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Comparison of techniques and grinding size to estimate digestibility of forage based ruminant diets

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Abstract

We compared prediction of *in vivo* dry matter (DMD) and neutral detergent fiber (aNDFD) digestibility by the following techniques: (1) Tilley and Terry two-stage *in vitro* (conventional *in vitro* or IVDMD), (2) Daisy^{II} *in vitro* (Daisy^{II}), and (3) filter bag *in situ* preceded by 48 h acid–pepsin treatment. In addition, we also evaluated the effects due to sample size (0.25 g *versus* 0.50 g) and Wiley Mill grinding size (1-mm *versus* 2-mm screens). In Experiment 1, fifteen forage species from mixed-conifer rangelands were used to evaluate digestion estimation techniques. Compared to IVDMD, Daisy^{II} and *in situ* techniques overestimated ($P<0.01$) DMD. In Experiment 2, we used meadow hay samples to compare the above techniques to *in vivo* DMD. *In situ* DMD, Daisy^{II} DMD, and IVDMD were greater ($P<0.01$) than *in vivo* DMD. In contrast, *in situ* aNDFD did not differ ($P=0.13$) from *in vivo* aNDFD for sheep. In Experiment 3, we used grasses, forbs, shrubs, and lichen in separate analyses to evaluate the interaction of Wiley Mill grind size (1-mm *versus* 2-mm) and digestibility technique. For grass hay, Daisy^{II} and *in situ* DMD were increased ($P<0.05$), and IVDMD was decreased ($P<0.05$), compared to *in vivo* DMD. Daisy^{II} and *in situ* aNDFD decreased ($P<0.05$), compared to

Abbreviations: ADF, acid detergent fiber; BW, body weight; CP, crude protein; CV, coefficient of variation; DM, dry matter; DMD, dry matter digestibility; GLM, general linear model; IVDMD, *in vitro* dry matter digestibility; MWU, modified wohlgemuth unit; aNDF, neutral detergent fiber assayed with a heat stable alpha amylase and expressed inclusive of residual ash; aNDFD, aNDF digestibility; SAS, statistical analysis system; S.E.M., pooled standard error of the least squares means

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in vivo aNDFD. In contrast, straw IVDMD and Daisy^{II} and *in situ* DM and aNDF digestibility were decreased (P<0.01) compared to *in vivo* DM and aNDF digestibility. Daisy^{II} and *in situ* digestibility estimates were greater (P<0.01) for grass hay milled at 1-mm *versus* 2-mm, while all digestibility estimates were higher (P<0.01) for straw ground at 1-mm. For the Daisy^{II} and *in situ* techniques, a 0.25 g sample resulted in greater (P<0.05) estimates of digestibility than a 0.5 g sample.

Digestibility values estimated by Daisy^{II} and *in situ* techniques were correlated ($r^2 = 0.58$–0.88) with values estimated by conventional *in vitro* and *in vivo* techniques, although in most cases, Daisy^{II} and *in situ* techniques overestimated DM and aNDF digestibility. The sieving off of different size particles in the ground forage sample, correcting for the fine particle losses from the filter bag during digestion, washing, and/or grouping the feeds into categories based on cell wall structure, and applying a corresponding correction factor may increase predictability and accuracy of Daisy^{II} technique.

Keywords: Digestion techniques; Filter bags; Grinding size; *In vitro* digestibility

1. Introduction

The two-stage technique (conventional *in vitro* or IVDMD) for *in vitro* digestion of forages developed by Tilley and Terry (1963) has been widely used because of its convenience and high degree of correlation to *in vivo* digestibility and accuracy (Tilley and Terry, 1963; Goldman et al., 1987; De Boever et al., 1988). However, the technique is time-consuming and labor intensive. Recently, a filter bag technique (Daisy^{II}) for analyzing forage *in vitro* dry matter (DM) and neutral detergent fiber (NDF) digestibility was developed by ANKOM Technology Corporation (Fairport, NY, USA). The technique entails digesting several forage samples in bags within glass jars which are rotated in an insulated chamber. This new approach must be validated on a wide variety of forages before becoming generally accepted.

Holden (1999) conducted an experiment with 10 feeds which were digested by the conventional *in vitro* technique or the Daisy^{II} technique and concluded that the Daisy^{II} technique can be used to improve labor efficiency in estimating DM digestibility. Vogel et al. (1999) recorded conflicting results when they used three different forages for comparing the conventional *in vitro* technique with the Daisy^{II} technique; with switchgrass (*Panicum virgatum* L.) and forage sorghum (*Sorghum bicolor* L.) both techniques resulted in similar dry matter digestibility (DMD) estimates, but with smooth bromegrass (*Bromus inermis* Leyss.) the Daisy^{II} technique increased DMD compared with conventional IVDMD.

Wilman and Adesogan (2000) compared conventional IVDMD estimates of 72 forage samples from two forage species (Italian ryegrass [*Lolium multiflorum* Lam.] and alfalfa [*Medicago sativa* L.]) to DMD estimates using the Daisy^{II} technique. They found that the conventional *in vitro* technique is likely to give more precise results than the Daisy^{II}. However, they also postulated that the use of the Daisy^{II}, rather than conventional *in vitro* technique, gave acceptable digestibility estimates for forage when the emphasis was on saving labor. Furthermore, Adesogan (2002) noted that the digestibility results obtained by the Daisy^{II} technique can be affected by sample size and processing method, the proximity of

the incubation jars to the heat source, and the extent to which individual bags are submerged throughout the incubation. There is an absence of *in vivo* data against which the Daisy^{II} technique can be validated.

Our objectives were to (i) compare digestibility estimates for forage based ruminant diets using the Daisy^{II} technique, and filter bag *in situ* DMD and aNDF digestibility (aNDFD) to *in vivo* and conventional *in vitro* DM and aNDF digestibility, (ii) evaluate effects of sample size and grinding size on digestibility estimates, and (iii) determine whether it is possible to predict conventional *in vitro*/*in vivo* digestibility with Daisy^{II} or filter bag *in situ* technique.

2. Materials and methods

2.1. Forages

We conducted three experiments to evaluate some common techniques used to estimate forage digestibility of grazing ruminants using 150 samples from cool-season (C_3) forage species or types. Plant nomenclature throughout our paper follows the recommendations of the USDA Natural Resources Conservation Service (USDA, NRCS 2005).

2.1.1. Experiment 1

One hundred forage samples including five grasses (California brome [*Bromus carinatus* H. & A], elk sedge [*Carex geyeri* Boott], pinegrass [*Calamagrostis rubescens* Bukl.], orchardgrass [*Dactylis glomerate* L.], and western fescue [*Festuca occidentalis* Walt.]); five forbs (western yarrow [*Achillea millefolium lanulosa* L.], tall annual willowherb [*Epilobium paniculatum* Nutt.], strawberry [*Fragaria* spp.], white hawkweed [*Hieracium albiflorum* Hook.] and lupine [*Lupinus* spp.]); four shrubs (low oregongrape [*Berberis repens* Lindl.], shinyleaf spirea [*Spiraea betulifolia lucida* Pall.], common snowberry [*Symphoricarpos albus* {L.} Blake] and big huckleberry [*Vaccinium membranaceum* Hook.]); and one lichen (tree hair lichen [*Bryoria fremontii* Tuck.]) species from a mixed-conifer rangeland were used to evaluate digestion estimation techniques.

We collected samples in late August, stored them in labeled paper bags in the field and oven-dried them at 50 °C for 48 h. Dried samples were ground through a Wiley Mill (Model #2, Arthur H. Thomas Co., Philadelphia, PA, USA) to pass a 1-mm screen. Techniques used to compare digestibility estimates were the conventional *in vitro* (Tilley and Terry, 1963), filter bag *in vitro* (Daisy^{II}), and filter bag *in situ* (*in situ*). In this study, each sample was replicated twice for each of the techniques evaluated. If the coefficient of variation within replicates for each technique of each sample mean was >4.0%, results were rejected and analyses reapplied to samples.

2.1.2. Experiment 2

Meadow hay samples from previously conducted *in vivo* digestibility trials were used to compare conventional *in vitro*, Daisy^{II}, and *in situ* techniques. Low-quality meadow hay was harvested from native flood meadows consisting of approximately 82% meadow foxtail (*Alopecurus pratensis* L.) with the majority of the remaining vegetation consisting of rushes (*Juncus* spp.), sedges (*Carex* spp.), and blue wild rye (*Elymus triticoides* Buckl.;

Wenick, 2000). We obtained *in vivo* apparent digestibility estimates from four wether sheep (Bohnert et al., 2002a) with an average body weight (BW) of 36 ± 1 kg and four steers (Bohnert et al., 2002b) with an average BW of 264 ± 8 kg, that consumed meadow hay without supplementation. Dried hay, ort, and fecal samples were ground in a Wiley Mill (1-mm screen size).

2.1.3. Experiment 3

We used meadow hay and fine fescue (*Festuca* spp.) straw samples from previously conducted (White, 2003) digestibility trials to compare conventional *in vitro*, Daisy^{II}, and *in situ* techniques. *In vivo* apparent digestibility data were from three steers (BW = 456 ± 6 kg) that had consumed each type of harvested forage. Samples were ground through either a 1- or 2-mm screen in a Wiley Mill to evaluate the effect of grind size on digestibility estimates. Also, two forbs (western yarrow and strawberry), two shrubs (red alder [*Alnus rubra* Bong.] and firmleaf willow [*Salix rigida*, Muhl.]), and tree hair lichen were ground through a 1- or 2-mm screen and subjected to ten consecutive conventional *in vitro*, Daisy^{II}, and *in situ* runs for DMD and aNDF digestibility estimates. In addition, for the Daisy^{II} and *in situ* techniques, two sample sizes (0.25 or 0.5 g) were used for the meadow hay and firmleaf willow to evaluate the effects of sampling size on digestibility estimates.

2.2. Techniques

2.2.1. Conventional in vitro

The technique for determination of conventional *in vitro* digestibility complied with the Galyean (1997) modification of the Tilley and Terry (1963) two-stage procedure. Twenty-four 50-mL Nalgene tubes were placed in a rack. Subsequently, 0.5 g of experimental samples were added to each of 20 tubes, 0.5 g samples from laboratory standards (grass hay) were added to 2 tubes and 2 tubes were used as blanks for the experiments. In each tube, 35 mL of a buffer–inoculum mixture as described by Marten and Barnes (1980) was added under purging with CO_2 and caped tightly with a rubber stopper/gas-release port (Galyean, 1997). Samples were incubated for 48 h in a water bath at 39 °C, followed by further digestion in an acid–pepsin solution containing 6.6 g/L pepsin (Catalog # P53-500, Fisher Scientific, Pittsburgh, PA, USA) and 0.1 N hydrochloric acid (35 mL of acid–pepsin solution was added to each tube) for 48 h in water bath at 39 °C. All tubes were mixed by swirling (Vortex Genie-2 Mixer, VWR Scientific, West Chester, PA, USA) them at 2, 4, 20, and 28 h after adding the buffer–inoculum and at 2, 4, and 6 h after adding acid–pepsin. After completion of the digestion, contents were filtered into pre-weighed standard coarse fritted disk gooch crucibles under mild vacuum, dried at 100 °C for 12 h, weighed for determination of DM, placed in a muffle furnace at 525 °C for at least 12 h, and reweighed to complete calculation.

2.2.2. Daisy^{II} in vitro

For the Daisy^{II} technique, Ankom filter bags (F57, 5.0 cm × 5.5 cm; ANKOM Technology Corporation, Fairport, NY, USA) and an incubator (Daisy^{II}; Ankom Technology Corporation, Fairport, NY, USA) were utilized. The incubator consists of a constant temperature cabinet that contains four glass fermentation jars that are placed on rotation racks

in the cabinet (Vogel et al., 1999). Sample sizes used were either 0.25 g (only for sample size trials of Experiment 3) or 0.5 g (for Experiments 1, 2, and 3) per bag with 24 bags per incubation jar. Each run contained one replicate of the experimental forage samples (20 samples) as well as two standards and two blank bags. Samples were heat sealed (Heat sealer #1915; ANKOM Technology Corporation, Fairport, NY, USA) in filter bags, placed in jars, and incubated for 48 h at 39 °C in a buffer-inoculum solution using techniques similar to those described in detail by Vogel et al. (1999) and Holden (1999). Briefly, buffer solution (1600 mL) and rumen inoculum (400 mL) were added to each jar, the jars purged with CO_2, and lids with gas relief valves were placed on the jars. After incubation, the buffer-inoculum was drained from the jars and the filter bags were gently squeezed against the sides of the jar to remove the gas trapped in the inflated bags. The bags were rinsed in jars with three changes of warm tap water (Holden, 1999) and then removed and boiled in a neutral detergent solution (Van Soest et al., 1991) for 80 min using an Ankom^{200} fiber analyzer (ANKOM Technology Corporation, Fairport, NY, USA). After the boiling period, NDF solution was drained from the reaction vessel and the reaction vessel was filled with 2 L of 95 °C distilled water. The top was left open and the samples were agitated for 5 min. The hot water rinse was repeated five times. Four millilitre of Ankom heat stable alpha amylase (ANKOM Technology FAA) with activity level of 340–374 MWU/mL was added to each of the first three rinses. Subsequently, filter bags were removed from jars and soaked in acetone for 5 min, air-dried, then stored for at least 12 h in a 100 °C oven, cooled in a desiccator, and weighed.

2.2.3. *Filter bag in situ*

Samples were weighed (0.25 g or 0.5 g; sample weight: bag surface area ratio was 8 and 16 mg cm^{-2} for the 0.25 g and 0.5 g samples, respectively) into acetone pre-rinsed, pre-weighed, and numbered Ankom filter bags (F57; ANKOM Technology Corporation, Fairport, NY, USA). Twenty-four sample bags (20 for samples, consisting of 10 from each sample size, 2 standards and 2 blanks) were placed in each jar in a Daisy^{II} incubator. The sample bags were incubated for 48 h at 39 °C in an acid–pepsin solution (same solution as described in Section 2.2.1) using a Daisy^{II} incubator. Sample bags were then removed, rinsed with warm tap water, placed in a polyester mesh bag (36 cm × 42 cm) and inserted into the rumen of two cannulated steers and incubated for 48 h. The cord length between cannula cap and anchor weight was 80 cm. After incubation, sample bags were removed and rinsed with tap water (39 °C) until the rinse water was clear. Subsequently, excess water was removed by gently pressuring and samples analyzed for NDF as described in Section 2.2.2.

2.2.4. *Preparation of the rumen inoculum*

Ruminal inoculum was obtained from two rumen cannulated steers consuming a moderate quality (86 g/kg CP, 690 g/kg aNDF; DM basis) meadow hay diet. Meadow hay was provided once daily. Ruminal contents were obtained approximately 30 min after feeding. Steers in this study were cared for in accordance with guidelines established by the Institutional Animal Care and Use Committee at Oregon State University. We collected approximately 4 L of rumen contents from each steer into an 8 L pre-warmed (39 °C) container. Ruminal contents were brought into the laboratory, immediately strained through four layers of cheesecloth into two 4 L conical flacks, and placed in a 39 °C water bath.

In addition, approximately 1 L of rumen contents were blended (Waring blender; Waring Products, New Hartford, CT, USA) at high speed for 30 s, strained through four layers of cheesecloth, and added to the conical flacks containing rumen inoculum. The blending action serves to dislodge particulate associated microbes and assures a representative microbial population for the *in vitro* fermentation (Holden, 1999). Under constant purging with CO_2 and mixing, rumen inoculum was divided into two pre-warmed flasks; one was used for the conventional *in vitro* and the second used for Daisy^{II} techniques.

2.2.5. Control of study accuracy and calculation

2.2.5.1. Control and corrections of results. Each rack, jar, and mesh bag/rumen was considered as a run for the conventional *in vitro*, Daisy^{II}, and filter bag *in situ* techniques, respectively. As described in Section 2.2, each run was comprised of 20 experimental samples, two standards (hay sample, with digestibility value pre-determined) and two blanks (empty bags). Blank bags and blank tubes were used to correct for bacterial contamination (Robertson et al., 1972). Each sample for Experiments 2 and 3 were analyzed with ten replicates ($n = 10$) in separate run for each of the techniques evaluated. For estimating mean values, we selected only those values that were within two standard deviations (error term between batch runs); otherwise the data were rejected and repeated analysis was undertaken. We expected that coefficients of variation of the means for standard samples would be at an acceptable level (CV < 4.0%). If means were outside two standard deviations of pre-determined values for standard samples in a particular run, all data from that particular run were discarded. However, this situation did not occur during the current study.

2.2.5.2. Calculation of dry matter and neutral detergent fiber digestibility. Conventional *in vitro* digestibility (IVDMD) was calculated as follows: $(1 - [\{\text{DM residue} - \text{ash residue}\} - \{\text{blank}_{\text{DM}} - \text{ash residue}_{\text{blank}}\}/\text{DM original}])$, where DM residues is the DM recovered after incubation, blank_{DM} is the DM recovered in the corresponding blank after the same fermentation time, ash residue is the ash after residue after combustion in a muffle furnace, and DM original is the DM of the substrate placed in the tube.

Daisy^{II} and *in situ* dry matter digestibility values (DMD) were calculated as follows: $(1 - ([W_3 - \{W_1 \times C_1\}] \times 1000)/(W_2 \times \text{DM}))$, where W_1 is the filter bag weight, W_2 is the sample weight (as is), W_3 is the final weight (filter bag + residue) after *in vitro* or *in situ* and sequential treatment with aNDF solution, C_1 is comparison of blank filter bag after and before digestion treatment weight, and DM is the dry matter content (g/kg) of samples.

Neutral detergent fiber digestibility (aNDFD) was calculated using the following equation: $(1 - (([W_3 - \{W_1 \times C_1\}] \times 1000)/(W_2 \times \text{aNDF})))$, where W_1 is the filter bag weight, W_2 is the sample weight (as is), W_3 is the final weight (filter bag + residue) after *in vitro* or *in situ* and sequential treatment with aNDF solution, C_1 is comparison of blank filter bag after and before digestion treatment weight, and sample aNDF content (g/kg in as is sample).

2.2.6. Variation within and between runs

The same technician performed all three experiments; therefore, technician bias was assumed to be only a minor source of variation of DM and aNDF digestibility estimates across the techniques tested. To evaluate precision of technique or estimate coefficient of

variation (CV, %) between runs, all raw values of tested forages obtained during Experiment 2 and 3 were utilized.

2.3. Analysis

The samples were analyzed according to AOAC (1990) for dry matter (DM; method ID 934.01), and crude protein (CP; method ID 942.01) content was determined by the Kjeldahl procedure (method ID 954.01) using a Kjeltec Auto System (Kjeltec Auto System, Büchi, Flawil, Switzerland). Neutral detergent fiber (aNDF) was determined according to Van Soest et al. (1991) with sodium sulfite and heat stable alpha amylase and expressed including residual ash. Acid detergent fiber (ADF) was determined according to AOAC (1990, method ID 954.01) and expressed with the inclusion of residual ash. The aNDF and ADF procedures were adapted for use in an Ankom^{200} Fiber Analyzer (ANKOM Technology Corporation, Fairport, NY, USA). Analyses were conducted with two replicates and acceptable coefficients of variation of analyses' means were <0.5%, <2.0%, <3.0%, and <3.0% for DM, CP, ADF, and aNDF, respectively. Chemical content was determined on a DM basis and expressed as g/kg of forage sample.

2.4. Statistical analysis

2.4.1. Experiment 1

Analysis of variance was performed on data using the General Linear Model (GLM) procedure of SAS (SAS, 2001) as a split plot design: $Y_{ijr} = \alpha_i + e_{ir}$; Y_{ijr} is the variable studied (DMD, aNDFD), α_i is the technique effect, and e_{jr} is the residual standard deviation used as the error term. Means were separated using preplanned pair-wise comparisons of LSMeans generated with the PDIFF and STDERR functions of SAS. Replicates of each forage type were considered the experimental units. Laboratory analyses' replicates within each sample were considered the observational units.

2.4.2. Experiment 2

Dry matter and aNDF digestibility estimates were analyzed using the GLM procedure of SAS (2001) appropriate for a split plot design: $Y_{ijr} = \alpha_i + \beta j + e_{ijr}$; where Y_{ijr} is the variable studied, α_i is the animal effect, βj is the technique effect, and e_{ijr} is the residual standard deviation used as the error term. LSMeans were separated using pre-planned orthogonal contrasts for a 2×4 (DMD) or a 2×3 (aNDFD) factorial design. Each trial or run was considered the experimental unit.

2.4.3. Experiment 3

Results were subjected to a 2-way analysis of variance (sampling/grinding size and technique) with GLM procedures of SAS (2001) as a split-plot design: the whole-plot experimental unit was grinding screen (1-mm and 2-mm) or sampling (0.25 g and 0.5 g) sizes and the sub-subplot experimental unit was research technique within the grinding screen/sampling sizes. The model used was: $Y_{ijr} = \alpha_i + \beta j + e_{ijr}$; where Y_{ijr} is the variable studied, α_i is the grinding/sampling sizes, βj is the technique effect, and e_{ijr} is the residual standard deviation used as the error term.

Each trial was considered as the experimental unit; therefore *in vivo* estimates included four replicates ($n = 4$), whereas conventional *in vitro*, Daisy^{II}, and *in situ* techniques included ten ($n = 10$) replicates per treatment combination. LSMeans were calculated and separated using selected pre-planned orthogonal contrast statements, depending on the response variable being evaluated. The results were considered significant at the $P<0.05$ level for all three experiments of this study. All data are presented as LSMeans ± S.E.M. For estimating coefficients of variation (CV, %) within runs, rack (conventional *in vitro*), jar (Daisy^{II}), and mesh bag (*in situ*) was considered the experimental unit. For estimates of precision or for estimates of coefficients of variation (CV, %) between replicates, sample/forage species was considered the experimental unit.

2.4.4. The regression analysis

The regressions between forage ADF and digestibility or between each pair of DM and aNDF digestibility values from the different techniques were obtained using the REG procedure of SAS (2001). Based on data obtained from Experiment 2 and 3, we also estimated correlations between *in vivo* DMD and DMD estimated by the conventional *in vitro*, Daisy^{II}, and *in situ* techniques. In addition, since our study covers a diverse range of forage samples, Spearman rank correlation (Altman, 1991) was used to determine if the techniques ranked the forage species/types samples in a similar order.

3. Results

3.1. Experiment 1

Chemical content and digestibility estimates for forage species by all tested techniques in Experiment 1 are presented in Table 1. Grasses contained lower ($P<0.001$) CP than lichens, forbs, and shrubs, which did not differ ($P>0.10$) from each other. Acid detergent fiber content was higher ($P<0.001$) for grasses compared to lichen with forbs and shrubs being intermediate. Across the forage species, ADF content ranged from 87 to 485 g/kg which indicated that our samples are diverse in terms of cell wall content. Compared to conventional *in vitro*, Daisy^{II} and the *in situ* technique overestimated ($P<0.05$) DMD. In addition, the difference in digestibility estimates between techniques appeared to be greatest with forbs, shrub, and lichen. In particular, incredibly high estimates of DMD were obtained on tree hair lichen using Daisy^{II} (0.935) and *in situ* (0.863) techniques. Mean values were overestimated by 23.6 and 16.4% unit, respectively compared to the conventional *in vitro* technique. The conventional *in vitro* and *in situ* techniques were similar ($P>0.05$) for two (California brome and elk sedge) of the five grass species, whereas the Daisy^{II} technique differed from conventional *in vitro* estimates for all forage species ($P<0.05$).

3.2. Experiment 2

Chemical composition of forages and digestibility estimates for this experiment are shown in Table 2. Sheep and steer *in vivo* DMD were higher ($P<0.05$) when estimated with conventional *in vitro*, Daisy^{II}, and *in situ* techniques. In turn, Daisy^{II} DMD esti-

Table 1
Chemical content (LSMeans ± standard error; g/kg, DM) and conventional *in vitro*, Daisy[II], and filter bag *in situ* dry matter digestibility (DMD) and Daisy[II], filter bag *in situ* neutral detergent fiber digestibility (aNDFD) for clipped forage samples from mixed-conifer rangelands in Experiment 1

Forages	*n*	Content			DMD			S.E.M.*	aNDFD		
		CP	ADF	aNDF	*In vitro*	Daisy[II]	*In situ*		Daisy[II]	*In situ*	S.E.M.*
Grasses											
California brome	8	60 ± 9	479 ± 16	665 ± 26	0.541[a]	0.621[b]	0.566[a]	24	0.434[a]	0.332[b]	17
Elk sedge	7	64 ± 4	407 ± 13	624 ± 11	0.571[a]	0.795[b]	0.593[a]	16	0.663[a]	0.333[b]	25
Orchardgrass	7	79 ± 6	380 ± 11	550 ± 07	0.637[a]	0.785[b]	0.729[c]	15	0.608[a]	0.494[b]	28
Pinegrass	7	74 ± 2	435 ± 50	587 ± 07	0.552[a]	0.793[b]	0.648[c]	8	0.639[a]	0.386[a]	15
Western fescue	7	43 ± 8	485 ± 15	710 ± 30	0.425[a]	0.526[b]	0.495[c]	19	0.331[a]	0.277[b]	10
Forbs											
Annual willowherb	5	91 ± 7	348 ± 21	456 ± 26	0.509[a]	0.699[b]	0.664[b]	23	0.330[a]	0.244[b]	24
Lupine	6	107 ± 9	288 ± 32	388 ± 40	0.752[a]	0.832[b]	0.777[a]	36	0.574[a]	0.425[b]	61
Strawberry	7	93 ± 3	177 ± 07	306 ± 17	0.566[a]	0.909[b]	0.870[c]	9	0.680[a]	0.546[b]	28
Western yarrow	4	92 ± 9	353 ± 23	383 ± 18	0.695[a]	0.805[b]	0.749[c]	13	0.483[a]	0.333[b]	22
White hawkweed	6	73 ± 6	351 ± 23	452 ± 23	0.640[a]	0.743[b]	0.705[c]	18	0.423[a]	0.335[b]	14
Shrubs											
Big huckleberry	7	88 ± 2	276 ± 14	321 ± 10	0.585[a]	0.775[b]	0.765[b]	12	0.291[a]	0.247[b]	14
Low oregongrape	6	106 ± 2	295 ± 79	399 ± 13	0.650[a]	0.749[b]	0.721[b]	10	0.372[a]	0.280[b]	19
Shinyleaf spirea	8	83 ± 2	278 ± 15	363 ± 13	0.558[a]	0.763[b]	0.747[b]	18	0.350[a]	0.299[b]	25
Snowberry	10	81 ± 2	263 ± 16	316 ± 13	0.661[a]	0.799[b]	0.789[b]	15	0.367[a]	0.322[b]	24
Lichen											
Tree hair lichen	5	101 ± 5	87 ± 11	360 ± 19	0.699[a]	0.935[b]	0.863[c]	11	0.796[a]	0.591[b]	36

[abc]LSMeans in the same row for either DMD or aNDFD with different superscripts differ ($P<0.05$).
* Standard error of the LSMeans.

Table 2
Chemical content (g/kg, DM), *in vivo*, conventional *in vitro*, Daisy^{II}, and filter bag *in situ* dry matter digestibility (DMD) and *in vivo*, Daisy^{II}, and filter bag *in situ* neutral detergent fiber digestibility (aNDFD) of meadow hay in Experiment 2

Animals	Content			DMD				aNDFD		
	CP	ADF	aNDF	*In vivo*	*In vitro*	Daisy^{II}	*In situ*	*In vivo*	Daisy^{II}	*In situ*
Wether	52	314	604	0.508^{a}	0.580^{b}	0.684^{c}	0.656^{d}	0.480^{a}	0.485^{a}	0.474^{a}
Steer	53	303	590	0.492^{a}	0.586^{b}	0.694^{c}	0.650^{d}	0.413^{a}	0.481^{b}	0.435^{c}
S.E.M.*	0	6	1	0.005	0.005	0.005	0.005	0.007	0.007	0.008
P**	0.46	0.27	0.15	0.05	0.49	0.25	0.45	<0.01	0.65	<0.01

^{a,b,c,d}LSMeans in the same row for either DMD or aNDFD with different superscripts differ (P<0.05).

* Standard error of the LSMeans; $n = 4$ for CP, ADF, aNDF, *in vivo* DMD, and *in vivo* aNDF; $n = 10$ for *in vitro*, Daisy^{II}, *in situ* DMD; and *in vitro*, Daisy^{II}, filter bag *in situ* aNDFD.

** Probability of *F*-test contrasts wether *vs.* steer.

mates were greater (P<0.05) than all other estimates of digestibility. *In vivo* DMD differed (P<0.05) between animal species, but the differences in techniques were not different (P>0.05) consistently across animal species. Daisy^{II} and *in situ* aNDF digestibility did not differ (P>0.05) from *in vivo* aNDF digestibility for wether diets. Although, for steer diets, both the Daisy^{II} and *in situ* techniques were greater (P<0.05) than *in vivo* aNDF digestibility estimates. No statistical difference (P>0.05) was detected with the Daisy^{II} technique between aNDF digestibility of diets of sheep and steers. However, *in situ* and *in vivo* techniques aNDF digestibility were lower (P<0.01) in steer diets as compared to sheep.

3.3. Experiment 3

Chemical composition and digestibility estimates for forages in this study are presented in Tables 3 and 4. For grass hay, Daisy^{II}, and *in situ* DMD estimates were higher (P<0.05) than *in vivo* and conventional *in vitro* DMD. In addition, IVDMD was lower (P<0.05) than *in vivo* estimates. In contrast, for grass straw, IVDMD, Daisy^{II} DMD, and *in situ* DMD were lower (P<0.01) compared to *in vivo* DMD. For grass hay and straw hay diets, aNDF digestibility were underestimated (P<0.05) by both Daisy^{II} and *in situ* techniques compared to *in vivo* estimates of digestibility.

Dry matter digestibility for the Daisy^{II} and *in situ* techniques were greater (P<0.01) for grass hay milled at 1-mm *versus* 2-mm. For grass straw, DMD estimates from the conventional *in vitro*, Daisy^{II}, and *in situ* techniques were greater (P<0.01) for 1-mm compared to 2-mm milling. In addition, aNDF digestibility was higher (P<0.05) for 1-mm milled samples of the two forage types using the Daisy^{II} and *in situ* techniques.

Daisy^{II} and *in situ* estimates were higher (P<0.05) than IVDMD for all clipped forage species (Table 4). Grinding diameter only influenced digestibility estimates for one forage species (strawberry) and that was only with the conventional *in vitro* technique.

Effects of sample mass on digestibility estimates are presented in Table 5. For grass hay, DM and aNDF digestibility estimates increased (P<0.05) when sample mass was reduced from 0.5 to 0.25 g in both the Daisy^{II} and *in situ* techniques. Likewise, for firmleaf willow,

Table 3
Chemical content (g/kg, DM), *in vivo*, conventional *in vitro,* Daisy^{II}, and filter bag *in situ* dry matter digestibility (DMD) and *in vivo*, Daisy^{II} and filter bag *in situ* neutral detergent fiber digestibility (aNDFD) of two harvested forages at two different grinding sizes in Experiment 3

	Size[***]	Content			DMD				S.E.M.[*]	aNDFD			S.E.M.[*]
		CP	ADF	aNDF	*In vivo*	*In vitro*	Daisy^{II}	*In situ*		*In vivo*	Daisy^{II}	*In situ*	
Grass hay		86							6				11
	1		345	583	0.624^{a}	0.533^{b}	0.708^{c}	0.728^{d}		0.562^{a}	0.519^{b}	0.521^{b}	
	2		356	615	0.624^{a}	0.519^{b}	0.667^{c}	0.698^{d}		0.581^{a}	0.479^{b}	0.498^{b}	
P[**]					0.98	0.15	<0.01	<0.01		0.06	<0.01	0.02	
Grass straw		36							7				8
	1		505	804	0.500^{a}	0.342^{b}	0.425^{c}	0.433^{c}		0.572^{a}	0.295^{b}	0.304^{b}	
	2		515	814	0.501^{a}	0.306^{b}	0.384^{c}	0.385^{c}		0.577^{a}	0.269^{b}	0.246^{c}	
P[**]					0.86	<0.01	<0.01	<0.01		0.61	0.02	<0.01	

^{a,b,c,d}LSMeans in the same row for either DM or aNDF digestibility with different superscripts differ (P<0.05).
[*] Standard error of the LSMeans; $n = 4$ for *in vivo* DMD, and *in vivo* aNDF; $n = 10$ for *in vitro*, Daisy^{II}, *in situ* DMD; and Daisy^{II}, filter bag *in situ* aNDFD.
[**] Probability of F-test contrasts sample grinding size 1-mm *vs.* 2-mm in the same forage species.
[***] Grinding screen size, diameter; 1 = 1-mm, 2 = 2-mm.

Table 4
Chemical content (g/kg, DM), conventional *in vitro,* Daisy[II], and filter bag *in situ* dry matter digestibility (DMD) and Daisy[II] and filter bag *in situ* neutral detergent fiber (aNDFD) digestibility of five clipped samples at two different grinding sizes in Experiment 3

	Size[***]	Content			DMD			S.E.M.[*]	aNDFD		
		CP	ADF	aNDF	*In vitro*	Daisy[II]	*In situ*		Daisy[II]	*In situ*	S.E.M.[*]
Strawberry		91						8			12
	1		246	286	0.590[a]	0.779[b]	0.816[c]		0.305[a]	0.388[b]	
	2				0.541[a]	0.761[b]	0.811[c]		0.200[a]	0.356[b]	
P[**]					<0.01	0.12	0.71		<0.01	0.01	
Western yarrow		99						6			10
	1		439	505	0.532[a]	0.639[b]	0.624[b]		0.285[a]	0.261[b]	
	2		432	487	0.546[a]	0.628[b]	0.641[c]		0.279[a]	0.274[a]	
P[**]					0.24	0.33	0.13		0.68	0.29	
Red alder		103						6			11
	1		269	364	0.547[a]	0.827[b]	0.821[b]		0.529[a]	0.486[b]	
	2		231	288	0.547[a]	0.837[b]	0.839[b]		0.439[a]	0.429[b]	
P[**]					0.99	0.40	0.11		<0.01	<0.01	
Firmleaf willow		75						6			14
	1		308	256	0.616[a]	0.865[b]	0.824[c]		0.479[a]	0.306[b]	
	2		298	243	0.612[a]	0.858[b]	0.825[c]		0.426[a]	0.326[b]	
P[**]					0.73	0.57	0.92		<0.01	0.22	
Three hair lichen		138						7			17
	1		114	319	0.666[a]	0.852[b]	0.866[b]		0.563[a]	0.591[b]	
	2		113	337	0.679[a]	0.867[b]	0.878[b]		0.607[a]	0.638[b]	
P[**]					0.27	0.19	0.33		0.01	<0.01	

[a,b,c,d]LSMeans in the same row for either DM or aNDF digestibility with different superscripts differ (P<0.05).
[*] Standard error of the LSMeans ($n = 10$).
[**] Probability of *F*-test contrasts sample grinding size 1-mm *vs.* 2-mm in the same forage species.
[***] Grinding screen size, diameter; 1 = 1-mm, 2 = 2-mm.

Table 5
Comparison of Daisy^{II}, and filter bag *in situ* dry matter digestibility (DMD) and Daisy^{II} and filter bag *in situ* neutral detergent fiber (aNDFD) digestibility estimates[****] of meadow hay and firmleaf willow using two different samples sizes

	Size[***]	DMD			aNDFD		
		Daisy^{II}	*In situ*	S.E.M.[*]	Daisy^{II}	*In situ*	S.E.M.[*]
Grass hay				6			10
	1	0.692[a]	0.700[a]		0.502[a]	0.516[a]	
	2	0.635[a]	0.651[a]		0.420[a]	0.458[a]	
P[**]		<0.01	<0.01		<0.01	<0.01	
Firmleaf willow				4			11
	1	0.867[a]	0.844[a]		0.500[a]	0.429[a]	
	2	0.815[a]	0.830[a]		0.370[a]	0.383[a]	
P[**]		<0.01	0.11		<0.01	<0.01	

[a,b]LSMeans in the same row for either DM or aNDF digestibility with different superscripts differ (P<0.05).
[*] Standard error of the LSMeans ($n = 10$).
[**] Probability of *F*-test contrasts sample size 0.25 *vs.* 0.5 g in the same forage species.
[***] Sample size; 1 = 0.25 g, 2 = 0.5 g.
[****]Estimates of *in vivo* digestibility of meadow hay were 0.492 and 0.413 for DMD and aNDFD, respectively.

reducing sample mass increased (P<0.05) DMD estimates for the Daisy^{II} technique and aNDF digestibility estimates for both techniques.

3.4. The relationships between digestibility values estimated by different techniques

Summarizing all three experiments, DM digestibility estimated by Daisy^{II} (IVDMD = 0.101 + 0.641 × Daisy^{II} DMD, $r^2 = 0.63$, $n = 115$, P<0.001) and by the *in situ* technique (IVDMD = 0.149 + 0.614 × *in situ* DMD, $r^2 = 0.58$, $n = 115$, P<0.001) was not much accurate but correlated with IVDMD (Fig. 1). The Daisy^{II} technique gave the best predictions for *in situ* DMD (*in situ* DMD = 0.27 + 0.904 × Daisy^{II} DMD, $r^2 = 0.81$, $n = 115$, P<0.001). The Spearman rank correlation coefficients (r^2) for all forage species/types ($n = 20$) were 0.62 (P<0.01), 0.58 (P<0.05), and 0.85 (P<0.001) for IVDMD *versus* Daisy^{II}, IVDMD *versus in situ*, and Daisy^{II} *versus in situ* digestibility "paired" estimates, respectively which indicated that these techniques ranked the samples in a relatively similar order (Table 6). Likewise, greater Spearman correlation coefficients were detected for aNDF digestibility estimates ($r^2 = 0.88$, $n = 20$, P<0.001) between the Daisy^{II} and *in situ* techniques. Overall, regressions were highly significant using different samples in terms of both botanical classification and chemical content but the relationship between digestibility values estimated by conventional *in vitro versus* Daisy^{II} or *in situ* techniques was not good enough to be used interchangeably for analysis of forage samples from mixed-conifer rangelands.

Based on three test diets (meadow hay, moderate quality hay, and grass straw), the correlations between *in vivo* and Daisy^{II} (*in vivo* DMD = 0.335 + 0.402 × Daisy^{II} DMD, $r^2 = 0.85$, $n = 24$, P<0.001) or *in vivo* and *in situ* (*in vivo* DMD = 0.352 + 0.373 × *in situ* DMD, $r^2 = 0.80$, $n = 24$, P<0.001) were slightly better than that between *in vivo* and IVDMD (*in vivo* DMD = 0.330 + 0.542 × IVDMD, $r^2 = 0.78$, $n = 24$, P<0.001). In similar fashion,

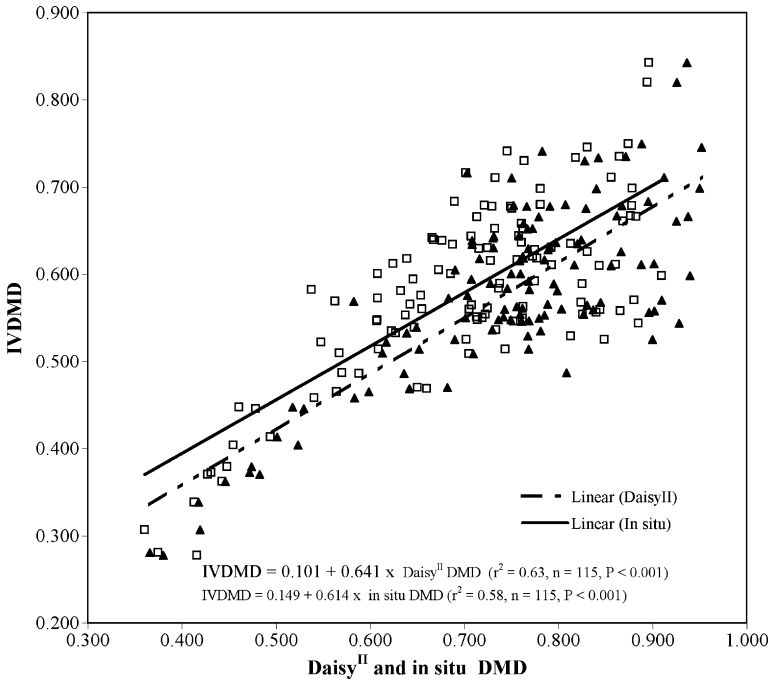

Fig. 1. The relationship between digestibility of dry matter (DMD) estimated by conventional *in vitro* (IVDMD, Y) on DMD estimated by Daisy^{II} (▲) and filter bag *in situ* (□) technique (X).

other studies (Gasa et al., 1989; Khazaal et al., 1993; Huntington and Givens, 1995; Ferret et al., 1997) have also documented that the *in situ* technique predicted *in vivo* digestibility with greater accuracy (higher r^2) than the *in vitro* techniques.

Based on the results of all three experiments it is clear that compared to IVDMD (IVDMD = 0.779 − 0.000589 × ADF, g/kg; $r^2 = 0.45$, $n = 115$, P<0.001), Daisy^{II} (Daisy^{II} DMD = 1.054 − 0.000912 × ADF, g/kg; $r^2 = 0.71$, $n = 115$, P<0.001), and *in situ* (*in situ* DMD = 1.042 − 0.00101 × ADF, g/kg; $r^2 = 0.86$, $n = 115$, P<0.001) DMD were more negatively correlated with forage ADF content (Fig. 2).

3.5. Variations within and between runs

According to data from Experiments 2 and 3, the average CVs of each run (within run, $n = 26$ for each technique) for DMD were 9.7%, 14.8%, and 14.9% for the conventional *in vitro*, Daisy^{II}, and *in situ* techniques, respectively. In our experiment, CVs of digestibility values estimated by the conventional *in vitro* technique were lower (P<0.05) compared to CVs of digestibility values estimated by the other techniques. Neutral detergent fiber digestibility CVs estimated by the Daisy^{II} (23.6%) and *in situ* techniques (23.1%) were not significantly different (P>0.05).

Using the pooled results of Experiments 2 and 3, the mean CVs of 55 DM digestibility samples across different runs (run-to-run fluctuations within each sample); ($n = 10$ for each

Table 6
Ranking order for forages based on dry matter digestibility (DMD) and neutral detergent fiber digestibility (aNDFD) values determined by the conventional *in vitro*, Daisy^{II}, and *in situ* techniques

Forages	Rank[a]				
	DMD			aNDFD	
	IVDMD	Daisy^{II}	*In situ*	Daisy^{II}	*In situ*
Grasses					
California brome	16	18	18	12	12
Elk sedge	11	8	17	3	10
Grass hay	17	17	12	7	3
Grass straw	20	20	20	20	18
Meadow hay	10	16	15	10	6
Orchardgrass	7	10	10	5	4
Pinegrass	14	9	16	4	8
Western fescue	19	19	19	17	17
Forbs					
Lupine	1	5	6	6	7
Strawberry	12	2	1	2	2
Western yarrow	3	6	8	9	11
White hawkweed	6	14	13	13	9
Shrubs					
Annual willowherb	18	15	14	18	20
Big huckleberry	9	11	7	19	19
Firmleaf willow	8	3	4	11	14
Low oregongrape	5	13	11	14	16
Red alder	15	4	3	8	5
Shinyleaf spirea	13	12	9	16	15
Snowberry	4	7	5	15	13
Lichen					
Tree hair lichen	2	1	2	1	1

[a] Ranking is achieved by giving the ranking '1' to the biggest digestibility value, '2' to the second biggest value so on. The smallest digestibility value will get the lowest ranking in the column.

CV) were 4.3%, 4.7%, and 4.7% for the conventional *in vitro*, Daisy^{II}, and *in situ* techniques, respectively. The CVs of aNDF digestibility were 12.4% and 12.2% for the Daisy^{II} and *in situ* techniques, respectively. Differences in the coefficient of variation of DMD or aNDFD were not detected (P>0.05) across the techniques. One criterion of an approach that has not been generally accepted is to minimize the required replicate number of samples without damaging the precision and accuracy of the results. We have estimated that in order to be within 10 units of mean digestibility values with 90% power at the 5% significance level (Kuehl, 2000), it would require a minimum of 4 (2–10), 4 (1–13), 6 (1–9), 29 (5–165), and 34 (16–189) replicates per sample for DM conventional *in vitro*, DM Daisy^{II}, DM *in situ*, aNDF Daisy^{II}, and aNDF *in situ* digestibility techniques, respectively.

When using 0.25 g sample for assay of digestibility estimation, to be within 10 units of mean digestibility with 90% power at the 5% significance level, the smallest required replicate numbers were 1, 4, 2, and 9 for Daisy^{II} DM, Daisy^{II} aNDF, *in situ* DM, and *in situ* aNDF digestibility, respectively. In contrast, when the sampling size was 0.5 g, these

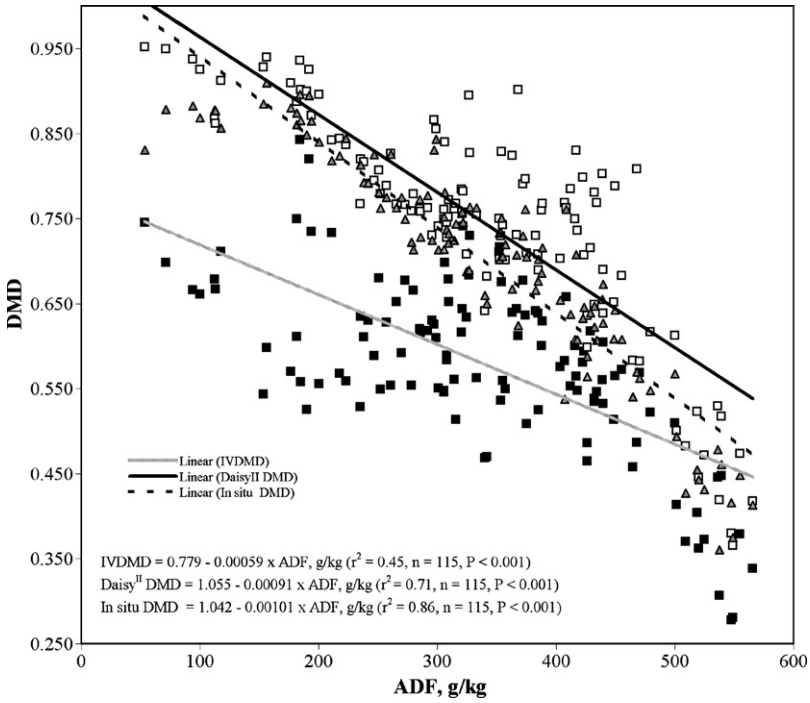

Fig. 2. The relationship between digestibility of dry matter (DMD) estimated by conventional *in vitro* (IVDMD, ■), Daisy^{II} (□), and filter bag *in situ* (▲) techniques (Y) on acid detergent fiber (ADF, X) of forages.

numbers reached 2, 10, 2, and 12 for Daisy^{II} DM, Daisy^{II} aNDF, *in situ* DM, and *in situ* aNDF digestibility, respectively, which is slightly higher than those with 0.25 g samples but is not considered to be of practical significance. However, as shown in Table 5, digestibility values obtained from a 0.5 g sample mass were closer to actual or *in vivo* DMD values, indicating that a 0.5 g sample mass may be more accurate.

4. Discussion

Different values for the conventional *in vitro* and filter bag based (Daisy^{II} and filter bag *in situ*) techniques seem to be related to sample particle size and cell wall structure of forages (Wilman and Adesogan, 2000; Adesogan, 2002, 2005). The filter bag based techniques tended to overestimate digestibility when forage was more finely ground (1-mm) compared to 2-mm ground forage. This may be caused by agitation during incubation, boiling in neutral detergent solution, and through rinsing of the filter bags with water after 48 h incubation. During this procedure a proportion of non-digestible fine particles may have been removed, reducing the weight of residue and increasing the estimate of digestibility compared to the conventional *in vitro* technique in which microbial matter and fine particles are retained. The porosity of the F57 filter bag is 30×10^{-3} mm (Ankom Technology Corporation, 1997);

therefore small particles of less than 30×10^{-3} mm in diameter can escape from the filter bag during processing of digestion. Further, Marinucci et al. (1992) speculated it is possible that relatively large particles can pass through the pores by exerting pressure on the loose fibers of the fabric. Adesogan (2005) also observed that predictions of *in vivo* digestibility from the filter bag technique were more accurate when forages were incubated in non-standard bags. However, when such non-standard bags are used results obtained will depend on pore size, seal treatment, and weave type.

In our study, digestion patterns of fine fescue grass straw (in which *in vivo* digestibility was higher compared to values estimated by the conventional *in vitro* technique) were similar to cereal straw digestion reported by Khazaal et al. (1993) and Adesogan et al. (1998). Despite this, other workers (Kitessa et al., 1999) indicate the conventional *in vitro* technique, although accurate for fresh grasses, is not suited for predicting the digestibility value of feeds like straws, which are generally bulky and very low quality. They gave two reasons for this conclusion: (i) 48 h incubation may be insufficient for cereal straws, and (ii) rumen microbial population may gradually shift to highly fibrolytic species when animals are retained on straw diets, an opportunity that may be very limited under *in vitro* conditions. Since our rumen inoculum donor steers were fed moderate quality hay and incubation time was 48 h, both aforementioned possibilities may apply to the grass straw digestion values estimated by the different techniques.

In general, our data suggest that differences in digestion estimates between the conventional *in vitro* and filter bag based techniques decrease as forage fiber/ADF composition increase. In our study, the Daisy^{II} and *in situ* techniques estimated true digestibility while the conventional *in vitro* estimated apparent digestibility. Therefore, theoretically, IVDMD should be expected to have lower values. However, as documented by Wilman and Adesogan (2000), digestibility estimates using filter bags result in apparent and true digestibility differences that are very low. It is also possible to convert IVDMD values to "true" IVDMD values using Van Soest et al. (1966) equation (true IVDMD = $0.162 + 0.92 \times$ IVDMD). Our results suggested that the difference in digestibility values estimated by the conventional *in vitro* and filter bag based techniques may vary among forages with different cell wall contents.

It should also be noted that when accuracy of the filter bag based technique is tested against the conventional *in vitro* technique, it is difficult to determine if a weaker correlation is due to problems with the Daisy^{II} or *in situ* technique (such as particle loss from the filter bag) or whether the conventional *in vitro* technique has a flaw. Ideally, results should be validated against *in vivo* measurements but these can also have methodological deficiencies (White and Ashes, 1999).

The variation in coefficients of digestibility values between samples within runs should indicate possible associative effects (synergistic *versus* antagonistic) between samples when they are incubated together (same jar or same mesh bag and rumen, for the Daisy^{II} and filter bag *in situ* techniques, respectively). Although some evidence exists that the microenvironment within bags can differ markedly from the environment of the incubation medium (Marinucci et al., 1992), the filter bag based technique is generally assumed to have conditions within bags similar to the conditions in the surrounding environment (Hvelplund and Weisbjerg, 2000). In contrast, in the conventional *in vitro* technique, because each sample is incubated in separate tubes, values should have more independence compared

to the other two techniques. In this study, within run CV of digestibility estimated by the conventional *in vitro* technique was lower ($P<0.05$) compared to the CV of digestibility estimated by other techniques. Several possible explanations may exist for this. First, our results suggest that when incubating different forages in the same place, the microenvironment inside jars or mesh bags may create a more diverse microenvironment, resulting in varied, but mostly increased digestibility values. In other words, synergistic associative effects may have existed. As our study indicated, another effect of the filter bag based techniques maybe the interaction among forage types (data not shown), resulting in higher CV values estimated with these techniques. Animal to animal variation may add to the variability of the *in situ* technique (Mehrez and Orskov, 1977). Since rumen fluid collected from animals is composited for the *in vitro* and Daisy^{II} techniques, this variation will not occur with conventional *in vitro* (Gulati et al., 1997) and Daisy^{II} techniques. Furthermore, the CV within runs did not differ between Daisy^{II} *versus in situ* technique, which is suggesting that animal to animal variation was small.

The coefficient of variation for DM digestibility of each forage sample across different runs (run-to-run/replicate variation within each sample) can be used to estimate the precision of each digestion technique. Our results are similar to those obtained by Wilman and Adesogan (2000), who demonstrated that the conventional *in vitro* technique is preferred because of better repeatability. Also, the required replicate number appears to be more consistent within different forages than with other techniques tested in this study. A general trend was that both Daisy^{II} and *in situ* techniques demanded unusually low replicate number (in most cases $n = 1$) to determine DMD of forbs, shrubs, and lichen. In contrast, when using this result for aNDF digestibility estimates, the required replicate number becomes much larger. This suggests that the Daisy^{II} and *in situ* techniques have some problems with estimation of aNDF and DM digestibility for forbs, shrubs, and lichen. This may be because these techniques might not be sensitive enough to detect run-to-run variation for these kinds of forages, although based on mathematical calculation of required replicate number, it can be erroneously (committing Type II errors) assumed that Daisy^{II} technique is more precise. In particular, for low ADF forages, both Daisy^{II} and *in situ* techniques failed to accurately estimate aNDF digestibility, and, as a result, required higher replicate numbers for sample accuracy. On the other hand, when determining forage aNDF digestibility, taking isolated aNDF as a sample instead of whole forage as a sample may increase magnitude of forage aNDF digestibility and repeatability (Varel and Kreikemeier, 1994; Kennedy et al., 1999). Therefore, for high quality forages Daisy^{II} and *in situ* techniques may not be the method of choice.

An unequal distribution of particles between different forages using the same grinding screen sizes may have contributed to forage type × research technique interactions (data not shown) in this study. Michalet-Doreau and Cerneau (1991) showed that plant material from different forage species ground through the same screen size could have a different distribution of particle sizes. This means that at the same screen size and at the same bag pore size, forages with different particle distribution after grinding will have different levels of mechanical loss of fine particles from filter bags (Kitessa et al., 1999). In addition, low ADF forages usually tend to get ground too much finer particles than high ADF forages (Mabjeesh et al., 2000).

Furthermore, according to Emanuele and Staples (1988), after grinding through a 2-mm screen, mean particle size of grass samples was greater than that of legume samples.

Similarly, results from our study suggest digestion values for samples ground through 1- and 2-mm screens were similar for forbs, shrubs, and lichen, but different for grass.

When choosing the appropriate grinding size, sample size or appropriate technique for estimating DM and aNDF digestibility, one should consider both precision and accuracy. Therefore, based on our data, a 0.5 g sample size seems more preferable than a 0.25 g sample size. We also felt that, when samples were 0.25 g, filter bag based techniques were not sensitive enough to estimate digestibility values accurately, particularly with low ADF forages. Lower sample size to surface area ratio facilitate more losses of undigested, soluble or fine particulate material through the pores of the bags (Mehrez and Orskov, 1977; Vanzant et al., 1998) which may cause overestimate digestibility in 0.25 g sample sizes. In order to explain why Daisy^{II} technique is getting more precise, but less accurate, when sampling size is decreased with low fiber forages, further investigation is warranted.

Based on this research it could be stated that, in general, results from the Daisy^{II} technique were more similar to the *in situ* technique, therefore digestibility values estimated by the Daisy^{II} and *in situ* techniques might be interchangeable using general predictive equations generated across different forages. Sieving off different size particles (Huntington and Givens, 1997) of the ground forage sample, correcting for the fine particle losses (Dhanoa et al., 1999) from the filter bag during digestion and washing, grouping the feeds into categories based on fiber fractions (summative and single fractions), applying a corresponding correction factor, and standardizing laboratory procedures may increase predictability and accuracy of the Daisy^{II} and *in situ* techniques.

5. Conclusion

Although IVDMD estimates are different than *in vivo* estimates of DMD, they are, generally, in closer agreement than the newer techniques. Sample size and grind size appear to have a large effect on filter bag based techniques, with larger sample size and greater grinding size lowering digestibility estimates. Daisy^{II} is an easier and less time-consuming *in vitro* technique of measuring forage digestibility than the conventional *in vitro* technique. However, for research involving wide ranges of forage quality and diverse forage species/types, some adjustments are necessary to ensure that estimates of digestibility are more biologically accurate. Our research suggests that laboratories that have access to fistulated animals may benefit from the use of filter bag *in situ* digestion techniques. This technique requires less labor and equipment than conventional *in vitro* techniques, and, like Daisy^{II} techniques, can process a large number of samples in a short time. In addition, filter bag *in situ* technique predictions of *in vivo* digestibility were more accurate than conventional *in vitro* and Daisy^{II} techniques.

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References

Adesogan, A.T., 2002. What are feed worth? A critical evaluation of selected nutritive value techniques. In: Proceedings of the Florida Ruminant Nutrition Symposium, Gainesville, FL, USA, pp. 33–47.

Adesogan, A.T., 2005. Effect of bag type on the apparent digestibility of feeds in ANKOM Daisy^{II} incubators. Anim. Feed Sci. Technol. 119, 333–344.

Adesogan, A.T., Givens, D.I., Owen, E., 1998. Prediction of the in vivo digestibility of whole crop wheat from in vitro digestibility, chemical composition, in situ rumen degradability, in vitro gas production and near infrared reflectance spectroscopy. Anim. Feed Sci. Technol. 74, 259–272.

Altman, D.G., 1991. Practical Statistics for Medical Research. Chapman & Hall, London, UK, p. 611.

Ankom Technology Corporation, 1997. Operator's manual: Ankom 200/220 fiber analyzer. Ankom Technol. Corp, Fairport, NY, USA.

AOAC, 1990. Official Methods of Analysis, 15th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Bohnert, D.W., Schauer, C.S., DelCurto, T., 2002a. Influence of rumen protein degradability and supplementation frequency on performance and nitrogen use in ruminants consuming low-quality forage: cow performance and efficiency of nitrogen use in wethers. J. Anim. Sci. 80, 1629–1637.

Bohnert, D.W., Schauer, C.S., Bauer, M.L., DelCurto, T., 2002b. Influence of rumen protein degradability and supplementation frequency on steers consuming low-quality forage: I. Site of digestion and microbial efficiency. J. Anim. Sci. 80, 2967–2977.

De Boever, J.L., Cottyn, B.G., Andries, J.I., Buysse, F.X., Vanacker, J.M., 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. Technol. 19, 247–260.

Dhanoa, M.S., France, J., Lopez, S., Dijkstra, J., Lister, S.J., Davies, D.R., Bannink, A., 1999. Correcting the calculation of extent of degradation to account for particulate matter loss at zero time when applying the polyester bag method. J. Anim. Sci. 77, 3385–3391.

Emanuele, S.M., Staples, C.R., 1988. Effect of forage particle size on in situ digestion kinetics. J. Dairy Sci. 71, 1947–1954.

Ferret, A., Gasa, J., Casanas, F., Bosch, L., Nuez, F., 1997. Prediction of voluntary intake and digestibility of maize silages given to sheep from morphological and chemical composition, in vitro digestibility, or rumen degradation characteristics. J. Anim. Sci. 64, 493–501.

Galyean, M.L., 1997. Laboratory Procedures in Animal Nutrition Research, 12th ed. West Texas A and M University, Division of Agriculture and Texas A and M Research and Extension Center, Amarillo, TX, USA.

Gasa, J., Castrillo, C., Baucells, M.D., Guada, J.A., 1989. By-products from the canning industry as feedstuff for ruminants: digestibility and its prediction from chemical composition and laboratory bioassays. Anim. Feed Sci. Technol. 25, 67–77.

Goldman, A., Genizi, A., Yulzari, A., Seligman, N.G., 1987. Improving the reliability of the two-stage in vitro assay for ruminant feed digestibility by calibration against in vivo data from a wide range of sources. Anim. Feed. Sci. Technol. 18, 233–245.

Gulati, S.K., Scott, T.W., Ashes, J.R., 1997. In vitro assessment of fat supplements for ruminants. Anim. Feed Sci. Technol. 64, 127–132.

Holden, L.A., 1999. Comparison of methods of in vitro dry matter digestibility for ten feeds. J. Dairy Sci. 82, 1791–1794.

Huntington, J.A., Givens, D.I., 1995. The in situ technique for studying the rumen degradation of feeds: a review of the procedure. Nutr. Abstr. Rev., Series B 65, 63–93.

Huntington, J.A., Givens, D.I., 1997. The studies on in situ degradation on feeds in the rumen: 1. Effect of species, bag mobility and incubation sequence on dry matter disappearance. Anim. Feed Sci. Technol. 64, 227–241.

Hvelplund, T., Weisbjerg, M.R., 2000. In situ techniques for the estimation of protein degradability and postrumen availability. In: Givens, D.I., Owen, E., Axford, R.F.E., Omed, H.M. (Eds.), Forage Evaluation in Ruminant Nutrition. CAB International, Wallington, UK, pp. 233–258.

Kennedy, P.M., Lowry, J.B., Conlan, L.L., 1999. Isolation of grass cell walls as neutral detergent fiber increases their fermentability for rumen micro-organisms. J. Sci. Food Agric. 79, 544–548.

Khazaal, K., Dentinho, M.T., Ribeiro, J.M., Orskov, E.R., 1993. A comparison of gas production during incubation with rumen contents in vitro and nylon bag degradability as predictors of the apparent digestibility in vivo and the voluntary intake of hays. J. Anim. Prod. 57, 105–112.

Kitessa, S., Flinn, P.C., Irish, G.G., 1999. Comparison of methods used to predict the in vivo digestibility of feeds in ruminants. Aust. J. Agric. Res. 50, 825–841.

Kuehl, R.O., 2000. Design of Experiments: Statistical Principles of Research Design and Analysis. Duxbury Press at Brooks/Cole Publishing, Pacific Grove, CA, USA.

Mabjeesh, S.J., Cohen, M., Arieli, A., 2000. In vitro methods for measuring the dry matter digestibility of ruminant feedstuffs: comparison of methods and inoculum source. J. Dairy Sci. 83, 2289–2294.

Marinucci, M.T., Dehority, B.A., Loerch, S.C., 1992. In vitro and in vivo studies of factors affecting digestion of feeds in synthetic fiber bags. J. Anim. Sci. 70, 296–307.

Marten, G.C., Barnes, R.F., 1980. Prediction of energy digestibility of forages with in vitro rumen fermentation and fungal enzyme systems. In: Pigden, W.G., Balch, C.C., Graham, M. (Eds.), Standardization of Analytical Methodology for Feeds. International Research Center, Ottawa, Ontario, Canada, pp. 61–71.

Mehrez, A.Z., Orskov, E.R., 1977. A study of the artificial fiber bag technique for determining the digestibility of feeds in the rumen. J. Agric. Sci. Cambr. 88, 645–650.

Michalet-Doreau, B., Cerneau, P., 1991. Influence of foodstuff particle size on in situ degradation of nitrogen in the rumen. Anim. Feed Sci. Technol. 35, 69–81.

Robertson, J.B., Van Soest, P.J., Torres, F., 1972. Substitution of filter paper for crucibles in the in vitro rumen true digestibility determination. J. Dairy Sci. 55, 1305–1307.

SAS, 2001. SAS/STAT Software. Release 8.02. SAS Institute Inc., Cary, NC, USA.

Tilley, J.M.A., Terry, R.A., 1963. A two-stage technique for the in vitro digestion of forage crops. J. Br. Grassl. Soc. 18, 104–111.

USDA Natural Resources Conservation Service, 2005. The PLANTS database, version 3.5. Baton Rouge, LA, USA, National Plant Data Center. Available at: http://plants.usda.gov. Accessed 18 October 2005.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Van Soest, P.J., Wine, R.H., Moore, L.A., 1966. Estimation of the true digestibility of forages by the in vitro digestion of cell walls. In: Proceedings of the 10th International Grassland Congress, Helsinki. Finnish Grassland Association, Helsinki, Finland, pp. 438–441.

Vanzant, E.S., Cochran, R.C., Titgemeyer, E.C., 1998. Standardization of in situ techniques for ruminant feedstuff evaluation. J. Anim. Sci. 76, 2717–2729.

Varel, V.H., Kreikemeier, K.K., 1994. Technical note: comparison of in vitro and in situ digestibility methods. J. Anim. Sci. 73, 578–582.

Vogel, K.P., Petersen, J.F., Masterson, S.D., Toy, J.J., 1999. Evaluation of a filter bag system for NDF, ADF, and IVDMD forage analysis. Crop Sci. 39, 276–279.

Wenick, J.J., 2000. The effect of grazing interval on forage quality and production of meadow foxtail. M.S. thesis. Oregon State University, Corvallis, OR, USA.

White, C.L., Ashes, J.R., 1999. A review of methods for assessing the protein value of grain fed to ruminants. Aust. J. Agric. Res. 50, 855–869.

White, J.J., 2003. Effects of forage quality and type of protein supplement on intake and digestibility in beef steers and performance of postpartum beef cows. M.S. thesis. Oregon State University, Corvallis, OR, USA.

Wilman, D., Adesogan, A., 2000. A comparison of filter bag methods with conventional tube methods of determining the in vitro digestibility of forages. Anim. Feed Sci. Technol. 84, 33–47.