

## COMPARISON OF TECHNIQUES AND GRINDING SIZE TO ESTIMATE DIGESTIBILITY OF FORAGE BASE RUMINANT DIETS

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**ABSTRACT:** The objectives of the following experiments were to evaluate DM and NDF digestibility estimates for the following techniques; DAISY<sup>II</sup> *in vitro*, filter bag *in situ* preceded by 48 h pepsin treatment, Tilley and Terry two stage *in vitro*, with *in vivo* digestibility. In addition, effects due to sample size (0.25 vs 0.50 g) and Wiley mill grind size (1mm vs 2mm) were also evaluated. In Exp. 1, 15 forage plants (five grasses, five forbs, four shrubs, and a lichen) from mixed conifer rangelands were used to evaluate digestion estimation techniques. Sample ranges for CP, ADF, and NDF were 2.7 to 14.5%, 5.3 to 53.9%, and 24.6 to 81.1%, respectively. Compared to IVDMD, *in situ* and DAISY<sup>II</sup> technique overestimated ( $P < 0.01$ ) DMD. In Exp. 2, meadow hay samples were used to compare the above techniques to *in vivo* digestibility. *In situ* DMD, DAISY<sup>II</sup> DMD, and IVDMD values were higher ( $P < 0.01$ ) than *in vivo* DMD values. In contrast, *in situ* NDF digestibility did not differ ( $P = 0.13$ ) from *in vivo* NDF digestibility. However, DAISY<sup>II</sup> NDF digestibility (0.25 g sample size) was greater ( $P < 0.01$ ) than *in vivo* NDF digestibility. In Exp. 3, two grasses, forbs, shrubs, and lichen were used to evaluate the interaction of Wiley mill grind size (1 vs 2 mm) and digestibility techniques. For grass hay, DAISY<sup>II</sup> DM and NDF, and *in situ* DM and NDF digestibilities were higher ( $P < 0.05$ ) and IVDMD was lower ( $P < 0.05$ ) compared to *in vivo* DM and NDF digestibility. In contrast, straw IVDMD, DAISY<sup>II</sup> DM and NDF, and *in situ* DM and NDF digestibilities were lower ( $P < 0.01$ ) compared to *in vivo* DM and NDF digestibility. DAISY<sup>II</sup> and *in situ* digestibility estimates were greater ( $P < 0.01$ ) for grass hay milled at 1 mm vs 2mm, and for straw samples all techniques' estimates were higher ( $P < 0.01$ ) for 1mm. For the DAISY<sup>II</sup> and *in situ* techniques using a 0.25 g sample resulted in higher ( $P < 0.05$ ) estimates of digestibility than a 0.5 mg sample. Summary, depending on chemical composition of forages the DAISY<sup>II</sup>, and *in situ* techniques may provide similar-, overestimate, or underestimate DM digestibility compared to IVDMD. For low ADF forages' DAISY<sup>II</sup> and *in situ* techniques failed to accurately estimate NDF digestibility.

Key Words: Ruminant, Digestion techniques, Forages

### Introduction

Because *in vivo* measurement of nutrient digestion is expensive, labor-intensive, and subject to error associated with markers and inherent animal variation, alternative *in*

*vitro* and *in situ* techniques have been developed. The two-stage technique 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. However, the method is slow and tedious. Recently, a filter bag technique (DAISY<sup>II</sup>) for analyzing forage *in vitro* DM digestibility was developed by ANKOM Technology Corporation (Fairport, NY).

Some studies on procedures for using the DAISY<sup>II</sup> method for analysis and its validation have been conducted. Holden (1999) conducted an experiment with ten feeds, which were digested by the two-stage *in vitro* method, the DAISY<sup>II</sup> method with the same types of feeds in a digestion vessel, and the DAISY<sup>II</sup> method with different types of feeds in a digestion vessel. They concluded that the DAISY<sup>II</sup> method can be used to increase labor efficiency in estimating DM digestibility, and forages and grains can be analyzed together in a single digestion vessel. Wilman and Adesogan (2000) compared two stage *in vitro* digestibility estimates on seventy-two forage samples belonging to two forage species to DM digestibility estimates using the DAISY<sup>II</sup> technique. They found that the two stage *in vitro* method is likely to give more precise results than the DAISY<sup>II</sup>, although it requires more labor costs. However, they also postulated that the use of the DAISY<sup>II</sup> rather than the two-stage *in vitro* method could give acceptable digestibility estimates for forages, if the emphasis is on saving labor rather than on obtaining the most precise results. Comparisons of the DAISY<sup>II</sup> technique directly to *in vivo* or *in situ* techniques have not been published to date.

The objective of this study was to compare digestibility estimates for forage based ruminant diets using the DAISY<sup>II</sup> technique, *in vitro*, and filter bag *in situ* DM and NDF digestibility to *in vivo* and two stage *in vitro* DM and NDF digestibility.

### Materials and Methods

Three experiments were conducted to evaluate techniques to estimate forage digestion for grazing ruminants. In experiment 1, 100 forage samples of 15 forage plant species (five grasses, five forbs, four shrubs, and a lichen) from a mixed conifer rangelands were used to evaluate digestion estimation techniques. Samples were collected in late August and oven-dried at 50°C for 48h. Filter bag *in vitro* (DAISY<sup>II</sup>), filter bag *in situ* (*in situ*) DM and NDF digestibility, and the two-stage *in vitro* DM

digestibility (IVDMD) techniques were used for each sample. Additionally, each sample was replicated for each of the techniques evaluated.

In experiment 2, meadow hay samples from a previously conducted digestive kinetics trials were used to compare *in vitro*, *in situ*, and DAISY<sup>II</sup> techniques to *in vivo* digestibility estimates. *In vivo* digestibility estimates were obtained from four steers (Bohnert et al., 2001a) and four lambs (Bohnert et al., 2001b) that were unsupplemented controls in the digestive kinetics studies.

In Experiment 3, meadow hay and fine fescue straw samples from a previously conducted (White et al. unpublished data) digestive kinetics trial were used to compare *in vitro*, *in situ*, and DAISY<sup>II</sup> techniques to *in vivo* digestibility estimates using three unsupplemented steers for each forage type for the *in vivo* estimates. Samples were ground through either a one or two mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) to evaluate the effect of grind size on digestibility estimates. In addition, two grasses, two forbs, two shrubs, and a tree hair lichen were ground at one and two mm and analyzed using the various techniques. For the Daisy<sup>II</sup> and *in situ* techniques two sample sizes (0.25 or 0.5) g were used for the meadow hay and willow samples to evaluate the effects of sample size on digestion estimates.

For the DAISY<sup>II</sup> technique ANKOM F57 filter bags and a DAISY<sup>II</sup> incubator (ANKOM Technology Corporation, Fairport, NY) were utilized. Sample sizes used were either 0.25 or 0.5 g per bag with 24 bags per incubation jar. Each run contained one replicate of the forage species as well as two standard (grass hay) and two blank bags. Samples were sealed in filter bags placed in jars, and incubated for 48 h in a rumen fluid/buffer solution. After incubation, samples were removed and boiled in a neutral detergent solution in an ANKOM<sup>200</sup> fiber analyzer.

For the *in situ* technique 0.25 or 0.5 g samples were sealed in ANKOM F57 filter bags and 24 bags were placed in each jar in a DAISY<sup>II</sup> incubator. The bags were incubated in 2 L of an acid pepsin solution for 48h at 39.5° C. The bags were then removed, rinsed, placed in a mesh bag and incubated in a steer rumen for 48h. After incubation, the bags were removed, rinsed, and NDF content was determined using an ANKOM<sup>200</sup> fiber analyzer.

We used the two-stage technique for IVDMD as described by (Tilley and Terry, 1963). Each 50-ml tube contained a 0.5 g of sample and 35 ml buffer/inoculum mixture (28 ml of the buffer solution and 7 ml of the rumen inoculum). Ruminal inoculum for all techniques was obtained from the same two steers consuming a meadow hay diet. All samples were analyzed for DM, OM (AOAC, 1990), CP (Kjeltec Auto System, Buch Co. Switzerland), NDF, and ADF (Ankom<sup>200</sup> fiber analyzer).

For experiment 2 and 3, DM and NDF digestibility were calculated with the following equation:  $DMD \text{ or } NDFD = ((\text{total forage offered} \times \text{percentage forage DM / NDF digestibility}) - (\text{total orts collected} \times \text{percentage orts DM / NDF digestibility})) \text{ divided by } (\text{total forage offered} - \text{total orts collected})$ .

Data were analyzed using the GLM procedure in SAS (SAS Inst. Inc., Cary, NC). LSMeans were used to

determine difference in digestibility technique means ( $P < 0.05$ )

## Results and Discussion

*Experiment 1:* Chemical composition and digestibility estimates for the forage species in exp. 1 are presented in Table 1. Grasses contained lower ( $P < 0.001$ ) CP, than lichens, forbs, and shrubs which did not differ ( $P > 0.1$ ) from each other. Acid detergent fiber content was higher ( $P < 0.001$ ) for grasses compared to lichen with forbs and shrubs being intermediate. Compared to IVDMD, the *in situ* method and DAISY<sup>II</sup> technique overestimated ( $P < 0.05$ ) DMD. In addition, the difference in digestibility estimates appeared to be greatest with the forb, shrub, and lichen forages. The *in situ* technique was similar to *in vitro* for two of the five grass species, whereas, the DAISY<sup>II</sup> technique differed from *in vitro* estimates for all forage species ( $P < 0.05$ ).

*Experiment 2:* Chemical composition of forages and digestibility estimates for exp. 2 are presented in Table 2. Sheep and steer *in vivo* DMD were overestimated ( $P < 0.05$ ) by *in situ* DMD, DAISY<sup>II</sup> DMD and IVDMD. In turn, Daisy<sup>II</sup> DMD estimates were higher than all other estimates of digestibility. *In vivo* DMD differed ( $P > 0.05$ ) among animal species, but the differences in techniques ( $P > 0.05$ ) were consistent across species. DAISY<sup>II</sup>, and *in situ* NDF digestibility were not different ( $P > 0.05$ ) from *in vivo* NDF digestibility for steer diets. However, for sheep diets, both DAISY<sup>II</sup> and *in situ* techniques were higher ( $P < 0.05$ ) than *in vivo* NDF digestibility estimates. DAISY<sup>II</sup> technique did not detect a difference ( $P > 0.5$ ) between sheep and steer diets NDF digestibility. However, *in situ* and *in vivo* techniques were lower ( $P < 0.001$ ) in steer diets as compared to sheep.

*Experiment 3:* Chemical composition and digestibility estimates for forages in exp. 3 are presented in Table 3. For grass hay, DAISY<sup>II</sup> and *in situ* DMD estimates were higher ( $P < 0.05$ ) than *in vivo* and *in vitro* estimates. In addition, *in vitro* digestibility was higher than *in vivo* estimates. In contrast, grass straw IVDMD, DAISY<sup>II</sup> DMD, and *in situ* DMD were lower ( $P < 0.01$ ) compared to *in vivo* DMD. For grass and straw diets, NDF digestibility was underestimated ( $P < 0.05$ ) by both DAISY<sup>II</sup> and *in situ* estimates as compared to *in vivo* estimates of digestibility.

Dry matter digestibility estimates of DAISY<sup>II</sup> and *in situ* techniques were greater ( $P < 0.01$ ) for grass hay milled at 1 mm vs 2mm. For grass straw, DM digestibility estimates from all techniques were higher ( $P < 0.01$ ) for 1mm compared to 2 mm milling. In addition, NDF digestibility was higher ( $P < 0.05$ ) for 1 mm milled samples for all techniques and forage types.

DAISY<sup>II</sup> and *in situ* estimates were also higher ( $P < 0.05$ ) than *in vitro* for all clipped forage species. Milling diameter only influenced digestibility estimates for one forage species and that was only with the *in vitro* technique.

Effects of sample size on digestibility estimates are presented in Table 4. For grass hay, both DM and NDF

digestibility estimates increased ( $P < 0.05$ ) when sample size was reduced from 0.5 to 0.25 g in both the DAISY<sup>II</sup> and *in situ* techniques. For willow, reducing sample size increased ( $P < 0.05$ ) DMD estimates for the DAISY<sup>II</sup> technique and NDF digestibility estimates for the DAISY<sup>II</sup> and *in situ* technique.

### Implications

Although the DAISY<sup>II</sup> and *in situ* techniques offer advantages in time and labor compared to *in vivo* and traditional *in vitro* techniques, they do not yield results that are comparable to traditional techniques. In most cases they overestimate DM and NDF digestibility compared to traditional *in vivo* and *in vitro* techniques. However, with extremely low-quality forages they appear to underestimate digestibility. Although IVDMD estimates are different than *in vivo* estimates of DMD, they are generally in closer agreement than the newer techniques. In addition, samples size and grind size appear to have large effects on filter bag based digestibility techniques in that larger sample sizes and greater milling sizes lower digestibility estimates.

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**Table 1;** Chemical composition (% DM), *in vitro*, DAISY<sup>II</sup>, and *in situ* DM and NDF digestibility (g/kg) for clipped forage samples.

Forages	n	Composition			DM digestibility			NDF Digestibility	
		CP	ADF	NDF	<i>In vitro</i>	DAISY <sup>II</sup>	<i>In situ</i>	DAISY <sup>II</sup>	<i>In situ</i>
<b>Grasses</b>									
<i>Bromus carinatus</i>	8	6.0±0.5	47.9±1.5	66.5±1.8	541±13 <sup>a</sup>	621±14 <sup>b</sup>	566±14 <sup>a</sup>	434±14 <sup>b</sup>	332±14 <sup>a</sup>
<i>Carex geyeri</i>	7	6.4±0.5	40.7±1.6	62.4±1.9	571±14 <sup>a</sup>	795±15 <sup>c</sup>	593±15 <sup>a</sup>	663±15 <sup>b</sup>	333±15 <sup>a</sup>
<i>Calamagrostis rubescens</i>	7	7.4±0.5	43.5±1.6	58.7±1.9	552±13 <sup>a</sup>	793±15 <sup>c</sup>	648±15 <sup>b</sup>	639±15 <sup>b</sup>	386±15 <sup>a</sup>
<i>Dactylis glomerata</i>	7	7.9±0.5	38.0±1.6	55.0±1.9	637±15 <sup>a</sup>	785±15 <sup>c</sup>	729±15 <sup>b</sup>	608±15 <sup>b</sup>	494±15 <sup>a</sup>
<i>Festuca occidentalis</i>	7	4.3±0.5	48.5±1.6	71.0±1.9	425±15 <sup>a</sup>	526±15 <sup>c</sup>	495±15 <sup>b</sup>	331±15 <sup>b</sup>	277±15 <sup>a</sup>
<b>Forbs</b>									
<i>Achillea millefolium</i>	4								
<i>lanulosa</i>		9.2±0.7	35.3±2.1	38.3±2.6	695±18 <sup>a</sup>	805±20 <sup>c</sup>	749±18 <sup>b</sup>	483±20 <sup>b</sup>	333±18 <sup>a</sup>
<i>Epilobium paniculatum</i>	5	9.1±0.6	34.8±1.9	45.6±2.3	509±18 <sup>a</sup>	699±18 <sup>c</sup>	664±18 <sup>c</sup>	330±18 <sup>b</sup>	244±18 <sup>a</sup>
<i>Fragaria. species</i>	7	9.3±0.5	17.7±1.6	30.6±1.9	566±15 <sup>a</sup>	909±15 <sup>d</sup>	870±15 <sup>c</sup>	680±15 <sup>b</sup>	546±15 <sup>a</sup>
<i>Hieracium albiflorum</i>	6	7.3±0.6	35.1±1.7	45.2±2.1	640±16 <sup>a</sup>	743±16 <sup>c</sup>	705±16 <sup>b</sup>	423±16 <sup>b</sup>	335±16 <sup>a</sup>
<i>Lupinus sp.</i>	6	10.7±0.6	28.8±1.7	38.8±2.1	752±16 <sup>a</sup>	832±16 <sup>b</sup>	777±16 <sup>a</sup>	574±16 <sup>b</sup>	425±16 <sup>a</sup>
<b>Shrubs</b>									
<i>Berberis repens</i>	6	10.6±0.6	29.5±1.7	39.9±2.1	650±16 <sup>a</sup>	749±16 <sup>bc</sup>	721±16 <sup>b</sup>	372±16 <sup>b</sup>	280±16 <sup>a</sup>
<i>Spiraea betulifolia lucida</i>	8	8.3±0.5	27.8±1.5	36.3±1.8	558±13 <sup>a</sup>	763±14 <sup>c</sup>	747±13 <sup>c</sup>	350±14 <sup>b</sup>	299±13 <sup>a</sup>
<i>Symphoricarpos albus</i>	10	8.1±0.4	26.3±1.3	31.6±1.6	661±12 <sup>a</sup>	799±13 <sup>c</sup>	789±12 <sup>bc</sup>	367±13 <sup>b</sup>	322±12 <sup>a</sup>
<i>Vaccinium</i>	7								
<i>membranaceum</i>		8.8±0.5	27.6±1.6	32.1±1.9	585±14 <sup>a</sup>	775±15 <sup>c</sup>	765±14 <sup>c</sup>	291±15 <sup>a</sup>	247±14 <sup>a</sup>
<b>Lichen</b>									
<i>Bryoria fremontii</i>	5	10.1±0.6	8.7±1.9	36.0±2.3	699±16 <sup>a</sup>	935 ±18 <sup>d</sup>	863±18 <sup>c</sup>	796±18 <sup>b</sup>	591±18 <sup>a</sup>

<sup>a,b,c</sup>Means in the same row for either DM or NDF digestibility with different superscripts differ ( $P < 0.05$ ).

**Table 2;** Chemical composition (% , DM),sheep and steer *in vivo*, *in vitro*, DAISY<sup>II</sup>, and *in situ* DM and NDF digestibility (g/kg, DM basis) of meadow hay.

Animals	Composition			DM Digestibility				NDF Digestibility		
	CP	ADF	NDF	<i>In vivo</i>	<i>In vitro</i>	DAISY <sup>II</sup>	<i>In situ</i>	<i>In vivo</i>	DAISY <sup>II</sup>	<i>In situ</i>
Sheep	5.2	31.4	60.4	508±6 <sup>a</sup>	580±6 <sup>b</sup>	684±6 <sup>c</sup>	656±6 <sup>d</sup>	480±6 <sup>a</sup>	485±6 <sup>a</sup>	474±6 <sup>a</sup>
Steer	5.3	30.3	59.0	492±6 <sup>a</sup>	586±6 <sup>b</sup>	694±6 <sup>c</sup>	650±6 <sup>d</sup>	413±6 <sup>a</sup>	481±6 <sup>b</sup>	435±7 <sup>c</sup>
<i>P</i> <sup>1</sup>	0.46	0.27	0.15	0.05	0.49	0.25	0.45	<0.01	0.65	<0.01

<sup>a,b,c,d</sup>Means in the same row for either DM or NDF digestibility with different superscripts differ (*P*<0.05).

<sup>1</sup> Probability of F-test contrasts sheep vs steer.

**Table 3;** Chemical composition (% , DM), *in vivo*, *in vitro*, DAISY<sup>II</sup>, and *in situ*, DM and NDF digestibility (g/kg) of forages of two harvested forages and five clipped samples milled at two different sizes.

Forages	Size <sup>2</sup>	Composition			DM Digestibility				NDF Digestibility		
		CP	ADF	NDF	<i>In vivo</i>	<i>In vitro</i>	DAISY <sup>II</sup>	<i>In situ</i>	<i>In vivo</i>	DAISY <sup>II</sup>	<i>In situ</i>
Grass hay	1	8.6	34.5	58.3	624±7 <sup>a</sup>	533±7 <sup>b</sup>	708±7 <sup>c</sup>	728±7 <sup>d</sup>	562±7 <sup>a</sup>	519±8 <sup>b</sup>	521±7 <sup>b</sup>
	2		35.6	61.5	624±7 <sup>a</sup>	519±7 <sup>b</sup>	667±7 <sup>c</sup>	698±7 <sup>d</sup>	581±7 <sup>a</sup>	479±8 <sup>b</sup>	498±7 <sup>b</sup>
<i>P</i> <sup>1</sup>					0.973	0.148	<0.001	0.003	0.059	0.001	0.019
Grass straw	1	3.6	50.5	80.4	500±7 <sup>a</sup>	342±7 <sup>b</sup>	425±7 <sup>c</sup>	433±7 <sup>c</sup>	572±7 <sup>a</sup>	295±8 <sup>b</sup>	304±7 <sup>b</sup>
	2		51.5	81.4	501±7 <sup>a</sup>	306±7 <sup>b</sup>	384±7 <sup>c</sup>	385±7 <sup>c</sup>	577±7 <sup>a</sup>	269±9 <sup>b</sup>	246±7 <sup>c</sup>
<i>P</i> <sup>1</sup>					0.863	0.002	<0.001	<0.001	0.610	0.021	<0.001
<i>A. millefolium lanulosa</i>	1	9.9	43.9	50.5		532±8 <sup>a</sup>	639±8 <sup>b</sup>	624±8 <sup>b</sup>		285±8 <sup>a</sup>	261±9 <sup>b</sup>
	2		43.2	48.7		546±8 <sup>a</sup>	628±8 <sup>b</sup>	641±8 <sup>c</sup>		279±11 <sup>a</sup>	274±9 <sup>a</sup>
<i>P</i> <sup>1</sup>						0.241	0.329	0.134		0.680	0.293
<i>Fragaria</i> species	1	9.1	24.6	28.6		590±8 <sup>a</sup>	779±8 <sup>b</sup>	816±8 <sup>c</sup>		305±10 <sup>a</sup>	388±9 <sup>b</sup>
	2		25.0	28.6		541±8 <sup>a</sup>	761±8 <sup>b</sup>	811±8 <sup>c</sup>		200±10 <sup>a</sup>	356±9 <sup>b</sup>
<i>P</i> <sup>1</sup>						<0.001	0.118	0.707		<0.001	0.007
<i>Alnus rubra</i>	1	10.3	26.9	36.4		547±8 <sup>a</sup>	827±9 <sup>b</sup>	821±8 <sup>b</sup>		529±9 <sup>a</sup>	486±8 <sup>b</sup>
	2		23.1	28.8		547±8 <sup>a</sup>	837±9 <sup>b</sup>	839±8 <sup>b</sup>		439±9 <sup>a</sup>	429±10 <sup>b</sup>
<i>P</i> <sup>1</sup>						0.987	0.403	0.109		<0.001	<0.001
<i>Salix rigida</i>	1	7.5	30.8	25.6		616±8 <sup>a</sup>	865±9 <sup>b</sup>	824±8 <sup>c</sup>		479±9 <sup>a</sup>	306±12 <sup>b</sup>
	2		29.8	24.3		612±8 <sup>a</sup>	858±9 <sup>b</sup>	825±8 <sup>c</sup>		426±9 <sup>a</sup>	326±11 <sup>b</sup>
<i>P</i> <sup>1</sup>						0.731	0.572	0.917		<0.001	0.222
<i>Bryoria fremontii</i>	1	13.8	11.4	31.9		666±8 <sup>a</sup>	852±8 <sup>b</sup>	866±8 <sup>b</sup>		563±9 <sup>a</sup>	591±9 <sup>b</sup>
	2		11.3	33.7		679±8 <sup>a</sup>	867±8 <sup>b</sup>	878±8 <sup>b</sup>		607±8 <sup>a</sup>	638±8 <sup>b</sup>
<i>P</i> <sup>1</sup>						0.274	0.192	0.333		0.003	<0.001

<sup>a,b,c,d</sup>Means in the same row for either DM or NDF digestibility with different superscripts differ (*P*<0.05).

<sup>1</sup>Probability of F-test contrasts sample grinding size 1 mm vs 2 mm in the same forage species.

<sup>2</sup>Milling diameter, 1= 1 mm, 2= 2 mm.

**Table 4;** Comparison of DAISY<sup>II</sup>, and *in situ* DM and NDF digestibility estimates of meadow hay using two different samples sizes to *in vivo* digestibility.

Forages	Sample size, g	DM Digestibility			NDF Digestibility		
		<i>In vivo</i>	DAISY <sup>II</sup>	<i>In situ</i>	<i>In vivo</i>	DAISY <sup>II</sup>	<i>In situ</i>
Meadow hay	0.25	467	692±6 <sup>a</sup>	700±6 <sup>a</sup>	426	502±6 <sup>a</sup>	516±6 <sup>a</sup>
	0.5		635±6 <sup>a</sup>	651±6 <sup>a</sup>		420±7 <sup>a</sup>	458±8 <sup>b</sup>
<i>P</i> <sup>1</sup>			<0.001	<0.001		<0.001	<0.001
<i>Salix rigida</i>	0.25		867±6 <sup>a</sup>	844±6 <sup>b</sup>		500±6 <sup>a</sup>	429±8 <sup>b</sup>
	0.5		815±6 <sup>a</sup>	830±6 <sup>a</sup>		370±9 <sup>a</sup>	383±8 <sup>a</sup>
<i>P</i> <sup>1</sup>			<0.001	0.112		<0.001	<0.001

<sup>1</sup>Probability of F-test contrasts 0.25 vs 0.5 g sample size in the same species forages