observed underestimation when the correction for bacterial contamination was omitted. However, two treatments (T1 and T4) were able to dislodge bacterial N fixed to incubated WB and so the Dg of N could be measured directly. In conclusion, bacterial contamination of concentrate feeds incubated in the rumen may be extensive. In this case Dg must be corrected for this contamination or treatments for decontamination can be applied to residues before determining the Dg of N.

Key Words: Nylon Bag, Microbial Contamination, Stable Isotope, Concentrates, Protein Degradation

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Introduction

The in situ technique is widely used for predicting the ruminal degradability of N in ruminant feeds. It is the official method in most of the new protein feeding systems in temperate countries (Jarrige, 1987). However, several factors have been shown to affect this technique. Mathers and Aitchison (1981) were the first to demonstrate that the residues from feeds incubated within nylon bags in the rumen were contaminated by microbial matter. The microbial contamination of forages incubated in the rumen is particularly high and a correction should be considered when predicting N degradability for these feeds (Kennedy et al., 1984; Olubobokun et al., 1990). In the case of concentrate feeds, it is considered as "nutritionally unimportant" (Mathers and Aitchison, 1981) and so is rarely measured. Few values of microbial contamination are in fact available, although a great diversity of concentrate feeds, differing by their fiber and N contents, may be incorporated into ruminant diets.

Different markers can be employed for measuring the microbial contamination of incubated feeds. The marker technique is nevertheless difficult and as for determining the microbial synthesis (Siddons et al., 1982), the results are probably affected by the type of

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marker used. An attractive alternative would be to develop some techniques able to dislodge microbes adhering to feed residues before the classic analysis. Mechanical agitation (e.g., blending for 1 min) has been widely adopted to extract bacteria from ruminal contents (Bryant and Burkey, 1953). Other techniques such as chilling (Dehority and Grubb, 1980) and stomaching (Merry and McAllan, 1983) are currently available.

The aim of the first experiment was to measure the effect of the microbial contamination of three concentrate feeds incubated in the rumen on the effective degradability of N. The second experiment was conducted to determine directly the degradability of DM and N corrected for microbial contamination with bags submitted to specific methods for dislodging bacteria fixed to residues of concentrate feeds incubated in the rumen.

Materials and Methods

Experiment 1

Nylon bag incubations were carried out in two ruminally cannulated (110 mm i.d.) steers each weighing approximately 880 kg and receiving twice a day (0800 and 1600) a mixed diet of 50:50 meadow hay:concentrate mixture (.20 barley, .20 wheat, .27 beet pulp, .21 gluten feed, .10 soybean meal, and .02 minerals). The DMI was maintained at 70 g/kg $BW^{.75}$. The steers were housed in individual stalls and had permanent access to fresh water and to trace mineral salt. Meat and bone meal (**MBM**), soybean meal (**SBM**), and wheat bran (**WB**; Table 1) were incubated in the rumen according to the method described by Ørskov and McDonald (1979). Duplicate bags were incubated in the rumen of each steer for 0, 2, 4, 8, 16, 24, and 48 h during two periods of 48 h. The bags (100 mm × 150 mm) were made of precision nylon cloth with apertures of 42.5 μm. They contained 5 g of air-dried sample (33 mg/cm^2) milled to pass a 2.0-mm screen (Cyclotec, Tecator) and were attached to PVC tubing, which was connected to the stopper of the ruminal cannula by an 80-cm fish line. Six bags were staggered on each tubing. The bags were introduced in reverse sequence and removed all at once before the morning feed. After withdrawal from the rumen, the bags were immediately rinsed lightly with cold tap water and then machine-washed (3 × 5 min). The bags were freeze-dried and weighed to determine the residual DM. The residues were ground to pass a 1-mm screen and pooled to obtain one residue per incubation time-animal-period and analyzed for N (Kjeldahl technique). Diet samples were analyzed for DM, OM, N (AOAC, 1984), NDF, and ADF (Goering and Van Soest, 1970).

Microorganisms were labeled by continuous intraruminal infusion of $(^{15}NH_4)_2SO_4$ (3 g/d, 99 atoms % excess) during 6 d. On d 1 and 2, whole ruminal contents (**WRC**, 800 g of wet weight) were collected from eight sites within the rumen of each steer every 8 h. During the incubation periods WRC were obtained from each steer before and 3, 6, and 9 h after the morning feeding on d 6, 5, 4, and 3, respectively. Liquid-associated (**LAB**) and solid-associated (**SAB**) bacteria were directly extracted according to the method described by Legay-Carmier and Bauchart (1989).

Table 1. Chemical composition (% of DM) of concentrate feeds incubated in the rumen

Feeds	OM	N	NDF	ADF
Wheat bran	93.7	2.8	46.7	13.2
Soybean meal	91.2	7.7	16.6	10.6
Meat and bone meal	59.7	9.1	—	—

The apparent DM and N degradabilities (**Dgapp**) were calculated as the percentage difference between the initial and the residual amounts in the bag after ruminal incubation. Degradability corrected for microbial contamination (**Dgcorr**) was calculated the same way but the microbial matter was subtracted from the residual amount. The proportions of microbial N and DM in the nylon bag residues (% of residual N or DM) were determined as follows: Microbial N % = (^{15}N excess in residue/^{15}N excess in LAB or SAB) × 100, and Microbial DM % = Microbial N % × % N in residue/% N in LAB or SAB.

The LAB and SAB isolated on d 3, 4, 5, and 6 were pooled by animal and source of bacteria to obtain a reference bacterial sample for the determination of the microbial contamination. Isotopic ratio was measured by mass spectrometry (VG SIRA 12, U.K.) following the determination of N by the method described by Bremner (1965). The ^{15}N excess was calculated assuming a natural abundance of .365 atoms %. Data of degradability were fitted to the nonlinear regression equation of Ørskov and McDonald (1979). The best-fit values of the nonlinear parameters were chosen using the Marquardt procedure (SAS, 1985). Effective degradability (P) was also calculated assuming a solid outflow rate constant from the rumen equal to .06/h. For each feed and incubation time, data were examined by analysis of variance in which the effects of correction for bacterial contamination, steers, periods, and their interactions were tested. Differences between means were compared using the Newman-Keuls test (Dagnelie, 1982). Significance to express differences among means was $P < .05$ unless otherwise stated following a significant F-test. Tests were performed using the GLM procedures (SAS, 1985).

Experiment 2

During four periods of 24 h, the concentrate feeds were incubated for 8, 16, and 24 h according to the

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same procedure as in Exp. 1, except that microorganisms were not labeled. After withdrawal, machine-washed bags were assigned to five treatments (four bags/treatment/incubation time/animal). Four treatments involved a chilling for 6 h at 4°C in a .9% (wt/vol) solution of NaCl (**T1**), NaCl .9% plus .05% commercial detergent (Superdecontamine 11 Intersciences, Brussels, Belgium, **T2**) or plus .01% sodium dodecyl sulfate (Fluka, **T3**) or plus .4% methyl cellulose 400 centipoises (Aldrich, **T4**). After chilling, bags were stomached (Laboblender 400, U.K.) for 5 min, then machine-washed and freeze-dried. A series of bags were only subjected to machine washing twice without chilling and stomaching (**T5**). The five treatments were also applied to feeds only machine-washed (time 0 h). The dried residues were weighed, milled to pass a 1-mm screen, and analyzed for N. Degradabilities of DM and N were then determined as previously described for Dgapp in Exp. 1. The efficiency of treatments to dislodge bacteria from incubated feeds was defined by comparing the degradabilities with Dgcorr measured during the first experiment. Data were analyzed as described for the first experiment. The tested effects were treatments, periods, and their interactions.

Results and Discussion

Experiment 1

The ^{15}N excess of bacteria increased rapidly during the first 48 h of infusion, then increased slowly afterward (Figure 1). The ^{15}N excess measured during the last 4 d equaled .2430 ± .0247 (SD) and .1935 ± .0261% (SD) for LAB and SAB, respectively. The results supported that marker:N ratio is higher in LAB than in SAB (Craig et al., 1987; Legay-Carmier and Bauchart, 1989; Cecava et al., 1990; Olubobokun and Craig, 1990). Variations of ^{15}N excess of bacteria have also been noted by others. Three to five preliminary days of infusion were required to reach the plateau enrichment of ^{15}N (Firkins et al., 1987), instead of the 2 d adopted by us and others (Petri and Pfeffer, 1987; Sadik et al., 1990). Several workers reported that the chemical composition of ruminal bacteria varies with postfeeding time (Craig et al., 1987; Cecava et al., 1990), level and frequency of feeding (Leedle et al., 1982; John, 1984), and specific rate and stage of growth of bacteria (Bates et al., 1985). As ^{15}N excesses in bacteria were certainly influenced by these factors, bacteria were harvested at different times after the morning feeding according to a standard procedure from fresh WRC. The bacterial pellets were always examined microscopically and found to be free of plant material and protozoa.

Uniform labeling of bacteria involved a constant and high incorporation of ^{15}N-ammonia into all kinds of ruminal bacteria. Although most species can utilize

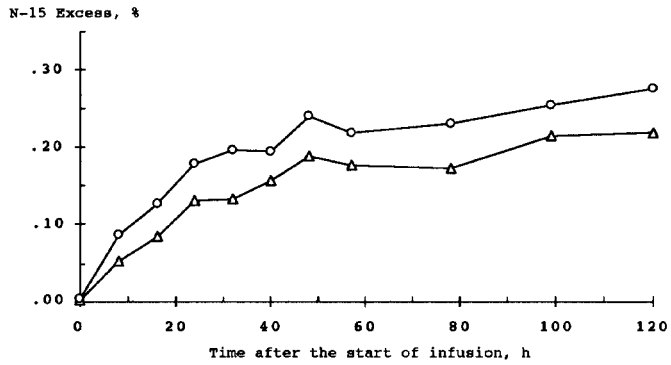

Figure 1. Change in enrichment of ^{15}N of liquid-associated (○) and solid-associated (△) bacteria after the start of continuous infusion of $(^{15}NH_4)_2SO_4$ in the rumen.

ammonia as the major source of N for their growth, there are some that are known to incorporate preferentially or specifically amino acids or small peptides (Wallace and Cotta, 1988). In this experiment, proportions of bacterial N derived from ammonia were not determined, but generally, it is assumed that 40 to 80% of bacterial proteins are derived from ammonia (Nolan and Leng, 1972; Steinhour et al., 1982; Firkins et al., 1987). If labeled ammonia was infused in a continuous manner, NH_3 concentration in the rumen and consequently $^{15}NH_3$:NH_3 ratios varied between the feedings, particularly with animals receiving only two meals (Bragg et al., 1986). Conversely, the availability of proteins depends on the degradability kinetics of the feed proteins and undoubtedly is known to vary with time after feeding. As pointed out by Firkins et al. (1987), ^{15}N excess of LAB would be more variable with time after feeding because these bacteria use more N from ammonia. Our results did not confirm this fact.

Estimates of the proportion of bacterial DM and N contamination of feed residues during incubation in the rumen are given in Figure 2. They were based on chemical composition of LAB and SAB isolated as previously described on d 3, 4, 5, and 6. The DM contamination was rapid but constant until 16 h of incubation and afterward depended on the tested feeds. It always represented less than 5% of the residual DM for MBM. In SBM residues, DM bacterial colonization peaked after 24 h of incubation and then slowly declined. For WB, bacterial contamination increased linearly with incubation time to reach 14.9% of the residual DM at 48 h. The proportion of residual N that is of bacterial origin depends on the extent of DM colonization and on the N concentration of bacteria and of the residues. Bacterial N represented less than 5% of the residual N at all times after ruminal exposure of MBM, because N concentrations of residues ranged between 5.8 and 7.8% and bacterial DM represented only 2 to 4% of residual DM. Bacterial

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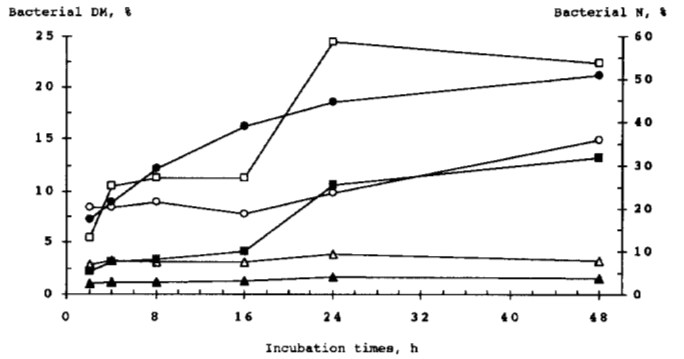

Figure 2. Bacterial DM (open symbols) and N (closed symbols) fixed to wheat bran (○, ●), meat and bone meal (△, ▲), and soybean meal (□, ■) incubated in situ in the rumen (means based on the chemical composition of LAB and SAB and expressed as percentage of residual DM or N).

N colonization of WB residues increased continuously along the incubation. The N concentration of WB (2.7%) was less than that of bacteria (6.5%). For SBM, N contamination progressed similarly to bacterial DM colonization up to 24 h of incubation but increased thereafter; N concentrations of the residues fell to 2.2% at 48 h of incubation.

The results in Table 2 show that the effect of bacterial contamination on the Dg N of concentrate feeds incubated in the rumen differed between feeds, supporting the conclusions of Yang (1991) with other concentrate feeds incubated in sheep and the results recently obtained by Wanderley et al. (1993) with corn grain and corn husks. The differences between Dgcorr and Dgapp were the greatest for WB and always for 4 and 8 h of incubation. The results can be explained by the chemical composition of concentrate feeds (Table 1). As shown for forages (Craig et al., 1987; Olubobokun et al., 1990; Wanderley at al., 1993), the high bacterial colonization of fibrous feeds (e.g., WB) led to the greatest underestimation of the effective degradability (**P**) of N when correction was omitted (Table 2). For concentrate feeds rich in protein and without fiber (e.g., MBM), such contamination may be nutritionally unimportant for the determination of P, confirming the results of Mathers and Aitchison (1981) obtained with fish meal. An intermediate situation was found with SBM for which the fiber content was higher than for MBM (Table 1), and although the bacterial DM contamination was the highest (except at 2 h, Figure 2), it had a small effect on the P of N. The fractional rate constant (c) of disappearance of the potentially degradable fraction (b) was significantly faster when degradabilities of feeds were corrected for bacterial contamination. This fact was also observed with forages by Nocek and Grant (1987). After the correction for bacterial contamination, the soluble (a) and the potentially degradable fractions (b) were significantly greater for WB. In the case of SBM, the greater value of the soluble fraction reduced the potentially degradable fraction, because the sum of the two fractions equaled 100%.

The Dgcorr of N based on LAB and SAB compositions were similar for MBM and SBM but significantly different for WB. The greater ratio ^{15}N:N in LAB was in fact partially compensated by the smaller N concentration in SAB (6 against 7%) and the bacterial N contamination was only important for WB residues.

Table 2. Apparent (App) and corrected (Corr) in situ N degradabilities (%) of concentrate feeds and parameter estimates of the equation of Ørskov and McDonald (1979)

	Meat and bone meal				Wheat bran				Soybean meal			
		Corr				Corr				Corr		
Item	App	SAB[a]	LAB[a]	SEM	App	SAB	LAB	SEM	App	SAB	LAB	SEM
Time												
2 h	38.6	40.2	39.9	.6	29.2^{x}	43.1^{y}	40.2^{y}	1.0	23.7^{x}	28.2^{y}	27.3^{y}	1.1
4 h	49.4^{x}	51.0^{y}	50.7^{y}	.3	50.3^{x}	62.1^{y}	59.7^{z}	.4	32.9^{x}	38.6^{y}	37.4^{y}	.7
8 h	51.7^{x}	53.2^{y}	52.8^{y}	.2	71.2^{x}	80.6^{y}	78.6^{y}	1.1	53.4^{b}	57.2^{b}	56.4^{b}	4.4
16 h	56.5^{x}	57.9^{y}	57.6^{z}	.1	86.5^{x}	92.4^{y}	91.2^{z}	.1	84.7	86.4	86.0	.5
24 h	59.1^{x}	60.8^{y}	60.5^{y}	.3	85.8^{x}	92.9^{y}	91.5^{z}	.1	93.8^{x}	95.5^{y}	95.2^{y}	.3
48 h	67.0^{x}	68.3^{y}	68.0^{y}	.1	86.6^{x}	94.3^{y}	92.7^{z}	.2	98.6^{x}	99.1^{y}	99.0^{y}	.1
Parameter[c]												
P	53.5^{x}	54.9^{y}	54.7^{y}	.2	69.9^{x}	78.1^{y}	76.4^{z}	.5	65.5^{x}	68.0^{y}	67.5^{y}	.4
a	30.0	30.4	30.2	1.0	23.2^{x}	25.6^{y}	25.2^{y}	.2	13.8^{x}	16.1^{y}	15.6^{y}	.6
b	32.3	33.5	33.1	1.3	65.7^{x}	69.1^{y}	68.2^{y}	.6	86.2^{x}	83.9^{y}	84.4^{y}	.6
c	.151^{x}	.175^{y}	.172^{y}	.008	.147^{x}	.186^{y}	.176^{y}	.006	.090^{x}	.097^{y}	.096^{y}	.001

[a]Degradability corrected for SAB and LAB contamination determined using ^{15}N.
[b]Value for one animal.
[c]P = effective degradability, %; a = rapidly soluble fraction, %; b = slowly degradable fraction, %; and c = fractional rate constant at which b is degraded, h^{-1}.
^{x,y,z}Means for each feed within a row lacking a common superscript letter differ ($P < .05$).

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Olubobokun and Craig (1990) reported that corrected Dg of forage DM were influenced by the microbial population harvested from the rumen, but on average the Dgcorr of N were not, although microbial contaminations were high. In their experiment, DAPA was used as microbial marker, a choice open to criticism (Broderick and Merchen, 1992), and the microbial population was divided into fluid, loosely, and firmly associated microorganisms. Protozoa present in fluid and loosely associated microorganisms certainly led to the overestimation of their importance in the washed bags and should diminish the difference in the chemical composition among the three fractions of microorganisms. As stated previously for forages (Olubobokun et al., 1990), differences in the present trial between corrected values based on LAB and SAB compositions were small (Table 2) compared with the difference between apparent and corrected Dg. Previous researchers have used LAB (Nocek and Grant, 1987) and SAB (Olubobokun et al., 1990) to quantify bacterial contamination. Only in the experiment of Kennedy et al. (1984) were bacteria really attached to the residues harvested and used as reference. They showed that the effect of bacterial sample (LAB or SAB) decreased with increasing incubation time.

Theoretically, SAB should have the same chemical composition as bacteria associated with feed particles and those remaining after the washing of the nylon bag. However, Yang (1991) showed that chemical composition of SAB differed with the size of colonized particle. Meyer and Mackie (1986) reported that the bags exerted a selection of the microorganisms based on their size and the activity of the microorganism depended on the feed enclosed in the bag and the bag porosity. Actually, classical methods allow 30 to 50% of SAB to be harvested from ruminal contents (Craig et al., 1987; Legay-Carmier and Bauchart, 1989). It was also assumed that the harvested bacterial sample was representative of the total SAB population in the rumen. If this assumption is false, a procedure for the higher recovery of SAB needs to be developed. The choice of the microbial sample used in our experiment is not without criticism because some protozoa and fungi were always present in the washed bag (Lindberg et al., 1984; Elliot et al., 1985) and were not included in our microbial sample despite their chemical compositions being different from those of bacteria. Nevertheless, the amount of these microbes in the bag was probably low.

Experiment 2

During the second experiment, five treatments were applied to the concentrate feeds incubated 8, 16, and 24 h in the rumen to dislodge the bacteria attached to the residues. After their applications, the degradability of feeds would be determined without making the correction for bacterial contamination. The efficiency of treatments was consequently defined by comparing the values with the results obtained during the first experiment.

Initially, all treatments were applied to unincubated concentrate feeds (0 h) to measure their effects on feed DM and N (Tables 3 and 4). Except for T5, the treatments applied to unincubated WB and MBM significantly increased DM and N losses compared to Dgapp. Treatments 1, 2, and 3 only increased significantly the DM degradability of SBM. This extra disappearance of DM and N could be explained by an increase in the soluble fraction of the feeds due to the

Table 3. Apparent (app) and corrected (corr) DM degradabilities (%) of concentrate feeds and DM degradabilities measured in situ after the treatments

Incubated feed[a]	Degradability		Treatment[c]					SEM
	App	Corr[b]	T1	T2	T3	T4	T5	
MBM 0 h	23.0^{d}	—	28.5^{e}	27.8^{e}	27.9^{e}	26.6^{e}	19.8^{f}	.7
MBM 8 h	37.3^{b}	38.9^{d}	39.0^{d}	39.4^{d}	40.4^{d}	40.5^{d}	37.9^{d}	.7
MBM 16 h	40.1^{d}	41.6^{de}	42.5^{de}	41.2^{de}	43.6^{e}	41.6^{de}	40.0^{d}	.6
MBM 24 h	41.7^{de}	43.6^{ef}	45.1^{fg}	47.3^{g}	45.5^{fg}	45.7^{fg}	40.7^{d}	.7
SBM 0 h	33.2^{d}	—	37.7^{e}	37.5^{e}	37.4^{e}	35.3^{de}	32.8^{d}	.7
SBM 8 h	60.4	65.2	61.9	65.7	64.6	65.5	55.1	2.2
SBM 16 h	83.6^{de}	85.8^{ef}	90.2^{f}	89.7^{f}	90.3^{f}	89.6^{f}	80.9^{d}	1.0
SBM 24 h	92.3^{d}	94.5^{de}	96.8^{e}	95.4^{de}	95.3^{de}	94.7^{de}	91.8^{d}	.9
WB 0 h	25.9^{d}	—	42.4^{e}	43.4^{e}	42.9^{e}	40.9^{e}	24.9^{d}	1.0
WB 8 h	60.1^{d}	64.4^{de}	62.6^{de}	67.6^{ef}	69.9^{f}	62.5^{de}	58.1^{d}	1.4
WB 16 h	70.3^{d}	73.1^{e}	72.5^{e}	72.9^{e}	73.3^{e}	72.5^{e}	69.6^{d}	.5
WB 24 h	71.7^{d}	75.0^{e}	75.8^{e}	75.1^{e}	75.2^{e}	74.3^{e}	72.9^{d}	.4

[a]MBM = meat and bone meal; SBM = soybean meal; WB = wheat bran.
[b]Degradability corrected for SAB contamination determined using ^{15}N.
[c]Treatments = chilling for 6 h at 4°C in a solution, respectively, of NaCl (T1), NaCl plus a commercial detergent (T2) or plus sodium dodecyl sulfate (T3) or plus methyl cellulose (T4) followed by a pummeling in a stomacher during 5 min; T5 = machine washing twice.
[d,e,f,g]Means for each feed within a row lacking a common superscript letter differ ($P < .05$).

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Table 4. Apparent (App) and corrected (Corr) N degradabilities (%) of concentrate feeds and N degradabilities measured in situ after treatments

Incubated feed[a]	Degradability		Treatment[c]					SEM
	App	Corr[b]	T1	T2	T3	T4	T5	
MBM 0 h	28.7[d]	—	35.8[e]	35.4[e]	34.9[e]	32.6[e]	26.7[d]	.7
MBM 8 h	51.7[d]	53.2[de]	53.3[def]	56.3[f]	55.9[ef]	54.8[def]	51.8[d]	.7
MBM 16 h	56.5[d]	57.9[def]	59.9[f]	58.3[ef]	59.8[f]	57.1[de]	55.9[d]	.5
MBM 24 h	59.1[d]	60.8[de]	62.2[ef]	64.4[f]	62.9[ef]	63.1[ef]	59.4[d]	.5
SBM 0 h	20.7	—	23.9	23.9	24.1	21.4	17.9	.9
SBM 8 h	53.4	57.2	55.7	60.5	58.9	58.5	46.4	3.8
SBM 16 h	84.7[d]	86.4[de]	92.8[e]	91.6[e]	92.7[e]	92.9[e]	77.0[d]	1.5
SBM 24 h	93.8[d]	95.5[de]	98.2[e]	97.0[e]	97.1[e]	96.5[e]	91.8[d]	1.1
WB 0 h	26.5[d]	—	52.5[e]	52.8[e]	49.8[e]	48.6[e]	28.7[d]	1.1
WB 8 h	71.2[d]	80.6[e]	83.4[e]	89.6[f]	90.5[f]	83.8[e]	73.0[d]	1.2
WB 16 h	86.5	92.4[e]	92.4[e]	93.1[e]	92.5[e]	92.1[e]	87.1[d]	.5
WB 24 h	85.8[d]	92.9[e]	93.1[e]	93.7[f]	92.7[eg]	92.4[g]	89.3[d]	.1

[a]MBM = meat and bone meal; SBM = soybean meal; WB = wheat bran.
[b]Degradability corrected for SAB contamination determined using ^{15}N.
[c]Treatments = chilling for 6 h at 4°C in a solution, respectively, of NaCl (T1), NaCl plus a commercial detergent (T2) or plus sodium dodecyl sulfate (T3) or plus methyl cellulose (T4) followed by a pummeling in a stomacher during 5 min; T5 = machine washing twice.
[d,e,f,g]Means for each feed within a row lacking a common superscript letter differ ($P < .05$).

chemical action of treatments and(or) by a greater number of fine feed particles (< 42.5 μm), which pass through the bag pores due to the physical effect of treatments. Indeed, mechanical treatments may (Cherney et al. 1990) or may not (Crawford et al., 1978) raise efflux of feed particles from the bags. Our results showed that machine washing twice (T5) did not significantly influence DM and N losses compared with the classic washing procedure (Dgapp, Tables 3 and 4). With the assumption that stomaching increased particle losses, N:DM ratios of removed and retained particles would be the same. These ratios equaled 11.4 and 9.2%, 5.0 and 7.7%, and 3.4 and 2.8% for MBM, SBM, and WB, respectively. Such values implied that N concentration of particles retained in the bags after the treatments was lower than that of removed particles for MBM and WB, but the opposite was found with SBM. Therefore, the treatments seem to have different effects on the DM and the N constituents of the feeds.

In our experiment, the bags were stomached after chilling in different solutions. This procedure may lead to an increase in the solubility of feed DM and N. Effectively, the feed solubility depends on the solvents used, including their chemical composition, ionic strength, or the ionic species contained in the solvent (Crooker et al., 1978; De Boever et al., 1984). The N solubility of MBM, SBM, and wilted grass silage increased in mean from 16% when bags were washed according to a standard procedure to 42 and 64% when bags were washed followed by soaking for 16 h in neutral detergent solution at 25 and 70°C, respectively (Hof et al., 1990). In our case, no significant difference in Dg of DM and N at 0 h was measured between Treatments 1, 2, 3, and 4 for the three tested feeds (Tables 3 and 4). Generally, T4 gave smaller values at 0 h than the other three treatments, probably due to the higher viscosity of the saline solution with methylcellulose. To distinguish the chemical from the physical effects of treatments, WB, SBM, and MBM unincubated in the rumen were chilled for 6 h at 4°C in the four solutions corresponding to Treatments 1 to 4. Results given in Table 5 were higher than the corresponding Dgapp of the feeds but lower than the degradabilities measured after the complete treatments (Dgcorr) at 0 h of incubation (Tables 3 and 4). If the chilling increased the DM and N solubilities of the feeds, the stomaching also led to greater amounts of feed particles passing through the bags, particularly for WB.

The degradabilities of incubated feeds for 8, 16, and 24 h show that the effects of the treatments depended on the feed and its incubation time in the rumen (Tables 3 and 4). Only T5 needed to be discarded because it was inefficient to reach the aim of the experiment. When bacterial DM contamination had no effect on the degradability (Table 3), T1 to T4 had a generally low effect on the DM Dg, except for SBM and MBM incubated 16 and 24 h, respectively. At these times and for these feeds, Dg measured after treatments were in most cases higher than Dgapp but similar to Dgcorr. For WB incubated 16 and 24 h, Dgcorr of DM were significantly higher than Dgapp and the four treatments resulted in greater Dg than Dgapp (Table 3). The same explanation can be made for the Dg of N obtained after Treatments 1 to 4 (Table 4). For MBM and SBM incubated 8, 16, and 24 h, the N Dg obtained after treatments were generally higher than Dgapp, but similar to Dgcorr, despite the fact that bacterial N contaminations were less important for these feeds. With regard to biological aspect, almost all these treatments overestimated, sometimes

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Table 5. DM and N solubilities (%) of concentrate feeds chilled in different solutions

Item	Treatment[a] T1	T2	T3	T4
DM solubility				
Meat and bone meal	26.1	26.4	24.9	26.2
Soybean meal	35.5	35.2	35.3	35.6
Wheat bran	29.4	27.3	29.7	31.4
N solubility				
Meat and bone meal	30.0	31.2	29.8	31.2
Soybean meal	24.2	23.7	25.6	25.3
Wheat bran	30.6	28.4	31.4	32.6

[a]Treatments = chilling for 6 h at 4°C in a solution respectively of NaCl (T1), NaCl plus a commercial detergent (T2) or plus sodium dodecyl sulfate (T3) or plus methylcellulose (T4) without pummeling.

significantly, the Dgcorr of MBM and SBM. On average, the overestimate of Dgcorr after the treatments was higher than the underestimate of N Dgcorr when bacterial contamination was not taken into account (Table 4). The treatments removed bacteria fixed to the residues, but also partially removed N feed residues from the bags. For these reasons, such treatments seem inappropriate for MBM and SBM at the tested times of incubation. However, the opposite was found for WB, probably because the bacterial N contamination and the difference between Dgapp and Dgcorr were high at these times of incubation. Nevertheless, T2 and T3 overestimated Dgcorr at 8, 24, and 8 h, respectively. For WB, T1 and T4 seem to reach the final objective of this experiment. Ould-Bah (1989) applied similar treatments to different green or dried forages and observed an increase in the Dg of N, which was greater when bacterial contamination was high. Nevertheless, he did not obtain an overestimate of Dgcorr after the treatments, because microbial matter was still present in the residues.

The chilling is efficient to remove bacteria adhering to feeds (Minato and Suto, 1978; Dehority and Grubb, 1980). Addition of surfactant salts to saline gave few advantages for improving the detachment of bacteria from feed residues (Leedle et al., 1987; Legay-Carmier and Bauchart, 1989; Ould-Bah, 1989). Chilling was successfully combined with the stomacher for extracting 20 to 70% of adherent bacteria from rumen contents (Merry and McAllan, 1983; Legay-Carmier and Bauchart, 1989). For these reasons, surfactant salts were combined in this experiment with the stomaching. These specific treatments seem inappropriate for SBM and MBM at the tested incubation times. For incubated WB, chilling in saline alone or with methyl cellulose followed by stomaching were efficient to dislodge fixed bacteria. Similar treatments should be tested with other feeds, with other concentrations of surfactant salts, or without the stomaching and their effects on the effective degradability of N need to be measured before validating or disproving this procedure.

Implications

As for forages, the in situ technique led to the underestimation of the N degradability of concentrate feeds due to bacterial contamination of the residues even after extensive washing. The effective degradability of N was consequently underestimated, particularly for concentrate feeds with low N and high fiber contents. To take into account the bacterial contamination, the ^{15}N technique can be used but it is laborious and expensive. When ruminal microorganisms were labeled with ^{15}N-ammonium, the reference sample of bacteria (liquid-associated vs solid-associated bacteria) had little effect on the correction of N degradability. In routine experiments, treatments for decontamination seem useful to dislodge bacterial DM fixed to the residues and consequently the degradability of DM can be measured directly after them. However, they generally tended to overestimate N degradability, particularly for residues with low level of contamination.

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