

## Effects of source and level of nitrogen, and changing buffer/ruminal fluid at 48 h on in vitro digestion of feedstuffs

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### Abstract

The effect of source and level of N in buffer solutions, and the effect of changing buffer/ruminal fluid at 48 h of incubation on in vitro digestion of different feedstuffs at 0–96 h were investigated in three experiments using ANKOM DAISY<sup>II</sup> incubators. In Experiment 1, bags containing corn and alfalfa grass silages were introduced into the digestion jars starting with the 96 h bags and ending with the 3 h bags. Two sources of N, trypticase A containing 13.1% total N and 4.6% amino N, and trypticase B containing 13.3% total N and 6.6% amino N, were added to the buffer solutions at the recommended rate of 2.5 g/l or 1.5 times the recommended rate. Trypticase B improved ( $P < 0.01$ ) in vitro true DM disappearance (IVTDMD) and in vitro neutral detergent fiber disappearance (IVNDFD) of feedstuffs at 72 and 96 h, and the higher level of N decreased ( $P < 0.05$ ) IVTDMD and IVNDFD at 72 h. In Experiment 2, which was conducted similarly to Experiment 1 without trypticase B or the higher level of N, buffer/ruminal fluid were changed at 48 h for treatment samples, while those for control samples were not changed. Changing buffer/ruminal fluid increased IVTDMD from 82.8 to 84.0% ( $P < 0.001$ ) at 72 h and from 85.5 to 86.1% ( $P < 0.05$ ) at 96 h, increased IVNDFD from 57.9 to 61.0% ( $P < 0.001$ ) at 72 h and from 64.8 to 66.3% ( $P < 0.05$ ) at 96 h, but had no effect on the rate of IVTDMD or IVNDFD. Experiment 3 was similar to Experiment 1 except that bags for all time points were placed into the digestion jars at the beginning of the digestion run, and the respective bags removed at each endpoint of digestion. Buffer/ruminal fluid were changed at 48 h for treatment samples, while those for control samples were not changed. Changing buffer/ruminal fluid had no effect on in vitro apparent DM disappearance (IVADMD), increased IVTDMD from 76.2 to 77.8% ( $P < 0.05$ ) at 72 h, increased IVNDFD from 58.6 to 61.0% ( $P < 0.001$ ) at 72 h, but had no effect on the rate of IVADMD,

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IVTDMD, or IVNDFD. Even though changing buffer/ruminal fluid increased *in vitro* disappearances at 72 and 96 h, the magnitudes were too small to be of practical relevance. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* *In vitro* disappearance; Buffer; Digestion kinetics

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## 1. Introduction

The method of *in vitro* dry matter disappearance (IVDMD) is extensively used to analyze feedstuffs (Moore and Mott, 1974; Holden, 1999; Robinson et al., 1999) because while it is relatively less labor-intensive and inexpensive compared to *in vivo* digestion trials, it is highly correlated to *in vivo* digestibility (Johnson et al., 1962; Deinum et al., 1968; Mandevu et al., 1999). The original *in vitro* method of Tilley and Terry (1963) and the *in vitro* buffer composition of McDougall (1948) have been modified over the years in terms of equipment and reagents that are used in the procedure (Van Soest et al., 1966; Goering and Van Soest, 1970; Marten and Barnes, 1980; Van Soest and Robertson, 1985).

One of the most significant recent modifications to the *in vitro* method has been the development of the DAISY<sup>II</sup> incubator (ANKOM Technology Corp., Fairport, NY, USA), which allows for multiple analysis of feed samples, thus reducing labor demands and potentially improving the precision of the assay (Holden, 1999; Robinson et al., 1999). Time-course disappearance curves for DM and neutral detergent fiber (NDF) of feedstuffs can be obtained by incubating samples at multiple times, and the data fitted to first order linear models (Smith et al., 1971; Cross et al., 1974; Mertens and Loften, 1980; Nocek and English, 1986), or nonlinear iterative least squares procedures (Ørskov et al., 1980) to describe the digestion kinetics of the feedstuffs.

The objectives of this study were to determine the effect of source and level of N in buffer solutions, and the effect of changing the buffer solutions and ruminal fluid (buffer/ruminal fluid) at 48 h, on the rate and extent of *in vitro* apparent DM disappearance (IVADMD), *in vitro* true DM disappearance (IVTDMD), and *in vitro* NDF disappearance (IVNDFD) of feedstuffs incubated for 0–96 h.

## 2. Materials and methods

### 2.1. Experiment 1

Feedstuffs used in this experiment were corn silage and alfalfa grass silage. The silages were dried at 60°C and ground to pass a 1 mm screen using a Wiley mill (model 3; Arthur H. Thomas Co., Philadelphia, PA, USA). Approximately 0.25 g of sample DM were weighed into 4.5 cm × 5.0 cm ANKOM dacron bags in triplicate. Bags were made from N-free, white polyester monofilament fabric with 57 µ pore size. Bags were heat-sealed and exposed to *in vitro* digestion for 0, 3, 6, 12, 24, 36, 48, 72, and 96 h using two ANKOM DAISY<sup>II</sup> incubators, each containing four 4 l digestion glass jars that rotated in a digestion

chamber maintained at 39.5°C. Two sources of N, trypticase A containing 13.1% total N and 4.6% amino N, and trypticase B containing 13.3% total N and 6.6% amino N, were added to the buffer solutions at the recommended rate of 2.5 g/l or 1.5 times the recommended rate. Buffer solutions in digestion jars in the first incubator contained trypticase A, while buffer solutions in jars in the second incubator contained trypticase B.

In order to test for variation between the two incubators in digestion of feedstuffs during the test-run, two laboratory standards, mixed cool season hay and wheat straw that had been grounded to pass a 1 mm screen using a Wiley mill were incubated at 30 h in both incubators in a pre-run.

Each digestion jar contained a total of 24 sample bags. The zero-hour bags were soaked in warm buffer solutions (39°C) without ruminal fluid for 15 min and washed to estimate disappearance of DM due to solubility and physical expulsion of DM during the washing procedure (Ørskov et al., 1980).

The *in vitro* buffer and mineral solutions used were a mixture of buffer solution, macromineral solution, micromineral solution, and reducing agents. The buffer solution contained 16 g of  $\text{NH}_4\text{HCO}_3$  and 140 g of  $\text{NaHCO}_3$  in 4 l of reverse osmosis water. The reverse osmosis water was prepared by filtering ordinary tap water through thin film cartridges and a deionizing resin bed using a model MP 750EH MacClean Water Treatment System (MacClean, Churubusco, IN, USA). The macromineral solution contained 22.8 g of anhydrous  $\text{Na}_2\text{HPO}_4$ , 24.8 g of anhydrous  $\text{KH}_2\text{PO}_4$ , and 2.4 g of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  in 4 l of reverse osmosis water. The micromineral solution contained 13.2 g of  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 1.0 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ , 10.0 g of  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , and 8.0 g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in 100 ml of reverse osmosis water. The reducing agents, which had to be prepared before each *in vitro* digestion run were solutions "A" and "B". To make solution "A", 0.625 g of  $\text{C}_3\text{H}_7\text{NO}_2\text{S} \cdot \text{HCl}$  (Cystein-HCl) were dissolved in 47 ml of reverse osmosis water and then 4.0 ml of 1.0 N NaOH were added. To make solution "B", 0.625 g of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  were dissolved in 47 ml of reverse osmosis water. The buffer solutions and other reagents were then mixed as follows for each digestion jar: 400 ml of buffer solution, 400 ml of macromineral solution, 0.2 ml of micromineral solution, 4.0 g of trypticase (pancreatic digest of casein), and 2 ml of aqueous resazurin solution (0.1% w/v) were mixed together and brought to 1.52 l with reverse osmosis water. Solutions "A" and "B" of reducing agents were combined and 80 ml of this solution added to each digestion jar, which was then purged with  $\text{CO}_2$  for approximately 1 min before being placed in the incubator. When jars turned translucent light-pink in color showing that the solutions had been sufficiently reduced, 400 ml of strained ruminal fluid was then added to each jar. Bags were then introduced into the digestion jars in reverse order, starting with bags for the longest time interval and ending with bags for the shortest time interval so that all samples could be removed from each digestion jar at the same time. Each time digestion jars were opened, they were purged with  $\text{CO}_2$  for approximately 1 min before being placed back in the incubator.

Ruminal fluid was collected into a thermos flask from a non-pregnant dry cow and strained through four layers of cheesecloth prior to mixing with buffer solutions. The cow was fed for *ad-libitum* intake a diet that was comprised of approximately 25.3% (DM basis) grass hay, 27.1% alfalfa grass silage, 40.3% corn silage, 0.9% phosphorus supplement,

0.2% selenium supplement, and 6.2% concentrate-mineral mixture, and contained 11.3% CP and 52.1% NDF. The concentrate-mineral mixture contained approximately 35% (DM basis) ground corn, 5% molasses, 20% soybean meal, 21% canola meal, 7% blood meal, 2% salt, and 10% mineral–vitamin supplements.

At the end of the *in vitro* digestion, each jar was half-filled with reverse osmosis water, covered, and vigorously shaken for 30 s. This was repeated twice or until the rinse-water was clear. The bags were then placed in reverse osmosis water containing 16 g of mild neutral detergent solution in a 4 l beaker on a stir plate for 15 min. Each bag was then rinsed thoroughly under a stream of reverse osmosis water from a tap, shaking each bag to make sure that particles were suspended. Bags were then placed in the ANKOM<sup>200/220</sup> Fiber Analyzer and digesta samples exposed to NDF extraction without sodium sulfite (Van Soest et al., 1991). The NDF residue was either expressed as a fraction of initial sample DM to determine IVTDMD, or as a fraction of initial sample NDF to determine IVNDFD on DM basis.

Ground samples of feedstuffs were also sent to Dairy One (Ithaca, NY, USA) and analyzed for DM by drying a 1 g sample in duplicate at 100°C in a conventional oven for 24 h, ash by burning a 2 g sample in duplicate at 600°C for 2 h in a muffle furnace (Method 942.05; AOAC, 1995), fat (Method 920.39; AOAC, 1995), N (Method 984.13; AOAC, 1995), NDF with residual ash (using  $\alpha$ -amylase and sodium sulfite), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest et al., 1991). The nonstructural carbohydrates were calculated as the difference between 100 and the sum of crude protein, NDF, fat, and ash. Analysis of Ca, P, Mg, K, Na, Fe, Zn, Cu, Mn, and Mo were conducted using a Thermo Jarrell Ash IRIS Advantage Inductively Coupled Plasma Radial Spectrometer (model ICAP 61; Thermo Jarrell Ash, Ithaca, NY, USA).

Treatments had a  $2 \times 2 \times 2$  factorial with feedstuffs, source of N, and level of N as the main variables. Data were analyzed using the General Linear Models procedures of SAS (1993).

## 2.2. Experiment 2

Experiment 2 was conducted similarly to Experiment 1 using one ANKOM DAISY<sup>II</sup> incubator, and without trypticase B or the higher level of N. Buffer/ruminal fluid in the first and second digestion jars were changed at 48 h of incubation, while those in the third and fourth digestion jars were not changed. Each digestion jar contained a total of 24 sample bags. At the end of the *in vitro* digestion, pH and temperature of the buffer/ruminal fluid mixture was measured before each jar was emptied and samples processed as described in Experiment 1.

Treatments had a  $2 \times 2$  factorial arrangement with feedstuffs and changing buffer/ruminal fluid as the main variables. Data were analyzed using the General Linear Models procedures of SAS (1993). The disappearance data were also fitted to the nonlinear equation  $p = a + b(1 - e^{-ct})$  of Ørskov et al. (1980) in NLIN program of SAS (1993) to calculate digestion constants, where  $a$  is the digestible fraction,  $b$  the slowly digestible fraction,  $(a + b)$  the potentially digestible fraction, and  $c$  is the rate of digestion for the slowly digestible fraction (or potentially digestible fraction for NDF).

### 2.3. Experiment 3

The same procedures used in Experiment 1 were followed in Experiment 3, except that the sample bags for all time points were placed into their respective jars in triplicate at the beginning of the digestion run, and then the respective bags removed at each endpoint of digestion. Feedstuffs used were corn silage, alfalfa grass silage, wheat straw, and mixed cool season grass hay. Two ANKOM DAISY<sup>II</sup> incubators were used in this experiment. The buffer/ruminal fluid in the second incubator were changed at 48 h of incubation, while those in the first incubator were not changed. Each digestion jar contained a total of 24 sample bags. Empty bags were also heat-sealed and included as blanks. Free ammonia N concentration in ruminal fluid, buffer solutions, and buffer/ruminal fluid in all jars was measured at the beginning of the digestion run, and at 6, 48 and 96 h of incubation. Approximately 45 ml of sample were centrifuged for 15 min at  $1500 \times g$  using a Beckman TJ-6 centrifuge with TH-4 rotor (Beckman Instruments, Palo Alto, CA, USA) leaving a clear layer of which a 10 ml aliquot was filtered through Whatman-540 ashless filter paper and the filtrate dispensed into a 15 ml conical tube. Approximately 6 ml of the filtrate drawn under a continuous flow aqueous system was analyzed for ammonia N using a Wescan Ammonia Analyzer (model 360; Wescan Instruments, Santa Clara, CA, USA). Ammonium chloride standards were used to develop the ammonium nitrogen concentration curve.

The hay and wheat straw standards were also incubated at 0, 3, 6, 12, 24, 30, 36, 48, 72, and 96 h in a pre-run to provide data for comparison with data from the respective feedstuffs. Two incubators were used with buffer/ruminal fluid in the second incubator being changed at 48 h of incubation, while those in the first incubator were not changed. This data set was needed to detect variation in digestion of feedstuffs during the study. The DM residue was expressed as a fraction of initial sample DM to determine IVADMD.

Data were analyzed using the General Linear Models procedures of SAS (1993) as a  $2 \times 4$  factorial with changing buffer/ruminal fluid and feedstuffs being the main variables. The DM and NDF disappearance data for all time points were used to calculate digestion constants as described in Experiment 2. Disappearance data for the hay and wheat straw standards were also analyzed as a split plot design in which the main variables were change of buffer/ruminal fluid and feedstuffs, with the subtreatments being run (pre-run and test-run), and feedstuffs  $\times$  change of buffer/ruminal fluid as the error term.

## 3. Results

### 3.1. Experiment 1

The chemical composition of feedstuffs used in the study (Table 1) were typical of reported values (NRC, 2001). There were no differences ( $P > 0.10$ ) in IVTDMD and IVNDFD of feedstuffs between the two incubators during the pre-run. In the test-run, corn silage had higher ( $P < 0.001$ ) IVTDMD at 96 h of incubation, and lower ( $P < 0.001$ ) IVNDFD at 72 h of incubation compared with alfalfa grass silage (Table 2). Trypsinase B

Table 1  
Nutrient composition of feedstuffs used in Experiments 1–3

	Corn silage	Alfalfa grass silage	Wheat straw	Mixed cool season grass hay
Organic matter (DM (%))	96.5	89.8	91.8	89.9
Crude protein (CP) (DM (%))	7.6	18.2	3.2	14.6
NDF (DM (%))	41.0	45.0	85.3	56.7
ADF (DM (%))	24.1	33.7	60.6	35.7
ADL (DM (%))	1.9	5.1	8.8	3.8
NSC (DM (%)) <sup>a</sup>	42.1	22.1	2.1	14.8
Fat (DM (%))	5.7	5.7	1.2	3.8
Ash (DM (%))	3.52	10.23	8.20	10.10
Calcium (DM (%))	0.23	1.25	0.40	0.52
Phosphorus (DM (%))	0.18	0.34	0.17	0.38
Magnesium (DM (%))	0.18	0.23	0.11	0.24
Potassium (DM (%))	0.81	2.82	1.15	2.86
Sodium (DM (%))	0.003	0.023	0.037	0.005
Iron (DM basis (mg/Kg))	42	96	47	103
Manganese (DM basis (mg/Kg))	23	25	16	18
Zinc (DM basis (mg/Kg))	17	24	12	26
Copper (DM basis (mg/Kg))	4	7	4	8
Molybdenum (DM basis (mg/Kg))	0.22	1.30	0.21	1.80

<sup>a</sup> Nonstructural carbohydrates were calculated as:  $100 - (\text{CP} + \text{NDF} + \text{fat} + \text{ash})$ .

Table 2  
In vitro disappearances (DM basis (%)) of corn silage and alfalfa grass silage at multiple times of incubation (Experiment 1)

Incubation time (h)	Feed (F)			Nitrogen source (S)			Nitrogen level (L)			P-values		
	Corn silage	Alfalfa grass silage	S.E.	A <sup>a</sup>	B <sup>b</sup>	S.E.	Low <sup>c</sup>	High <sup>d</sup>	S.E.	F	S	L
IVTDMD												
0 <sup>e</sup>	63.5	57.5	1.83	60.7	60.2	1.83	60.4	60.5	1.83			
72	83.4	83.4	0.14	82.9	83.8	0.14	83.6	83.2	0.14	0.985	<0.001	0.037
96	86.8	84.9	0.12	85.4	86.2	0.12	85.7	85.9	0.12	<0.001	<0.001	0.319
IVNDFD												
0	7.8	-0.1	4.62	4.5	3.2	4.62	3.8	3.9	4.62			
72	58.1	60.9	0.34	58.4	60.6	0.34	60.1	58.9	0.34	<0.001	0.001	0.033
96	66.7	64.4	0.30	64.6	66.5	0.30	65.3	65.7	0.30	<0.001	<0.001	0.375

<sup>a</sup> Trypticase (pancreatic digest of casein) containing 13.1% total nitrogen and 4.6% amino nitrogen.

<sup>b</sup> Trypticase (pancreatic digest of casein) containing 13.3% total nitrogen and 6.6% amino nitrogen.

<sup>c</sup> Trypticase (pancreatic digest of casein) added to the buffer solutions at the recommended rate of 2.5 g/l.

<sup>d</sup> Trypticase (pancreatic digest of casein) added to the buffer solutions at 1.5 times the recommended rate.

<sup>e</sup> The zero-hour bags were soaked in warm buffer solution (39°C) for 15 min and washed to estimate disappearance of DM due to solubility and physical expulsion of DM during the washing procedure.

improved IVTDMD ( $P < 0.001$ ) and IVNDFD ( $P < 0.01$ ) of feedstuffs at 72 and 96 h, and the higher level of N decreased IVTDMD and IVNDFD at 72 h ( $P < 0.05$ ).

### 3.2. Experiment 2

The buffer/ruminal fluid in the control digestion jars and in the digestion jars in which buffer/ruminal fluid were changed had respectively, a pH of  $7.12 \pm 0.098$  and  $7.12 \pm 0.091$  at the beginning of the digestion run, and a pH of  $6.90 \pm 0.022$  and  $6.91 \pm 0.040$  at the end of the digestion run. Corn silage had a higher ( $P < 0.001$ ) IVTDMD at 96 h of incubation compared to alfalfa grass silage (Table 3).

Changing buffer/ruminal fluid increased IVTDMD from 82.8 to 84.0% ( $P < 0.001$ ) at 72 h and from 85.5 to 86.1% ( $P < 0.05$ ) at 96 h. The IVNDFD differed between the two feedstuffs at 72 and 96 h ( $P < 0.01$ ). Changing buffer/ruminal fluid increased IVNDFD from 57.9 to 61.0% ( $P < 0.001$ ) at 72 h and from 64.8 to 66.3% ( $P < 0.05$ )

Table 3

In vitro disappearances (DM basis (%)) and digestion constants of corn silage and alfalfa grass silage incubated in buffer/ruminal fluid that were changed (C) or not changed (UC) at 48 h (Experiment 2)

	Feed (F)				S.E.	P-values		
	Corn silage		Alfalfa grass silage			F	C	F × C
	C	UC	C	UC				
<b>IVTDMD</b>								
Incubation time (h)								
0 <sup>a</sup>	63.3	63.6	57.3	57.7	2.0			
72	84.1	82.7	83.9	82.8	0.3	0.990	<0.001	0.535
96	87.1	86.5	85.2	84.6	0.3	<0.001	0.030	0.901
Digestion constants <sup>b</sup>								
<i>a</i> (%) <sup>c</sup>	61.9	61.9	55.2	56.0	0.6	0.001	0.500	0.500
<i>b</i> (%) <sup>d</sup>	37.6	33.5	33.2	30.4	3.5	0.400	0.200	0.200
<i>c</i> (h <sup>-1</sup> ) <sup>e</sup>	0.012	0.014	0.030	0.032	0.003	0.001	0.500	0.500
<b>IVNDFD</b>								
Incubation time (h)								
0	7.5	8.1	-0.6	0.4	5.0			
72	59.9	56.3	62.1	59.6	0.7	<0.001	<0.001	0.442
96	67.5	65.9	65.1	63.7	0.7	0.001	0.028	0.838
Digestion constants								
<i>a</i> + <i>b</i> (%) <sup>f</sup>	83.7	76.7	68.4	64.5	3.1	0.001	0.100	0.100
<i>c</i> (h <sup>-1</sup> )	0.019	0.021	0.039	0.042	0.003	0.001	0.500	0.500

<sup>a</sup> The zero-hour bags were soaked in warm buffer solution (39°C) for 15 min and washed to estimate disappearance of DM due to solubility and physical expulsion of DM during the washing procedure.

<sup>b</sup> Digestion constants were determined by fitting in vitro disappearance data to the nonlinear equation  $p = a + b(1 - e^{-ct})$  of Ørskov et al. (1980) using the nonlinear program of SAS (1993).

<sup>c</sup> Readily digestible fraction.

<sup>d</sup> Slowly digestible fraction.

<sup>e</sup> Rate of digestion of the slowly digestible fraction of DM or total potentially digestible fraction of NDF.

<sup>f</sup> Total potentially digestible fraction.

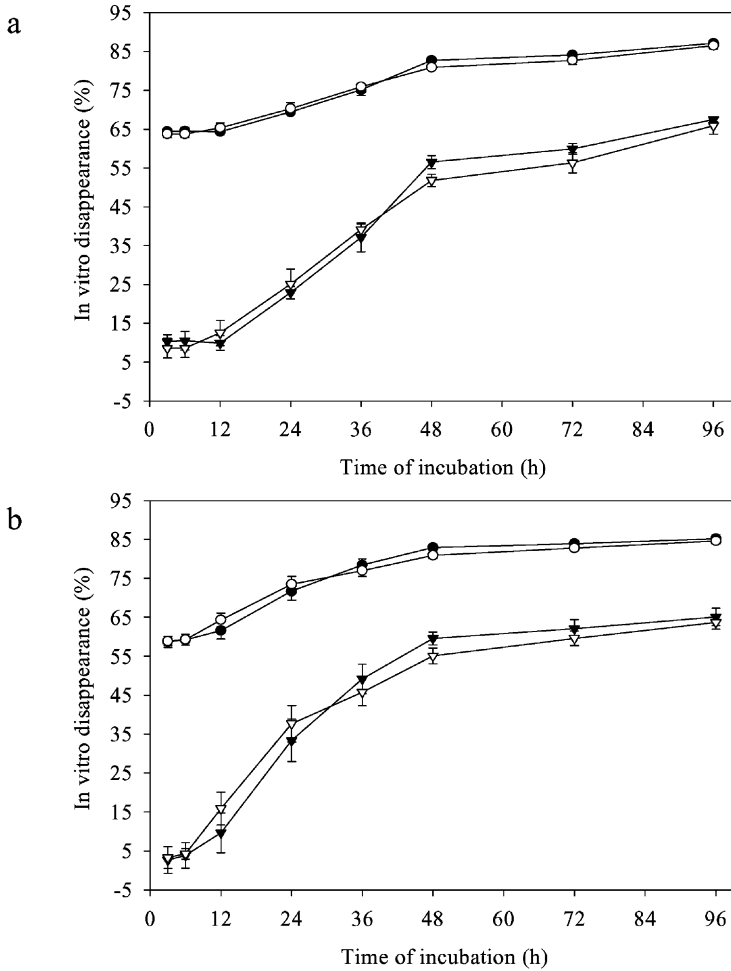


Fig. 1. IVTDMD of samples incubated in buffer/ruminal fluid that were changed (●) or not changed (○) at 48 h, and IVNDFD of samples incubated in buffer/ruminal fluid that were changed (▼) or not changed (▽) at 48 h for (a) corn silage or (b) alfalfa grass silage (Experiment 2).

at 96 h. Fig. 1 illustrates that the numerical differences in IVTDMD and IVNDFD from changing buffer/ruminal for corn silage and alfalfa grass silage, were small and of little possible biological relevance. Changing buffer/ruminal fluid had no effect on the rate and extent of IVTDMD or IVNDFD.

### 3.3. Experiment 3

Statistical analysis of IVADMD, IVTDMD, and IVNDFD data from the pre-run and test-run for the hay and wheat straw standards showed no variation in digestion of the feedstuffs between the two runs ( $P > 0.13$ ) at all times of incubation.

During the test-run, the buffer/ruminal fluid in the control digestion jars and in the digestion jars in which buffer/ruminal fluid were changed had respectively, a pH of  $7.02 \pm 0.012$  and  $6.99 \pm 0.003$  at the beginning of the digestion run; a pH of  $6.98 \pm 0.107$  and  $6.99 \pm 0.164$  at the end of the digestion run; and ammonia N concentration of

Table 4

In vitro disappearances (DM basis (%)) and digestion constants of corn silage, alfalfa grass silage, wheat straw, and mixed cool season grass hay (Hay) incubated in buffer/ruminal fluid that were changed (C) or not changed (UC) at 48 h (Experiment 3)

	Feed (F)								S.E.	P-values		
	Corn silage		Alfalfa grass silage		Wheat straw		Hay					
	C	UC	C	UC	C	UC	C	UC		F	C	F × C
<b>IVADMD</b>												
Incubation time (h)												
0 <sup>a</sup>	48.9	50.0	39.9	39.5	7.8	8.1	24.7	25.2	0.5			
72	79.2	78.4	76.7	75.0	43.8	43.1	72.5	71.4	0.9	<0.001	0.128	0.936
96	82.0	82.9	77.0	74.9	45.5	46.0	77.1	76.4	2.0	<0.001	0.616	0.415
Digestion constants <sup>b</sup>												
a (%) <sup>c</sup>	45.5	45.0	36.8	36.6	4.9	7.0	19.9	21.5	1.9	0.001	0.500	
b (%) <sup>d</sup>	36.4	37.8	39.5	38.9	42.6	41.1	54.7	52.0	2.4	0.100	0.400	0.500
c (h <sup>-1</sup> ) <sup>e</sup>	0.040	0.036	0.091	0.086	0.034	0.028	0.061	0.057	0.008	0.400	0.500	0.500
<b>IVTDMD</b>												
Incubation time (h)												
0	64.9	69.0	58.0	57.5	18.5	19.1	39.7	41.3	1.1			
72	86.9	84.9	85.6	84.4	52.8	51.9	85.8	83.9	0.9	<0.001	0.023	0.900
96	86.1	87.9	84.8	84.6	52.8	54.0	86.5	88.7	0.9	<0.001	0.065	0.563
Digestion constants												
a (%)	62.3	64.7	55.1	54.8	14.3	17.7	35.1	38.0	1.2	0.001	0.200	
b (%)	26.2	28.8	30.2	30.2	41.4	39.2	52.0	49.2	2.3	0.500	0.400	0.500
c (h <sup>-1</sup> )	0.032	0.018	0.063	0.061	0.034	0.027	0.053	0.045	0.006	0.500	0.500	0.500
<b>IVNDFD</b>												
Incubation time (h)												
0	-0.1	3.3	-1.1	-2.3	0.2	0.6	-11.6	-8.7	0.3			
72	62.7	57.0	65.3	62.3	42.1	40.9	73.7	74.3	0.8	<0.001	0.001	0.007
96	60.5	65.7	63.4	62.8	42.0	43.6	75.0	79.0	1.8	<0.001	0.062	0.407
Digestion constants												
(a + b) (%) <sup>f</sup>	68.0	72.1	64.5	63.5	45.2	46.5	76.0	78.3	3.4	0.001	0.500	0.500
c (h <sup>-1</sup> )	0.031	0.025	0.063	0.063	0.035	0.029	0.053	0.044	0.006	0.500	0.500	0.500

<sup>a</sup> The zero-hour bags were soaked in warm buffer solution (39°C) for 15 min and washed to estimate disappearance of DM due to solubility and physical expulsion of DM during the washing procedure.

<sup>b</sup> Digestion constants were determined by fitting in vitro disappearance data to the nonlinear equation  $p = a + b(1 - e^{-ct})$  of Ørskov et al. (1980) using the nonlinear program of SAS (1993).

<sup>c</sup> Readily digestible fraction.

<sup>d</sup> Slowly digestible fraction.

<sup>e</sup> Rate of digestion of the slowly digestible fraction of DM or total potentially digestible fraction of NDF.

<sup>f</sup> Total potentially digestible fraction.

520 and 487 ppm at 6 h, 683 and 652 ppm at 48 h, and 707 and 716 ppm at 96 h of incubation. The ammonia N concentration in ruminal fluid, buffer solutions, and the buffer/ruminal fluid mixture at the beginning of the digestion run, respectively were 153, 272, and 296 ppm. Feedstuffs had different ( $P < 0.001$ ) IVADMD, IVTDMD, and IVNDFD at 72 and 96 h of incubation (Table 4). Changing buffer/ruminal fluid had no effect on IVADMD, but increased IVTDMD from 76.2 to 77.8% ( $P < 0.05$ ) at 72 h of incubation.

Changing buffer/ruminal fluid increased IVNDFD from 58.6 to 61.0 ( $P < 0.01$ ) at 72 h of incubation. Time-course disappearance curves for IVADMD, IVTDMD, and IVNDFD for all feedstuffs followed the same trend shown in Fig. 1 for corn silage and alfalfa grass silage samples incubated in buffer/ruminal that were changed or not changed at 48 h of incubation. Changing buffer/ruminal fluid had no effect on the rate and extent of IVADMD, IVTDMD, or IVNDFD calculated using the nonlinear equation of Ørskov et al. (1980). The readily digestible fractions for IVADMD and IVTDMD were close to the disappearances and total potentially digestible fractions for IVADMD, IVTDMD, and IVNDFD were close to the respective 96 h disappearance for each feedstuffs and respective treatment.

#### **4. Discussion**

Differences in IVADMD, IVTDMD, and IVNDFD among feedstuffs are probably due to the differences in fiber, lignin, and nonstructural carbohydrate levels among the feedstuffs. Even though statistically significant, differences in IVTDMD and IVNDFD between trypticases A and B, and between the low and high levels of N, were too small to be of practical significance.

The IVNDFD at 72 h of digestion were similar to reported values for the comparable forages (Smith et al., 1971; Cross et al., 1974; Mertens and Loften, 1980). The zero-hour disappearance, the component that is soluble or fine enough to escape from the bags simply by soaking and washing, is used to calculate the readily digestible fraction (Ørskov et al., 1980). It is often assumed that the readily digestible fraction is degraded rapidly in the rumen by the microorganisms (Ørskov et al., 1980). The rates and extents of fiber digestion for feedstuffs were similar to reported values determined *in vitro* for comparable forages (Mertens, 1973; Mertens and Loften, 1980).

Increases in IVTDMD and IVNDFD at 72 and 96 h of incubation in Experiment 2 and at 72 h of incubation in Experiment 3 from changing buffer/ruminal fluid at 48 h of incubation were likely too small to be of practical relevance. In addition, lack of treatment effects on rate and extent of DM or NDF digestion, calculated using the nonlinear iterative least squares estimating procedures, makes it unnecessary to change buffer/ruminal fluid when samples are incubated for >48 h.

The method of introducing bags into the digestion jar did not have an effect on the digestion profiles of the test samples. Increases in ammonia N concentration with increase in time of incubation may be reflective of the depletion of fermentable carbohydrates. After exhaustion of fermentable substrate, microbial turnover begins and an increasing amount of ammonia becomes available.

## 5. Conclusions

The trypticase that contained a higher content of total N and amino N tended to improve IVTDMD and IVNDFD of feedstuffs at longer incubation times, but increasing the concentration of N above the recommended level did not improve IVTDMD and IVNDFD of feedstuffs. Even though changing buffer/ruminal fluid at 48 h of incubation increased IVTDMD and IVNDFD of feedstuffs incubated for 72 and 96 h, the magnitudes were too small to be of practical relevance. Changing buffer/ruminal fluid at 48 h had no effect on the rate and extent of IVADMD, IVTDMD, or IVNDFD.

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