

Abstract

Various assays are used to evaluate the intestinal digestibility of dry matter (DM) and nitrogen (N) in ruminant feeds (Casamiglia and Stern, 1995; Gargallo et al. 2006). The objective of this study was to evaluate *in vitro* (IV) intestinal digestibility of DM and N of seven feeds (two animal proteins, two soy products, corn silage, alfalfa silage and DDG) following *in situ* (IS) or ruminal IV procedures. We wanted to determine if ruminal IV or IS exposure or length of exposure altered the IV intestinal digestibility and further, if the pore size of the incubation bag impacted variation of the assay. Samples were placed in three pore size (15, 25 or 50 μm) bags (5 x 5 cm) and incubated for 12-h or 24-h ruminal digestion either IS or IV (Daisy™ incubator, Ankom, Macedon, NY) followed by IV intestinal digestion (Gargallo et al. 2006). Empty bags were incubated for correction. Data were analyzed as a factorial design using GLM in SAS and Tukey's method to separate overall means and Duncan's method to separate individual treatment means. Incubation time in the rumen or rumen fluid impacted digestibility, but there was no difference in IV intestinal digestibility based on ruminal exposure time (Table 1). Digestibility of DM and N_{DM} were significantly lower in the 25 μm pore bags; however no differences among bag pore sizes were detected for IV intestinal digestion parameters. The effect of bag pore size for ruminal IV or IS digestion was not linear, due to bag material characteristics and will be discussed.

KEYWORDS: *in vitro*, *in situ*, intestinal digestion

Introduction

Several ruminant formulation systems (CNCPS, Tylutki et al 2008; NRC, 2001) balance for metabolizable protein (MP). MP is the product of feed protein from rumen undegraded feed, microbial protein and endogenous protein and is variable depending on the rate and extent of digestion of the feedstuffs in the rumen, the amount microbial protein and the intestinal digestibility of the undegraded feed. These systems use global intestinal digestibility values for undegraded feed, however we know that feeds vary greatly by amino acid in their digestibility.

Mobile bag techniques have traditionally been used to measure intestinal digestion. Recently assays with *in situ* (IS) ruminal digestion followed by two additional *in vitro* (IV) steps to closely simulate physical conditions in the rumen were developed as an alternative to duodenal cannulated animals for intestinal digestion (Casamiglia and Stern, 1995; Gargallo et al. 2006). Further evaluation of these methods is necessary to understand variation due to method or feed in order to use these methods to establish feed library values for formulation systems. The objective of this study was to evaluate IV intestinal digestibility of DM and N in ruminant feeds following 12- or 24-h IS or IV ruminal digestion in three pore size bags (15, 25 and 50 μm).

Materials and Methods

SAMPLES: Silages were ground in a Wiley mill through a 1mm screen, remaining feeds were used as received.

- Animal proteins: ProVAAL (Venture Milling), ruminant fishmeal (Omega Protein)
- Poet HP DDG (Dakota Gold, Sioux Falls, SD)
- Mercer soy (Mercer Landmark, Mercer, OH), SoyBest (West Point, NE)
- Corn silage
- Alfalfa silage

BAGS, pore size (μm): 5 x 5 cm (Ankom Technology)

- 15 – mesh
- 25 - fiber (acetone rinsed)
- 50 (\pm 15) - *in situ*

INCUBATIONS: To control for within feed bias, all bags for a feed were incubated at the same time

RUMINAL: Parallel 12- and 24-h ruminal IV and IS incubations were run with both time points inserted simultaneously

- in vitro* (IV): Ankom Daisy™ incubator
- in situ* (IS): Bags split between 2 cows with each feed/time point in separate mesh bags

After completion of ruminal digestion, bags were removed, rinsed in cold tap water until it ran clear (de Boer et al., 1987) and drained. Bags for ruminal degradation determination were dried overnight at 106°C and weighed. Bags for sequential intestinal degradation were frozen at -20°C by feed until assayed

INTESTINAL: *in vitro* using Daisy™ incubator (Gargallo et al., 2006)

- HCl containing pepsin (1 g/L; P7000, Sigma, St. Louis, MO) for 1-h; drained and rinsed with tap water

- Phosphate buffer (pH 7.75) containing pancreatin (3g/L; P7545, Sigma) and 50 ppm thymol for 24-h; drained, rinsed and dried overnight at 106°C

Dry matter and N were obtained for all samples; N was measured by block digestion and steam distillation with automatic titration (Tecator Digestor 20 and Kjeltac 2300 Analyzer, Foss Analytical AB, Höganäs, Sweden; AOAC 2001.11).

STATISTICAL ANALYSIS: Data were analyzed as a factorial design using GLM in SAS (SAS Inst., Inc., Cary, NC) and Tukey's method to separate overall digestion times and bag means and Duncan's method to separate individual treatment means. Significance was considered when ($P < 0.05$) with trends when ($0.05 < P < 0.10$).

Results

Table 1. Overall ruminal and intestinal dry matter and N disappearances of feeds incubated *in vitro* or *in situ* for 12 or 24 hrs for ruminal digestibility in three different pore size bags (mean \pm SD).

	RumDig.	Rum N Dig	IntDig ¹	Int N Dig. ¹
n	164	163	159	159
RumDig/time ²				
IV12	0.42 \pm 0.14 ^a	0.45 \pm 0.17 ^a	0.79 \pm 0.18	0.94 \pm 0.07
IS12	0.42 \pm 0.13 ^a	0.31 \pm 0.17 ^b	0.82 \pm 0.18	0.93 \pm 0.08
IV24	0.46 \pm 0.13 ^a	0.49 \pm 0.17 ^a	0.79 \pm 0.17	0.94 \pm 0.07
IS24	0.53 \pm 0.17 ^b	0.43 \pm 0.23 ^a	0.85 \pm 0.15	0.94 \pm 0.08
Bag pore size, μm				
15	0.47 \pm 0.14 ^{at}	0.42 \pm 0.18 ^a	0.82 \pm 0.18	0.96 \pm 0.06
25	0.38 \pm 0.13 ^b	0.33 \pm 0.19 ^b	0.79 \pm 0.18	0.93 \pm 0.08
50	0.52 \pm 0.15 ^{at}	0.49 \pm 0.18 ^a	0.84 \pm 0.16	0.94 \pm 0.09

¹Represents cumulative digestion from rumen and intestine. ²IV, *in vitro*; IS, *in situ*; 12-, 24-h. ^{ab}Means (Tukey's) with different letters are significant, $P < 0.05$. ^tMeans with common superscript show trends, $P < 0.10$.

Table 2. Ruminal dry matter disappearance of feeds incubated *in vitro* or *in situ* for 12 or 24 hrs for ruminal digestibility in three different pore size bags.

Trt ²	Pore	Feed ¹							
		overall	ProV	FishM	DDG	MerS	SoyB	CornS	AlfS
IV12	15	0.41 ^{cde}	0.64 ^c	0.26 ^{de}	0.28 ^{gh}	0.46 ^e	0.55 ^{cde}	0.30 ^{ef}	0.45 ^e
	25	0.39 ^{de}	0.50 ^e	0.23 ^{ef}	0.33 ^{efg}	0.42 ^f	0.54 ^{def}	0.28 ^f	0.43 ^e
	50	0.47 ^{bcd}	0.70 ^b	0.33 ^{cd}	0.33 ^{efg}	0.52 ^d	0.61 ^b	0.33 ^{de}	0.48 ^d
IS12	15	0.46 ^{cd}	0.37 ^h	0.41 ^b	0.43 ^{bc}	0.50 ^d	0.51 ^{ef}	0.35 ^{cd}	0.62 ^c
	25	0.33 ^e	0.30 ^f	0.18 ^f	0.25 ^h	0.37 ^g	0.43 ^g	0.34 ^d	0.47 ^d
	50	0.48 ^{bcd}	0.47 ^f	0.29 ^{de}	0.41 ^{bcd}	0.59 ^b	0.59 ^{bcd}	0.39 ^c	0.73 ^b
IV24	15	0.45 ^{cd}	0.60 ^d	0.27 ^{de}	0.36 ^{def}	0.52 ^d	0.60 ^{bc}	0.34 ^d	0.44 ^e
	25	0.41 ^{cde}	0.58 ^d	0.21 ^{ef}	0.31 ^{gh}	0.46 ^e	0.59 ^{bcd}	0.28 ^f	0.43 ^e
	50	0.52 ^{abc}	0.74 ^a	0.38 ^{bc}	0.39 ^{cde}	0.54 ^c	0.63 ^b	0.46 ^b	0.48 ^d
IS24	15	0.57 ^{ab}	0.43 ^g	0.73 ^a	0.48 ^b	0.60 ^b	0.61 ^b	0.46 ^b	0.77 ^a
	25	0.41 ^{cde}	0.32 ⁱ	0.18 ^f	0.37 ^{def}	0.52 ^d	0.49 ^f	0.35 ^{cd}	0.61 ^c
	50	0.62 ^a	0.51 ^e	0.36 ^{bc}	0.76 ^a	0.70 ^a	0.70 ^a	0.52 ^a	0.77 ^a
overall	0.46	0.51 ^c	0.28 ^f	0.40 ^d	0.51 ^c	0.57 ^a	0.36 ^e	0.56 ^b	

¹Feeds: ProVAAL; FishMeal; DDG; MercerSoy; SoyBest; corn silage; alfalfa silage. ²IV, *in vitro* (Daisy); IS, *in situ*; 12-, 24-h. ^{a-h, A-F} Means (Duncan's) with different letters in same column are significant, $P < 0.05$.

Table 3. Ruminal N disappearance of feeds incubated *in vitro* or *in situ* for 12 or 24 hrs for ruminal digestibility in three different pore size bags.

Trt ²	Pore	Feed ¹							
		overall	ProV	FishM	DDG	MerS	SoyB	CornS	AlfS
IV12	15	0.45 ^{bc}	0.66 ^c	0.26 ^{cde}	0.18 ^{bcd}	0.40 ^{bc}	0.53 ^{bc}	0.56 ^b	0.56 ^c
	25	0.43 ^{bc}	0.51 ^e	0.26 ^{de}	0.15 ^{bcd}	0.39 ^{cd}	0.53 ^{bc}	0.69 ^a	0.57 ^c
	50	0.49 ^{abc}	0.73 ^b	0.35 ^{bc}	0.24 ^b	0.47 ^a	0.60 ^{abc}	0.44 ^c	0.59 ^c
IS12	15	0.35 ^{cd}	0.37 ^g	0.43 ^b	0.18 ^{bcd}	0.21 ^f	0.24 ^e	0.41 ^{cd}	0.60 ^c
	25	0.19 ^e	0.28 ^h	0.15 ^f	0.04 ^c	0.05 ^g	0.13 ^f	0.33 ^{de}	0.36 ^e
	50	0.39 ^c	0.46 ^f	0.32 ^{cd}	0.27 ^b	0.35 ^{de}	0.35 ^d	0.33 ^{de}	0.74 ^b
IV24	15	0.48 ^{abc}	0.60 ^d	0.30 ^{cd}	0.20 ^{bc}	0.44 ^{ab}	0.60 ^{abc}	0.66 ^a	0.55 ^c
	25	0.44 ^{bc}	0.59 ^d	0.24 ^{de}	0.14 ^{bcd}	0.41 ^{bc}	0.59 ^{abc}	0.55 ^b	0.56 ^c
	50	0.54 ^{ab}	0.75 ^b	0.39 ^b	0.39 ^b	0.48 ^a	0.63 ^a	0.68 ^a	0.60 ^c
IS24	15	0.43 ^{bc}	0.39 ^g	0.67 ^a	0.22 ^{bc}	0.33 ^e	0.34 ^d	0.36 ^{cde}	0.76 ^{ab}
	25	0.25 ^{de}	0.29 ^h	0.18 ^{ef}	0.08 ^{cb}	0.24 ^f	0.19 ^{ef}	0.30 ^e	0.48 ^d
	50	0.60 ^a	0.99 ^a	0.39 ^b	0.61 ^a	0.47 ^a	0.51 ^c	0.40 ^{cd}	0.82 ^a
overall	0.42	0.55 ^b	0.30 ^f	0.22 ^g	0.34 ^e	0.44 ^d	0.47 ^c	0.60 ^a	

¹Feeds: ProVAAL; FishMeal; DDG; MercerSoy; SoyBest; corn silage; alfalfa silage. ²IV, *in vitro* (Daisy); IS, *in situ*; 12-, 24-h. ^{a-h, A-G} Means (Duncan's) with different letters in same column are significant, $P < 0.05$.

Table 4. Estimated intestinal dry matter digestibility of feeds incubated *in vitro* or *in situ* for 12 or 24 hrs for ruminal digestibility and then *in vitro* for intestinal digestion in three different pore size bags.

Trt ²	Pore	Feed ¹							
		overall	ProV	FishM	DDG	MerS	SoyB	CornS	AlfS
IV12	15	0.79	0.97 ^{cd}	0.98 ^a	0.72 ^{efg}	0.89 ^{bc}	0.87 ^e	0.46 ^{de}	0.63 ^{fg}
	25	0.78	0.95 ^d	0.97 ^a	0.70 ^g	0.82 ^{de}	0.84 ^e	0.44 ^{ef}	0.62 ^g
	50	0.81	0.98 ^b	0.99 ^a	0.77 ^{def}	0.88 ^{cd}	0.89 ^{de}	0.49 ^c	0.65 ^f
IS12	15	0.82	0.97 ^{bcd}	0.99 ^a	0.82 ^{bc}	0.95 ^{ab}	0.92 ^{bc}	0.35 ^h	0.74 ^d
	25	0.79	0.93 ^h	0.93 ^b	0.69 ^g	0.89 ^{bc}	0.92 ^{bc}	0.41 ^g	0.68 ^e
	50	0.84	0.97 ^{bcd}	0.98 ^a	0.85 ^{ab}	0.91 ^{abc}	0.94 ^{ab}	0.48 ^{cd}	0.77 ^c
IV24	15	0.81	0.95 ^{fg}	0.98 ^a	0.74 ^{defg}	0.92 ^{abc}	0.88 ^e	0.53 ^b	0.65 ^f
	25	0.76	0.96 ^{defg}	0.94 ^b	0.69 ^g	0.81 ^e	0.87 ^e	0.42 ^g	0.63 ^{fg}
	50	0.82	0.98 ^{bc}	0.98 ^a	0.78 ^{cde}	0.88 ^{cd}	0.91 ^{cd}	0.49 ^c	0.69 ^e
IS24	15	0.86	0.95 ^{efg}	0.98 ^a	0.87 ^{ab}	0.97 ^a	0.96 ^a	0.57 ^a	0.81 ^b
	25	0.82	0.95 ^{fg}	0.97 ^a	0.80 ^{cd}	0.89 ^{bc}	0.92 ^{bc}	0.43 ^g	0.78 ^c
	50	0.89	0.99 ^a	0.95 ^b	0.92 ^a	0.96 ^a	0.95 ^a	0.58 ^a	0.85 ^a
overall	0.81	0.96 ^b	0.97 ^a	0.79 ^e	0.89 ^d	0.90 ^c	0.47 ^g	0.71 ^f	

¹Feeds: ProVAAL; FishMeal; DDG; MercerSoy; SoyBest; corn silage; alfalfa silage. ²IV, *in vitro* (Daisy); IS, *in situ*; 12-, 24-h. ^{a-h, A-G} Means (Duncan's) with different letters in same column are significant, $P < 0.05$.

Table 5. Estimated intestinal disappearance of feed N after incubation *in vitro* or *in situ* for 12 or 24 hrs then *in vitro* for intestinal digestion in three different pore size bags.

Trt ²	Pore	overall	Feed ¹						
			ProV	FishM	DDG	MerS	SoyB	CornS	AlfS
IV12	15	0.96	0.99 ^a	0.99	0.96 ^{ab}	0.99	0.99	0.87 ^a	0.91 ^{cd}
	25	0.94	0.97 ^b	0.99	0.94 ^b	0.98	0.97	0.78 ^{bcd}	0.88 ^e
	50	0.94	0.99 ^a	0.99	0.96 ^{ab}	0.99	0.99	0.78 ^{bcd}	0.89 ^e
IS12	15	0.95	0.99 ^a	0.99	0.96 ^{ab}	0.99	0.99	0.80 ^{abc}	0.94 ^{ab}
	25	0.91	0.96 ^c	0.97	0.89 ^c	0.98	0.98	0.71 ^d	0.89 ^{de}
	50	0.93	0.98 ^b	0.99	0.97 ^a	0.99	0.99	0.70 ^d	0.91 ^{de}
IV24	15	0.95	0.98 ^b	0.99	0.96 ^{ab}	0.99	0.99	0.83 ^{ab}	0.90 ^{de}
	25	0.93	0.97 ^b	0.98	0.94 ^b	0.98	0.97	0.81 ^{ab}	0.89 ^e
	50	0.94	0.98 ^a	0.99	0.96 ^{ab}	0.98	0.99	0.77 ^{bcd}	0.91 ^{cd}
IS24	15	0.95	0.97 ^b	0.98	0.97 ^a	0.99	0.99	0.80 ^{abc}	0.96 ^a
	25	0.93	0.96 ^c	0.99	0.94 ^b	0.98	0.99	0.72 ^d	0.93 ^{bc}
	50	0.95	0.99 ^a	0.99	0.98 ^a	0.99	0.98	0.73 ^{cd}	0.95 ^a
overall	0.94	0.98 ^b	0.99 ^a	0.95 ^a	0.99 ^a	0.99 ^a	0.77 ^c	0.91 ^b	

¹Feeds: ProVAAL; FishMeal;