

## Measurement error in the estimation of intake from herbage patches by non-fistulated heifers on apparently uniform swards

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**Summary** — We examined a field-based micro-sward methodology to study the plant-animal interface, using non-fistulated animals grazing small, carefully prepared patches of herbage. The study focused on the error introduced by the indirect estimation of herbage intake from a patch. In each of two experiments, 102 patches (height 0.3 m, area 0.5 m<sup>2</sup>), each with an adjacent estimation area, were created in apparently uniform areas of a sown alfalfa sward. Of these, 72 patches were grazed (30 bites) individually by eight heifers, and 30 were used as a calibration set. Dry matter intake from a grazed patch was determined by the difference method whereby residual herbage mass was measured directly by clipping, and initial herbage mass was based on the mass of herbage clipped in the estimation area. Four methods were used to compute initial herbage mass of a patch; two incorporated information from the calibration patches in which both the patch and the estimation area were clipped. Measurement error was determined by applying the four computational methods to the calibration sets. The calibration set enabled us to detect and correct for a significant estimation bias. This resulted in very different bite weight estimates from those that would have been obtained otherwise. Measurement error was lowest for estimations of initial patch biomass based on a regression equation derived for the calibration sets. Measurement error estimated from the calibration sets was of similar order of magnitude to the total error (within-animal) variance component of the grazing trials.

**intake / patch / estimation / cattle**

**Résumé** — Erreur dans la mesure de l'ingéré par des génisses non-fistulées sur des « carrés » d'herbe apparemment uniforme. L'utilisation de microparcelles d'herbe est d'un développement

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récent dans l'étude du comportement alimentaire de ruminants au pâturage et de leur ingestion. Dans ces études, on réduit l'échelle d'observation temporelle et spatiale en utilisant des carrés d'herbe (*patches*), ce qui permet d'observer de façon très précise le processus d'ingestion. Pour étudier l'interface végétal-animal, nous avons testé une méthodologie basée sur des petits carrés d'herbe à champ ouvert, préparés très soigneusement, et broutés par des animaux non-fistulés. Le but de l'étude était de connaître l'erreur introduite par l'estimation indirecte de l'ingéré d'herbe. Dans chacun des deux essais, 102 carrés apparemment uniformes de luzerne (hauteur 0,3 m, surface 0,5 m<sup>2</sup>, densité 825 g MS m<sup>-3</sup>) ont été construits, chacun ayant une surface d'estimation-témoin adjacente. Parmi ces 102 carrés, 72 ont été broutés (30 coups de dent) individuellement par huit génisses, et les 30 autres ont été utilisés pour la calibration. La quantité de matière sèche (MS) ingérée d'un carré brouté a été estimée comme la différence entre la masse d'herbe résiduelle mesurée par coupe directe après broutage et celle de la surface témoin adjacente. Pour la calibration, le carré essai et son témoin adjacent ont été coupés. Quatre méthodes ont été utilisées pour déterminer la masse initiale d'herbe des carrés, dont deux incluant des informations issues des carrés de calibration. L'erreur de mesure a été déterminée par l'application des quatre méthodes de calcul au système de calibration en calculant des quadratiques moyens. Les composantes de la variance ont été déterminées par analyse de la variance de l'ingéré. Sans l'information fournie par la calibration, le poids d'un coup de dent était 1,1 g MS. Avec cette information, le poids d'un coup de dent a été évalué à 0,55 g MS. La cause essentielle de cette différence est la sous-estimation de la masse d'herbe du carré initial. Le système de calibration nous a permis de détecter et corriger ce biais. Une méthode de régression linéaire simple a entraîné la plus petite erreur d'estimation du quadrat central. Les composantes de la variance résiduelle (intra-animal) de l'ingéré étaient du même ordre de grandeur que l'écart quadratique moyen obtenu par régression. Nos estimations de la variance intra-animale sont donc des surestimations, et celles de la variance interanimale ne sont biaisées automatiquement dans aucune direction. Il est donc important d'inclure un système de calibration dans le protocole expérimental pour estimer « par différence » le poids d'herbe ingérée d'un carré : cela permet de détecter des biais de mesure et d'établir des coefficients de correction. Cela a également permis d'agrandir le choix des méthodes de calcul de la somme des carrés des écarts. Nous en concluons que les études sur carrés d'herbe à champ ouvert basées sur la méthode « des différences » ne sont pas assez exactes pour estimer la variance intra- et interanimale des coups de dents. Cependant, cette méthodologie permet d'estimer de façon significative l'ingéré moyen et le poids des coups de dent et peut être utilisée quand des effets importants des traitements expérimentaux sont attendus.

#### **ingestion / microparcelle / estimation / bovin**

## **INTRODUCTION**

The accurate determination of herbage intake by a grazing animal has always been a methodological challenge in studies of the plant-animal interface. The only direct method of measurement is by use of an oesophageal fistula (Van Dyne and Torell, 1964). This method can determine intake over a short collection period with negligible error. However, fistulation is not always a feasible option, not least because of animal welfare regulations currently in effect in a number of countries.

An innovative approach to the study of the plant-animal interface has been to reduce

the spatial and temporal scale of observation by using single patches of herbage, or micro-swards. The pioneering study of Black and Kenney (1984) presented sheep with boards that had plant units threaded through an array of regularly-spaced holes. The methodology was developed further and adapted for use with cattle by Laca et al (1992). One of the advantages of hand-constructed micro-swards is the ability to achieve a high degree of uniformity in the horizontal plane. As a result, the dry matter (DM) difference method can yield an accurate estimate of intake from a micro-sward. This method estimates pre-grazing DM herbage mass by clipping an area of

micro-sward not offered to the animal, and measures directly the post-grazing DM herbage mass by clipping the entire micro-sward. In the hand-constructed micro-sward methodology of Laca et al (1992), the DM difference method yielded a more accurate estimate of intake than did the fresh weight difference method in which the entire micro-sward assembly was weighed before and after grazing with correction for moisture loss and DM content.

The hand-constructed micro-sward methodology may be unmatched in terms of spatial uniformity, but it does have potential drawbacks and is exceedingly labor intensive. Other methodologies have used micro-swards sown in trays (Illius et al, 1992), turves cut from the field (Hughes et al, 1991; Newman et al, 1992), and micro-swards created directly in the field (Burlison et al, 1991). Creating micro-swards in the field has the advantage of providing the most natural foraging arena of all micro-sward methodologies, and may be the only feasible option when larger scales involving animal movement and multi-patch arenas are being used. (Note that in multi-patch arenas fistulation can not provide direct measurement of intake on a patch by patch basis.) Since some compromise in horizontal uniformity is inevitable at the field scale, measurement error in the estimation of herbage intake from a patch will place limits on the sensitivity of such studies.

We examined the problem of measurement error in two field-based micro-sward experiments. Sown swards under intensive management were used, within which apparently uniform areas were selected carefully for the creation of patches and the estimation of initial herbage mass. In order to test this methodology rigorously, the experiments minimized sources of variation by repeatedly grazing patches of uniform structure. Thus the analysis of variance focused on the within- and between-animal variation in bite weight.

## MATERIALS AND METHODS

### Field preparation

Field experiments were conducted at The Volcani Center, Bet Dagan, Israel, on an area of 2.1 ha situated near the central dairy unit. The field was sown in November 1994 to barley, alfalfa and annual ryegrass in three strips. All field preparations aimed to achieve the greatest possible uniformity.

The field was divided by electric fence across the three crops into an experiment section of 1.4 ha, and a smaller area of 0.7 ha on which the animals grazed as a group. Patches for both experiments were constructed on a 15 × 60-m hand-weeded strip of alfalfa in the experiment section of the field. This area was cut and irrigated before each patch experiment.

### Animals

Eight Israeli-Holstein heifers from the dairy herd of The Volcani Center, Bet Dagan, of average age 420 ( $\pm 10$ ) days, were selected without prior inspection and housed as a group near the experimental field on 1 February 1995.

The animals were trained to be approached, haltered and led by rope. From 28 February the animals grazed the electrically-fenced section of the field for 1–2 h daily. The animals were accustomed to being led away from the group to a separate area, where they would graze a single patch of herbage, and then returned to the group.

Grazing was usually during the period 08.00–10.00 h. On return from pasture the heifers received *ad libitum* the standard total mixed ration supplied by the dairy unit. Feed was available at the feeding gates the next morning before the animals were let out to graze. Thus hunger was not induced prior to grazing.

Mean live weight ( $\pm$  SD) of the heifers increased linearly from 379  $\pm$  15 kg on 20 March 1995 to 482  $\pm$  15 kg on 11 July 1995. Average date of first conception by artificial insemination (AI) for the group was 14 February 1995.

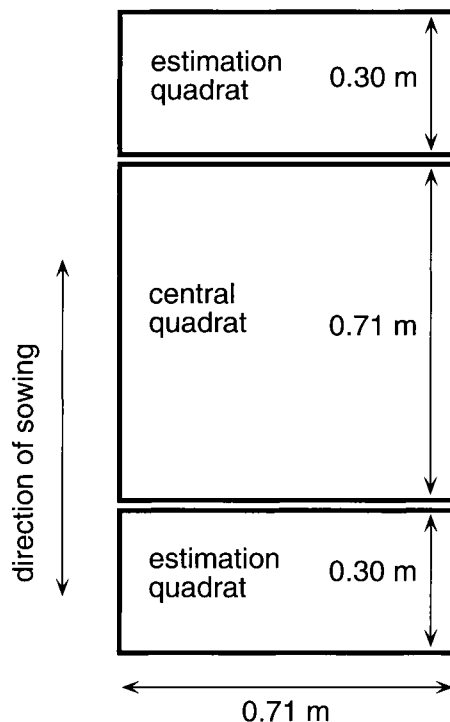
## Experimental protocol

The experiment estimated the mass of herbage removed from a patch of alfalfa in the course of 30 bites. This estimation was conducted three times per day for each of eight heifers on three non-consecutive days, giving a total of 72 patches for grazing. The experiment was conducted twice. Patches were grazed on 23, 29 May and 1 June in experiment 1, and on 4, 6 and 10 July in experiment 2. Patches were always prepared the day before they were grazed. Animals grazed the electrically-fenced section of the field for 1–2 h daily on the intermediate days of the experimental periods and during the period between the two experiments.

To create a patch, an area of approximately  $1 \times 1.5$  m was visually selected for horizontal uniformity of cover, and height slightly greater than 0.30 m. A square 'central quadrat' (the patch to be grazed) of internal area  $0.5 \text{ m}^2$  ( $0.71 \times 0.71$  m) was placed on the ground with minimum disturbance of the canopy, in the middle of the selected area. Two 'estimation quadrats', of internal dimensions  $0.71 \times 0.30$  m each, were used for each patch. These were placed adjacent to two opposite sides of the central quadrat, across the same drill lines as those in the central quadrat (fig 1). The total area used for estimation was split this way to allow for any non-apparent gradients in herbage density across the central quadrat. Quadrats were precisely welded using 12 mm construction iron.

Herbage around the central and estimation quadrats was removed to ground level. Herbage in the quadrats was clipped to a uniform height of 0.30 m. Herbage in the estimation quadrats was then clipped at ground level for drying and weighing. Flat-profile high-grade shears were used throughout, with due care to minimize operator bias and soil contamination.

The following day, at about 08.00 h, the eight heifers were led into the fenced section of the field. The first heifer in a randomized sequence was immediately separated from the group, led through a gate to the experimental area, and allowed to graze a patch. The target bite number was 30, though animals occasionally stopped grazing earlier. The characteristic tearing sound generated by the severance of a bite was the basis for counting bites. The actual bite number was counted and the grazing session was recorded on video. The heifer was then returned to the group to graze, and the next heifer in the cycle



**Fig 1.** Dimensions and spatial arrangement of quadrats used in the construction of grazed and calibration patches. Surrounding herbage was removed to ground level. For grazed patches, estimation quadrats were clipped, the central quadrat was grazed (30 bites) and its residual herbage was clipped. For calibration patches, both estimation and central quadrats were clipped.

was separated and led to a fresh patch. Three cycles of eight heifers were completed on a grazing day, with the heifer sequence re-randomized each cycle. Average time requirement was 45 min per cycle of eight heifers.

The heifers were returned to the animal shed at the end of the three cycles of patch grazing. The residual herbage on each grazed patch was then clipped at ground level and bagged for drying.

An additional set of 30 'calibration' patches was prepared in the same way as the set of grazed patches, ie, central plus two estimation quadrats. The calibration patches were interspersed randomly among the grazed patches and their preparation was spread over the same experiment days.

Both the estimation quadrats and the entire central quadrat of the calibration patches were clipped at ground level and bagged separately for drying. Even though every effort was made in the methodology to ensure that the calibration and grazed patches were identical, they are nevertheless independent data sets from a statistical point of view.

All clipped herbage was dried at 60°C for at least 3 days and weighed. In experiment 2, each patch was sampled for DM content determination immediately before grazing, and residual herbage height was measured at three grazed points in the patch immediately after grazing.

### Analysis of calibration patch data

Four methods of estimating the initial mass of a patch were compared. In all methods, herbage mass of the estimation quadrats was multiplied by a factor of 71/60, which is the ratio of central:estimation quadrat area, prior to the estimation procedure.

We define:

- $C_c$  measured herbage mass of central quadrat of area  $0.71 \times 0.71$  m of the  $c$ -th patch of the calibration set of patches, in grams DM;
- $\tilde{C}_c$  predicted herbage mass of central quadrat of the  $c$ -th patch of the calibration set of patches, in grams DM;
- $E_c$  measured herbage mass of estimation quadrats of total area  $0.60 \times 0.71$  m, multiplied by 71/60, of the  $c$ -th patch of the calibration set of patches, in grams DM;
- $n_{cal}$  number of patches in the calibration set for the entire experiment;
- $b_{C,E}$  regression coefficient of  $C$  on  $E$ ;
- $a$   $Y$ -intercept of above regression

In estimation method 1 (EM1)  $\tilde{C}_c$  was set equal to the herbage mass of the estimation quadrats matched to the same patch ('match estimate'). Thus:

$$\tilde{C}_c = E_c$$

In EM2  $\tilde{C}_c$  was set equal to the mean of all estimation quadrats in the experiment ('estimate by mean').

$$\tilde{C}_c = \sum E_c / n_{cal}$$

In EM3  $\tilde{C}_c$  was predicted by the least squares regression equation of the true mass of the cen-

tral quadrat on the mass of the estimation quadrats ('regression estimate'). We did not use bivariate analysis (model II regression) since we wish to use the resulting equation predictively (Sokal and Rohlf, 1981).

$$\tilde{C}_c = a + b_{C,E} E_c$$

In EM4 the herbage mass of the estimation quadrats of a patch was multiplied by the ratio of mean central quadrat mass to mean estimation quadrat mass ('ratio estimate').

$$\tilde{C}_c = E_c \sum C_c / \sum E_c$$

The sum of squared deviations from the true mass of the central quadrat was computed for each method, and the mean squares compared. In principle, the method with the lowest mean square is expected to introduce the minimum measurement error. For the regression estimate (EM3), the sum of squared deviations is the residual sum of squares of the regression analysis.

### Analysis of grazed patch data

Intake was computed as the initial minus the terminal herbage mass on a  $0.5 \text{ m}^2$  patch. The predicted initial mass of each grazed patch ( $\tilde{C}_g$ ) was estimated using the four methods described above, but based on the measured herbage mass of the estimation quadrats of the grazed patches ( $E_g$ ):

$$\tilde{C}_g = E_g \quad (\text{EM1})$$

$$\tilde{C}_g = \sum E_g / n_{grz} \quad (\text{EM2})$$

$$\tilde{C}_g = a + b_{C,E} E_g \quad (\text{EM3})$$

$$\tilde{C}_g = E_g \sum C_c / \sum E_c \quad (\text{EM4})$$

where  $n_{grz}$  is the number of patches grazed for the entire experiment. Note that EM1 and EM2 are independent of the calibration set.

In experiment 2, the herbage removed from the patch for DM determination was subtracted from initial mass estimates for intake determination. Bite weight was defined as intake divided by number of bites removed from the same patch. The video record was used to identify grazing sessions in which all 30 bites were removed in a single uninterrupted bout. Biting rate was computed for these sessions only.

Variance components were derived from the analysis of variance of intake. Categorical variables examined were animal (1-8), day of experiment (1,2,3), within-day cycle (1,2,3), and the interaction between animal and within-day cycle. Animal and day of experiment were defined as

random variates. Animals did not always remove 30 bites and for this reason bite number was included as a covariate in the analyses of variance.

The experimental technique controls only the dimensions of a patch but not the bulk density of herbage in it. For the purposes of establishing variance components, it is necessary to include bulk density in the analysis of variance, since it is a primary determinant of bite weight (Laca et al, 1992). Bulk density is equal to the initial estimated biomass of a patch divided by patch volume. Since patch volume is a constant, the initial estimated patch biomass was used as a covariate in the analysis, instead of bulk density. This is legitimate even though the initial estimated patch biomass is used in the calculation of the dependent variable (intake or bite weight). The artificial covariance due to the calculations, if present, should be negative, whereas we obtained positive covariances. This indicates that the association due to bulk density derived from the mechanics of grazing. Other covariates examined were number of bites removed from the patch, time of grazing, and DM content of the herbage (experiment 2).

## RESULTS

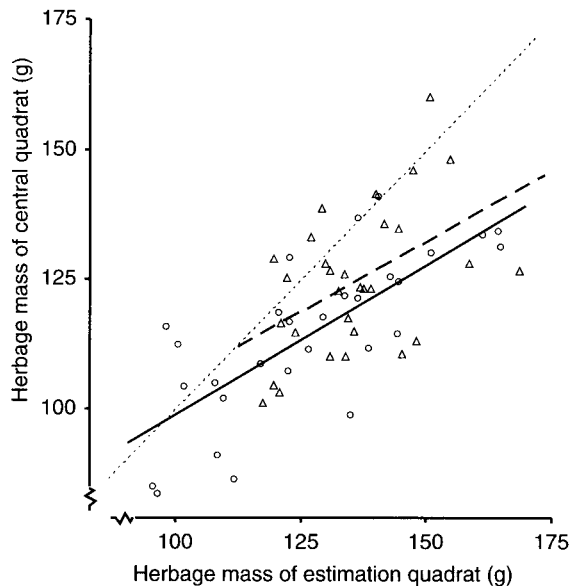
To avoid confusion and the erroneous scaling of variances, all results are reported on a per

0.5 m<sup>2</sup> basis, the area of the central quadrat. All herbage mass values are on a DM basis.

### Calibration patches

Mean herbage mass ( $\pm$  SD) of the central and estimation quadrats of the calibration patches in experiment 1 was 114.4 ( $\pm$  15.7) and 127.0 ( $\pm$  20.3) g, respectively. Corresponding values for experiment 2 were 124.5 ( $\pm$  13.9) and 135.8 ( $\pm$  12.5) g, respectively. The mean differences between estimation and central quadrats for the two experiments were both highly significant using paired *t*-tests. Thus the match estimate (EM1) and the estimate by mean (EM2) are biased and over-estimate the initial mass of the central quadrats.

Figure 2 shows the relationship between herbage mass of the central and estimation quadrats. In both experiments, slopes of the regression lines were significantly different from zero. Note that only about one-fifth of the points lie above the line  $y = x$  of 45° slope. Mean predicted central quadrat mass must be equal to the mean of the estimation



**Fig 2.** Measured herbage mass (DM) of the central quadrat versus that of paired estimation quadrats in the calibration studies of experiment 1 ( $\circ$  = data, — = regression) and experiment 2 ( $\Delta$  = data, - - - = regression).  $x = y$  shown as - - - - -. Regression coefficients for experiment 1: 40.8 (intercept) and 0.58 (slope). Corresponding values for experiment 2: 50.9 and 0.54, respectively.

quadrats for EM1 and EM2, and to the mean of the central quadrats for EM3 and EM4. Table I compares the calibration mean square of error (MSE) introduced by each estimation method in the estimation of the herbage mass of the central quadrat. For EM1 and EM2 the MSE consists of the squared bias plus the calibration variance. The regression method yielded the lowest measurement error.

### Grazed patches

Both mean herbage mass in the estimation quadrats of the grazed patches and mean residual mass in the grazed patch were

**Table I.** Residual mean square (RMS) of herbage mass DM of the central quadrat of the calibration patches obtained by four methods of estimation, in experiment 1 ( $n = 29$ ) and experiment 2 ( $n = 30$ ).

Estimation method	df	RMS	
		Experiment 1	Experiment 2
EM1: match estimate	$n$	334	302
EM2: estimate by mean	$n$	398	315
EM3: regression estimate	$n-2$	113	152
EM4: ratio estimate	$n-1$	151	169

Units are  $g^2$ . RMS is based on herbage mass per  $0.5 m^2$  for central and estimation quadrats.

**Table II.** Intake and bite weight in experiment 1 and experiment 2 using four estimation methods for initial herbage mass of the grazed patch ( $\tilde{C}_g$ ).

Estimation method	Variable	Experiment 1		Experiment 2	
		Mean	SD	Mean	SD
EM1	$E_g$	137.7	18.3	139.9	15.5
	$C_{residual}$	106.0	16.9	108.6	15.5
	Bites	28.3	4.8	29.6	1.7
	$\tilde{C}_g$	137.7	18.3	139.9	15.5
	Intake	31.6	15.3	31.3	13.8
EM2	Bite weight	1.16	0.59	1.07	0.49
	$\tilde{C}_g$	137.7	–	139.9	–
	Intake	31.6	16.9	31.3	15.5
EM3	Bite weight	1.16	0.62	1.07	0.53
	$\tilde{C}_g$	120.6	10.6	126.7	8.4
	Intake	14.6	13.2	18.1	12.4
EM4	Bite weight	0.52	0.46	0.62	0.43
	$\tilde{C}_g$	124.0	16.5	128.2	14.2
	Intake	18.0	14.5	19.7	13.3
	Bite weight	0.65	0.51	0.67	0.46

EM1, match estimate; EM2, estimate by mean; EM3, regression estimate; EM4, ratio estimate. Standard deviations are based on  $n = 72$  (experiment 1) and  $n = 71$  (experiment 2). All herbage mass values are grams DM.  $E_g$  is measured herbage mass of the estimation quadrats of the grazed patches.  $C_{residual}$  is measured residual herbage mass after grazing.  $E_g$ ,  $\tilde{C}_g$  and  $C_{residual}$  are on  $0.5 m^2$  basis.

remarkably similar for the two experiments (table II). Hence, for methods that estimate the initial mass of the grazed patch solely from the estimation quadrat mass (EM1 and EM2), the intake estimates are similar in experiment 1 and experiment 2. Methods EM1 and EM2 yield higher estimates of the initial mass of the grazed patch than do methods EM3 and EM4. Since other values in the computation of intake are the same, methods EM1 and EM2 yield higher estimates of intake as well. However, because of the relatively large residual mass that is being deducted, the effect of the estimation method on intake is amplified.

Table III shows the analysis of variance model selected for both experiments, using estimation method 3. The animal  $\times$  within-day cycle interaction was dropped from the model since it was not significant in experiment 1 and resulted in negative variance component estimates in experiment 2. We

were unable to detect any significant between-animal variation in either experiment. Day of experiment proved highly significant in both experiments, whereas within-day cycle was not significant. Bite number was significant in experiment 1, where its variability was greater. Initial herbage mass was significant in experiment 1 only, where it was also slightly more variable.

Dry matter content of the grazed herbage in experiment 2 increased with day of experiment but not with time within-day. Thus there was no advantage to using this variable in the model over day of experiment. Similarly, time of grazing did not improve the explanation of variance over within-day cycle.

The variance component estimates are given in table IV for both experiments. Using the method recommended by Sokal and Rohlf (1981), the 95% confidence inter-

**Table III.** Analysis of variance of intake and bite weight of a heifer from a 0.5 m<sup>2</sup> patch of alfalfa in the course of ca 30 bites in experiment 1 and experiment 2. Initial herbage mass of the grazed patch estimated by regression (EM3). Each factor in the model is adjusted for all other factors ('type III').

Source	Experiment 1			Experiment 2		
	Mean square	df	P	Mean square	df	P
<i>Intake</i>						
Animal	200	7	0.1764	153	7	0.3137
Day of experiment	1229	2	0.0003	1060	2	0.0007
Within-day cycle	285	2	0.1228	58	2	0.6346
Number of bites	680	1	0.0265	< 1	1	0.9649
Initial mass	694	1	0.0250	65	1	0.4789
Error	131	58	–	127	57	–
<i>Bite weight</i>						
Animal	0.238	7	0.2132	0.188	7	0.2752
Day of experiment	1.401	2	0.0006	1.239	2	0.0006
Within-day cycle	0.311	2	0.1640	0.076	2	0.5978
Number of bites	0.133	1	0.3763	0.241	1	0.2048
Initial mass	0.771	1	0.0358	0.079	1	0.4658
Error	0.167	58	–	0.146	57	–



**Table IV.** Intake and bite weight variance component estimates for animal, day of experiment and residual (within-animal), based on expected mean squares, in experiment 1 and experiment 2.

Component	Experiment 1		Experiment 2	
	$g^2$	%	$g^2$	%
<i>Intake</i>				
Animal	7.9	3.8	3.0	1.7
Day of experiment	68.4	33.0	41.5	24.2
Residual	131.1	63.2	127.0	74.1
<i>Bite weight</i>				
Animal	0.0081	3.2	0.0047	2.4
Day of experiment	0.0769	30.5	0.0486	24.3
Residual	0.1670	66.3	0.1464	73.3

val of the residual (within-animal) variance for intake is  $L_1 = 94$  and  $L_2 = 196$  for experiment 1, and  $L_1 = 91$  and  $L_2 = 190$  for experiment 2, where  $L_1$  and  $L_2$  are the lower and upper bounds. For the between-animal variance, the limits are:  $L_1 = 0$  and  $L_2 = 78$  for experiment 1, and  $L_1 = 0$  and  $L_2 = 57$  for experiment 2. Confidence limits for the estimate of the variance due to measurement error (for EM3, table I) are:  $L_1 = 71$  and  $L_2 = 209$  for experiment 1, and  $L_1 = 96$  and  $L_2 = 278$  for experiment 2.

The residual (within-animal) variance for intake consists of the calibration error introduced into the initial mass estimation and the random within-animal between-patches deviation. However, the residual (within-animal) variance component estimates for intake were of similar magnitude to the residual mean squares obtained for estimation method 3 (table I). This means that the noise introduced by measurement error was large relative to the variability in foraging parameters. Thus our estimates of within-animal variance are over-estimates, whereas those of between-animal variance are not necessarily changed in any one direction. Confidence limits (95%) for overall mean bite weight (by EM3) are:  $L_1 = 0.41$  and

$L_2 = 0.63$  for experiment 1, and  $L_1 = 0.52$  and  $L_2 = 0.72$  for experiment 2.

### Biting rate

An analysis of biting rate of uninterrupted grazing sessions only, yielded a significant animal variance component in both experiments. Average biting rate was 39 bites  $\text{min}^{-1}$  in experiment 1. Percent of variation between and within animals was 26 and 74, respectively. Coefficient of variation (CV;  $100 \times \sqrt{\text{variance component estimate}} \div \text{overall mean}$ ) for between- and within-animal variation was 13% and 23%, respectively. In experiment 2, average biting rate was 45 bites  $\text{min}^{-1}$ , and percent of variation between and within animals was 46 and 54, respectively. CV for between- and within-animal variation was 22% and 23%. Day of experiment was not significant for biting rate.

### DISCUSSION

In the two experiments reported here, heifers (approximately 450 kg live weight) grazed 30 bites from a patch of alfalfa of area

0.5 m<sup>2</sup>, height 0.30 m and bulk density ca 825 g DM m<sup>-3</sup>. In the absence of the calibration patches, these experiments would have estimated bite weight at ca 1.1 g DM, since one could use only EM1 or EM2 to compute the initial mass of the grazed patches. When information based on a statistically independent set of patches was used, a lower estimate of ca 0.55 g DM was obtained. The results are quite similar for EM3 and EM4, suggesting that the primary factor at play here is apparent over-estimation (ie, mean estimation mass > mean central mass) and the correction for that in the form of the ratio estimate (EM4) or by regression (EM3).

Given that the regression method yielded the lowest residual mean square, the large scatter and the strong deviation from the 1:1 line seen in figure 2 may be unexpected. However, in the context of a relatively small area of land growing a crop of uniform appearance, the degree of relatedness between adjacent spots will depend on the spatial pattern of heterogeneity over that area. If the spatial scale of change is very small, there need not be any relationship at all. This would yield an intercept equal to the mean and slope zero. The best estimate is then obtained using the mean (ie, EM2). As the degree of spatial relatedness at the 0.5–1.0 m scale increases, so the intercept will depart from the average, and the slope will increase so as to add a fraction of the estimate (EM3). By extension, complete relatedness between adjacent areas (with no bias) is equivalent to an intercept of zero and slope unity, which is EM1.

The statistical analyses and variance component estimates were not sensitive to estimation method. However, even our lowest estimate of measurement error was high in relation to the variances being determined. Thus it is important to establish the limits to uniformity that can be attained under field conditions. In December 1995 we conducted a calibration study on an area of ryegrass

sown at high seed density in the experimental field. Crop height exceeded 0.25 m 49 days after sowing, and 30 calibration patches were clipped at a height of 0.25 m over the next few days. Mean herbage mass DM (0.5 m<sup>2</sup> basis) for the estimation quadrats was 109 g (range 92–130) and for the central quadrats, 105 g (range 81–121). Mean squares for estimation error by the four methods were: EM1, 153; EM2, 117; EM3, 98; EM4, 135. Direct estimation from the adjacent area (EM1) introduced the highest measurement error. Regression yielded the lowest error, although the slope was no longer significantly different from zero. Thus we did achieve a higher level of uniformity in the field. We nevertheless did not obtain a large reduction in the absolute error level introduced by estimation compared with the values given in table I.

Despite the measurement error, some effects were found to be statistically significant. In experiment 1, bulk density (represented by initial patch mass in our case) was significant in the analysis of intake and bite weight. Bite number was significant in the analysis of intake but not in that of bite weight. This is consistent with minimal feedback of patch depression on bite weight (Laca et al, 1994) over the first 30 bites, and with the top grazing horizon not being grazed completely. Within-day cycle was not significant in either experiment. This is in contrast to the large effect of time at pasture on bite weight reported by Dougherty et al (1987, 1989). In those studies, however, hunger was induced by overnight fasting prior to grazing. Hunger was not induced in the present study. It is also noted that within-day cycle was not significant in the analysis of biting rate in either experiment.

The two experiments yielded surprisingly similar variance component estimates, given the very large noise supposedly added by the estimation problem. The very wide confidence intervals given above would also seem to weigh against obtaining similar val-

ues twice in succession. If these similar estimates are indeed representative of the true values, we might estimate the variance added by measurement error to be ca 100 g<sup>2</sup>. The variance components for between animals, between days and within animals are then ca 5, 50 and 30 g<sup>2</sup> (6%, 61% and 33% of the combined total), respectively. The analysis of biting rate, in which measurement error can be assumed to be negligible, revealed a larger percent of variation for the between-animal source.

## CONCLUSIONS

The inclusion of calibration measurements in the experimental protocol proved important in the estimation of intake from a patch by the difference method. The calibration set detected measurement bias and provided a coefficient for its correction. It expanded the number of alternative computational methods for estimation, and enabled their mean square error to be computed. The mean square error of the selected computational method is then an estimate of the contribution of measurement error to the total mean square error in the analysis of intake.

The mean square error introduced by the estimation of initial patch biomass from adjacent areas depends on the spatial pattern of heterogeneity in the field. It would appear difficult to reduce its value below ca 100 g<sup>2</sup> for an initial patch biomass in the range 100–200 g, and for a total area in the order 100 m<sup>2</sup>.

The sensitivity of intake to estimation method is large at low levels of patch depletion, ie, when the residual patch biomass is high. Under such conditions, field-scale patch-oriented studies that use the difference method have insufficient resolution to provide reliable estimates of the within- and between-animal variances in bite weight. However, the methodology yielded a meaningful estimate of the average intake and bite

weight, and can probably be used where treatment effects are expected to be large. It remains to be seen whether an additional non-destructive variable (eg, capacitance, light penetration) can further reduce measurement error on apparently uniform swards.

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