

**1Monitoring and assessment of ingestive chewing sounds for prediction of herbage
2intake rate in grazing cattle**

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18*Short title: Acoustic monitoring of intake rate in grazing cattle*

19Abstract

20 Accurate measurement of herbage intake rate is critical to advance knowledge of
21the ecology of grazing ruminants. This experiment tested the integration of behavioral and
22acoustic measurements of chewing and biting to estimate herbage dry matter intake
23(DMI) in dairy cows offered micro-swards of contrasting plant structure. Micro-swards
24constructed with plastic pots were offered to three lactating Holstein cows (608 ± 24.9 kg

25of body weight) in individual grazing sessions (N = 48). Treatments were a factorial
26combination of two forage species (alfalfa and fescue) and two plant heights (tall = $25 \pm$
273.8 cm and short = 12 ± 1.9 cm) and were offered on a gradient of increasing herbage
28mass (10 to 30 pots) and number of bites (approximately 10 to 40 bites). During each
29grazing session, sounds of biting and chewing were recorded with a wireless microphone
30placed on the cows' foreheads and a digital video camera to allow synchronized audio
31and video recordings. Dry matter intake rate was higher in tall alfalfa than in the other 3
32treatments (32 ± 1.6 vs. 19 ± 1.2 g/min). A high proportion of jaw movements in every
33grazing session (23 to 36%) were compound jaw movements (chew-bites) that appeared
34to be a key component of chewing and biting efficiency and of the ability of cows to
35regulate intake rate. Dry matter intake was accurately predicted based on easily
36observable behavioral and acoustic variables. Chewing sound energy measured as
37energy flux density (EFD) was linearly related to DMI, with 74% of EFD variation
38explained by DMI. Total chewing EFD, number of chew-bites and plant height (tall vs.
39short) were the most important predictors of DMI. The best model explained 91% of the
40variation in DMI with a coefficient of variation of 17%. Ingestive sounds integrate valuable
41information to remotely monitor feeding behavior and predict DMI in grazing cows.

42

43**Keywords:** Acoustic analysis, Ingestive behavior, Chewing, Chew-bite, Ruminants

44**Implications**

45 Herbage intake of grazing cattle can be estimated easily and accurately enough for
46practical purposes, through concurrent measurements of chewing behavior and sounds.
47Energy flux density of chewing sounds was the best single predictor of the short-term
48herbage intake of dairy cows offered experimental swards. Further validation of the

49present technique is necessary to assess herbage intake in noisy, natural environments
50and over prolonged time periods.

51

52Introduction

53 Most grazing systems seek efficient herbage utilization and animal production by
54practices that are both economically and ecologically sound. Consistent with this goal is
55the need to routinely monitor, assess and manage relationships between grazing
56resources, herbage intake and animal production. Grazing involves nested feeding
57choices within specific domains of time and space (Bailey *et al.*, 1996) and herbage
58intake is the consequence of several underlying trade-offs that directly or indirectly
59influence intake rate (Laca, 2008). Hence, herbage intake rate by livestock is an essential
60quantity for management that necessitates improved measurement techniques.

61 Grazing animals generally prefer the forages they can eat faster (Black and
62Kenney, 1984) and the rate of herbage consumption can vary widely with plant structure
63(i.e. height and bulk density) and coupled chemical and physical attributes of forages,
64such as dry matter content, type and amount of fiber, particle size, and resistance to
65fracture. These characteristics can significantly affect the effort necessary to crop and
66chew a bite, and hence, herbage intake rate (Inoué *et al.*, 1994, Benvenuti *et al.*, 2006,
67Galli *et al.*, 2006).

68 Most models of ruminant intake rate predict feed ingestion as a function of two
69mutually exclusive actions, biting and chewing. So, time per bite is the sum of time
70invested in jaw movements for biting and chewing (Laca and Demment, 1991). This
71assumption implies negligible average search cost per bite, so that any increase in time
72per bite is function of the chewing requirements per bite and hence bite mass (Laca *et al.*,
731994). Consequently, extensive research has been conducted to examine major

74determinants of bite mass, but given past methodological difficulties in measuring jaw
75movements precisely, comparatively less effort was made to quantify variations in time
76per bite and its effects on intake rate.

77 Biting and chewing sounds can reveal important features of the foraging behavior
78of free-ranging (WallisDeVries and Laca, 1998) and stall-fed (Galli *et al.*, 2006) animals.
79Indeed, studies using acoustic methods have found that cattle (Laca *et al.*, 1994, Ungar *et*
80*al.*, 2006), giraffe (Ginnett and Demment, 1995) and sheep (Galli *et. al.*, 2011) use
81discrete jaw movements to chew, bite, or to simultaneously chew and bite on the same
82jaw opening-closing cycle (i.e. chew-bite jaw movement). Acoustic biotelemetry
83successfully discriminated ingestive bites and chews of grazing cattle (Laca and
84WallisDevries, 2000), and has been successfully applied to monitor the timeline and
85extent of eating activity in both grazing sheep (Klein *et al.*, 1994, Galli *et al.*, 2011) and
86cattle (Ungar and Rutter, 2006).

87 Today, acoustic biotelemetry has promising applications as a reliable on-farm
88monitoring system to estimate rumination activity in grazing (Watt *et al.*, 2015) and non-
89grazing (Schirmann *et al.*, 2009) cattle, and there is potential for additional automation of
90the analysis of ingestive sounds to further monitor grazing activity (Milone *et al.*, 2012).
91Moreover, the energy contained in chewing sounds appears to be linearly related to the
92intake and characteristics of grazed forages, suggesting that dry matter intake (DMI)
93could be predicted fairly well based on measurable chewing sound parameters. Laca and
94WallisDeVries (2000), found that intake of steers offered experimental turfs of setaria
95(*Setaria lutescens*) was accurately predicted by the total energy flux of chewing sounds,
96the energy flux of chewing sounds per chew, and the average intensity of chewing sounds
97($R^2= 0.90$; $CV= 17\%$). More recently, Galli *et al.* (2011) found that the best predictors of
98DMI in sheep ($R^2= 0.92$; $CV= 18\%$) grazing experimental micro-swards of orchardgrass

99(*Dactylis glomerata*) and alfalfa (*Medicago sativa*) were the chewing sound energy per
100bite and the total energy flux of chewing sounds, two acoustic variables that provided
101combined information of intake rate and eating time.

102 The present study builds upon previous experimental findings of acoustic
103monitoring and was specifically designed to validate the integration of ingestive sounds
104and behavioral variables for estimation of DMI in dairy cows offered different sets of
105experimental micro-swards of alfalfa and fescue (*Festuca arundinacea*) varying in
106herbage mass. Specific objectives were: (1) to examine likely determinants of bite mass,
107bite rate and intake rate; (2) to examine variation in chewing energy sound flux as a
108function of sward characteristics, herbage mass intake, ingestive behavior and associated
109biting and chewing sound data, and (3) to test predictions of DMI based on behavioral
110and acoustic variables obtained from ingestive sound data.

111**Materials and methods**

112 All feeding trials were performed at the Campo Experimental J. Villarino, Facultad
113de Ciencias Agrarias, Universidad Nacional de Rosario, Argentina (33°01'00" S 60°53'00"
114W). The approach integrated the use of micro-swards of alfalfa and fescue for direct
115measurement of herbage intake, and recording of ingestive sounds. Animal handling and
116experimental procedures were reviewed and approved by the Committee on Ethical Use
117of Animals for Research of the Universidad Nacional de Rosario.

118 *Experimental procedure*

119 Micro-swards were established using alfalfa or fescue sown in 4-liter plastic pots,
120firmly attached with metallic clamps to iron holders bolted to a wooden baseboard (Figure
121). Treatments were a 2 x 2 factorial combination of two plant species (fescue or alfalfa)
122and two plant heights (short or tall) offered in sets of 10, 16, 24, or 30 pots from which an

123animal was allowed to remove 10, 20, 30 or 40 bites. This design allowed a gradient of
124DMI level for which predictive DMI models were developed and tested. Both tall (intact)
125and short (cut to 50% of tall) plants were in a vegetative state (based on Kalu and Fick,
1261981, for alfalfa and Moore *et al.*, 1991, for fescue), and were intentionally manipulated to
127generate micro-swards that cows could eat with negligible displacement (i.e. small
128feeding stations). Potted plants were kept in an outdoor nursery near the experimental
129site and were irrigated and fertilized with urea (a single application with a dose equivalent
130to 50 kg/ha) to ensure adequate growth. Each day, about 80 to 100 alfalfa and fescue
131pots with plants of homogeneous herbage mass and height were selected and
132transported to the experimental barn where grazing sessions took place.

133 Three placid multiparous lactating Holstein cows (608 ± 24.9 kg) previously trained
134to graze micro-swards and to wear acoustic equipment were used. By the time this study
135started all cows were very well accustomed to the experimental procedures. Cows were
136guided with a halter and rope, and were allowed to take up to 10, 20, 30 or 40 bites, as
137micro-sward size increased. This grazing prescription was used to minimize differences in
138herbage depletion among treatments that otherwise could affect intake rate (Laca *et al.*,
1391994). Ten to twelve grazing sessions were performed between 09:00 and 16:00 h each
140day. The order of treatments and cows - were randomized with the restriction that all four
141treatments (species x height) and three cows were observed each day. Cows were milked
142twice daily and grazed a mixed sward of alfalfa and fescue near the experimental site
143where they had ad libitum access to fresh water and shade. Animals were fasted for 1 h
144before grazing sessions. All grazing sessions were conducted inside a closed barn to
145minimize environmental background noises such as wind, machinery or neighboring
146animals.

147 *Video and sound recording*

148 Grazing sessions were recorded using a Sony CCD-TR517 camcorder. Sounds of
149biting and chewing were recorded with a remote wireless microphone (Nady Systems 151
150VR). The microphone was protected by half of a rubber foam ball, placed inwards on the
151animal's forehead and fastened to the halter where a transmitter was attached (See
152Supplementary Figure S.1 for more details).. Two microphones were used and were
153randomly rotated among cows during the study.

154 *Measurements and calculations*

155 Herbage DMI was determined as the difference between forage mass before and
156after grazing. Each pot was weighed individually with 0.1 g accuracy using a digital scale
157(Setra 140 CP). Two ungrazed pots (control pots) were weighed before and after each
158grazing session to estimate evapotranspiration losses. Plant height was measured before
159and after grazing in five extended stems (in alfalfa) or leaves (in fescue) in a randomly
160selected subset of pots. After each grazing session, representative samples of grazed
161forage were obtained by hand plucking of control pots and offered pots that were not
162grazed. Samples were oven-dried at 65°C, weighed and analyzed for neutral detergent
163fiber content (NDF; Robertson and Van Soest, 1980).

164 Sound tracks from video recordings were digitized and analyzed using Cool Edit
165Pro V.2. software (Syntrillium Software Corporation, 2002). Sound sampling rate was
16644.100 kHz, and sample size (resolution) was 16 bits. A total of 48 individual grazing
167sessions were recorded and processed. One signal from a cow grazing short alfalfa had
168to be discarded because it was distorted by an unknown source of noise. Two different
169sets of variables were obtained from the analysis of recordings: behavioral measurements

170from sounds (BMS) and acoustic measurements of ingestive sounds (AMS) as detailed
171below.

172 *Behavioral measurements from sounds.*

173 Number of bites and eating time were used to calculate intake rate (DMI per eating
174time), bite rate (number of bites per eating time) and bite mass (DMI per number of
175bites). Eating time (T) started with the grasping of the first bite and lasted until all
176prescribed number of bites were removed and swallowed. Bites were identified and
177counted by the characteristic ripping sound produced during the grasping and severance
178of standing herbage, chews were identified and counted by the characteristic grinding
179sound of masticatory jaw movements, and composite chew-bites were identified anytime
180a chew followed and partially overlapped with a bite on the same jaw movement.

181 Chewing and biting sounds were classified and analyzed as in previous studies
182(Galli et al., 2006, Galli et al., 2011) to obtain number of bites (B), number chews (C,
183includes exclusive chews and chews of chew-bites), number of chew-bites (ChB), biting
184time (TB) and chewing time (TC). Total jaw movements (TJM) was $B + C - ChB$, total jaw
185movement rate was TJM / T , chew rate (C_T) was C / T , chew per bite was C / B and
186exclusive chews per bite was $(C - ChB) / B$. Jaw movements that did not produce any
187detectable sound signal were disregarded and ignored in calculations. The number of
188chews per g DMI was C / DMI , and the number of chews per g NDF intake (NDFI) was $C /$
189NDFI.

190 *Acoustic measurement of sound.*

191 Acoustic measurements were used to estimate the energy flux density (EFD) of
192biting and chewing sounds. Acoustic energy flux density (EFD) is the product of the
193acoustic intensity and the duration of the sound. The EFD is mechanistically linked to the
194amount of forage being severed and/or progressively crushed in a given jaw movement.

195The average intensity (in decibels) of bites ($\log VB$) and chews ($\log VC$) were measured by
 196the statistics option of Cool Edit Pro, and other variables were calculated as Galli *et al.*
 197(2011):

$$198 \quad \text{Biting intensity (fW/m}^2\text{), } VB = 10^{(\log VB/10)} \times I_{\text{ref}} \quad (1)$$

$$199 \quad \text{Chewing intensity (fW/m}^2\text{), } VC = 10^{(\log VC/10)} \times I_{\text{ref}} \quad (2)$$

$$200 \quad \text{Biting total EFD (pJ/m}^2\text{), } EB = VB \times TB \quad (3)$$

$$201 \quad \text{Chewing total EFD (pJ/m}^2\text{), } EC = VC \times TC \quad (4)$$

$$202 \quad \text{Biting duration (ms), } TB_B = TB / B \quad (5)$$

$$203 \quad \text{Chew duration (ms), } TC_C = TC / C \quad (6)$$

$$204 \quad \text{Biting EFD (fJ/m}^2\text{) per bite, } EB_B = EB / B \quad (7)$$

$$205 \quad \text{Chewing EFD (fJ/m}^2\text{) per chew, } EC_C = EC / C \quad (8)$$

$$206 \quad \text{Chewing EFD (fJ/m}^2\text{) per bite, } EC_B = EC / B \quad (9)$$

$$207 \quad \text{Chewing EFD (fJ/m}^2\text{) per unit intake, } EC_I = EC / \text{DMI} \quad (10)$$

$$208 \quad \text{Chewing EFD (fJ/m}^2\text{) per unit eating time, } E_T = EC / T, \quad (11)$$

$$209 \quad \text{Chewing EFD (fJ/m}^2\text{) per unit NDF intake, } EC / \text{g NDF} \quad (12)$$

210where VB and VC are average intensities in W/m^2 of bites and chews, $\log VB$ and $\log VC$
 211are the average intensities in dB of bites and chews, I_{ref} is the reference intensity in air
 212(arbitrarily was assumed to be 1 pW in order to have meaningful dimensions), chewing
 213time and biting time are the duration of the signal excluding all “silences” between chews
 214or bites. Chew duration and biting duration are measures of the time during which
 215herbage is being either crushed or severed, and are not therefore an exact measure of
 216the total time spent on either a single chew or bite event. For example, total time per
 217chew is composed of both a chew duration and silence time between chews. Chewing
 218EFD per unit of eating time is equivalent to the gross average intensity when the
 219“silences” are included in the analysis of a given chewing signal. Formulas 1 to 4 were
 18

220 adapted from (Charif et al., 1995). Characteristic sounds of bites, chews and chew-bites
221 were described using average sound properties of 60 events.

222 *Statistical analysis*

223 A mixed model was used for ANOVA analyses of behavioral measurements from
224 sounds (BMS) and acoustic measurements of sound (AMS) variables. Fixed effects were
225 forage species (alfalfa vs. fescue), plant height (tall vs. short), and the interaction between
226 both factors. The random effect was the combination of microphone, animal and day. The
227 model also included the actual DMI as a continuous covariate because by design, this
228 variable was controlled by the predefined number of bites (approximately 10 to 40) and
229 micro-sward size (10 to 30 pots). The use of DMI as a covariate applies only to the
230 ANOVA for effects on behavioural and acoustic measurements. It is important to
231 emphasize that none of the models to predict intake or intake rate uses information about
232 DMI. The use of DMI as covariate in the statistical analysis with ANOVA allowed control of
233 confounding effects associated with the offering of micro-sward treatments.

234

235 Forage characteristics were modeled as a factorial of forage species x plant height
236 with day (from 1 to 5) as a continuous covariate. Differences among least squares means
237 were compared by a protected Tukey-Kramer HSD test with significant effects determined
238 using a F-test ($P < 0.05$). Residuals plots were examined to check deviations from
239 linearity and logarithmic transformation (\log DMI) was used when data did not meet
240 assumptions for normal distribution ($P < 0.01$; Shapiro–Wilk test) or homogeneous
241 variance ($P < 0.05$, Levene test). All statistical analyses were performed with JMP® 12
242 software (SAS Institute Inc., 2015). Differences among sounds of bites, chews and chew-

243bites were compared by a protected Tukey-Kramer HSD with significant effects
244determined using a F-test ($P < 0.05$).

245 Variables calculated from sound tracks were divided into BMS and AMS variables
246to compare predictions of DMI based on different sets of variables. Dry matter intake was
247regressed on BMS, AMS or both sets of variables, by using a variable model selection
248based on the lower Akaike information criterion (AIC), a measure of the relative quality of
249statistical models for a given set of data (SAS Institute Inc., 2015). All possible models
250including one to ten variables were explored. In addition, selected models were further
251tested with the inclusion of categorical effects for plant species (alfalfa vs. fescue) and
252plant height (tall vs. short), respectively. Categorical effects were determined and
253interpreted as deviation units from the overall intercept, where the effects for the
254alternative factor (fescue plants or short plants) have the exact same absolute value but
255with opposite sign. External validation of models was assessed by *K*-fold adjusted cross-
256validation (SAS Institute Inc., 2015). Path analysis (Li, 1975) was used to evaluate and
257describe direct and indirect effects of plant treatments on intermediate chewing variables
258and total chewing EFD. Chewing sound energy was described as a function of its three
259components: chewing intensity, chewing duration and number of chews per g DMI.

260Results

261 *Forage characteristics*

262 Fescue pots had 38 % more herbage biomass than alfalfa pots (6.5 vs. 4.7 g DM
263per pot, $P < 0.001$). Similarly, herbage mass was 51 % greater in tall than short plants
264(7.5 vs. 3.7 g DM per pot, $P < 0.001$). Alfalfa and fescue did not differ in height (18 cm, P
265 > 0.05), but in both species short plants were 52 % shorter than tall plants (25 vs. 12 cm,
266 $P < 0.001$). Dry matter content did not differ ($P > 0.05$) among treatments and was on

267average 190 ± 10 g DM per kg. Fiber content (NDF) was lower in alfalfa than in fescue
268(360 vs. 631 g per kg, $P < 0.001$), but similar between short and tall plants (490 g per kg,
269 $P > 0.05$). See Supplementary Table S.1 for more details.

270 *Ingestive behavior*

271 On average, grazing sessions lasted 61.4 s (from 19 to 121 s) and cows removed
27225 bites (from 9 to 48) and consumed 23 g of dry matter (from 4 to 52 g). The actual
273number of bites was slightly different from the number of bites predefined by design. This
274was due to inherent difficulties of aurally assessing and controlling the harvest of an exact
275number of bites during a grazing session. Intake rate was affected ($P < 0.01$) by an
276interaction between plant species and plant height due to a greater ($P < 0.05$) intake rate
277in tall alfalfa than in the other 3 micro-sward treatments (Table 1). Similarly, a significant
278($P < 0.05$) interaction between species and plant height was observed in bite mass, due
279to greater ($P < 0.05$) bite mass in tall vs. short micro-swards and in short fescue vs. short
280alfalfa (Table 1).

281 Bite rate was greater ($P < 0.05$) in alfalfa than fescue (Table 1) and was not
282affected ($P > 0.05$) by plant height ($P > 0.05$). Number of chews per g of DMI was greater
283($P < 0.05$) in fescue than alfalfa, but both species had a similar ($P > 0.05$) number of
284chews per g of NDF intake (Table 1). Time per bite was longer in fescue than alfalfa (2.88
285vs. 2.01 s), and about the same ($P > 0.05$) between the plant height treatments (2.40 s).
286There were no significant differences in total jaw movement rate among all 4 treatments
287(57 movements per min, $P > 0.05$), but chewing rate (51 vs. 44 per min), jaw movements
288per bite (2.97 vs. 1.85), chews per bite (2.60 vs. 1.45), and the number of exclusive
289chews per bite (1.97 vs. 0.85) were higher ($P < 0.05$) in fescue than in alfalfa. Number of
290chew-bites per bite was different ($P < 0.05$) between plant height treatments (0.65 vs.

2910.54 for tall and short, respectively), but it was not affected ($P > 0.05$) by plant species. Proportion of total jaw movements involving chew-bites was greater in alfalfa than in fescue (0.33 vs. 0.23, $P < 0.05$) and was about the same (0.27, $P > 0.05$) for both plant height treatments. See Supplementary Table S.2 for more details.

295 *Biting and chewing sounds.*

296 Exclusive bites and chews, and compound chew-bites were accurately distinguished by their sound characteristics (Figure 2). Bites had greater ($P < 0.05$) average intensity (values dB), and were louder ($P < 0.05$, 28.2 ± 3.42 vs. 4.0 ± 0.74 fW/m²) and shorter ($P < 0.05$, 178 ± 9.1 vs. 252 ± 64.7 ms) than chews. Short plants produced greater ($P < 0.05$) chewing EFD per g of DMI than tall plants, whereas fescue plants had greater ($P < 0.05$) chewing EFD per bite, biting intensity and biting duration than alfalfa (Table 2). All treatments produced similar ($P > 0.05$) chewing EFD per g of NDF intake (4.86 ± 1.73 fJ/m²). Neither chewing EFD per unit eating time (0.83 ± 0.22 fJ/m²) nor chewing EFD per chew (1.00 ± 0.22 fJ/m²) differed significantly ($P > 0.05$) among treatments.

306 Total energy flux density (EFD) of chewing sounds was linearly related to DMI ($P < 0.0001$), with 74% of the total variation in chewing EFD explained by differences in DMI (Figure 3). Different direct and indirect effects and correlations between plant treatments, chewing intensity, chewing duration and chewing EFD per mass were detected (Figure 4). Plant treatments affected the number of chews per unit DMI, which in turn had a positive direct effect on the final chewing EFD per unit DMI. Conversely, neither chewing sound duration nor intensity were influenced by plant treatments but both were negatively correlated with number of chews per unit DMI, indirectly reducing chewing EFD per unit DMI.

315 *Prediction of dry matter intake*

316 The best predictive model of DMI ($R^2= 0.86$) combining BMS and AMS variables
317 included 2 predictors, chewing total EFD and number of chew-bites (Table 3, bottom). The
318 best predictive model for DMI based on AMS variables ($R^2= 0.84$) included variables
319 chewing total EFD, chewing EFD per bite, chewing EFD per unit of eating time and
320 chewing EFD per chew (Table 3, upper). The best model using only BMS variables ($R^2=$
321 0.83) included number of chew-bites and chewing time (Table 3, middle). Predictions of
322 DMI based on BMS, AMS, or combinations of both sets of variables were significantly
323 improved by inclusion of categorical effects for plant height and species (Table 3). Models
324 that included categorical plant effects as well as BMS and AMS variables explained up to
325 91% of DMI variance (Figure 5). The best models based on a single predictor included
326 number of chew-bites ($R^2= 77\%$), total chewing EFD ($R^2= 72\%$) or chewing time ($R^2=$
327 65%).

328 **Discussion**

329 The experiment was designed to examine the main determinants of intake rate,
330 and to predict herbage DMI based on easily observable behavioral and acoustic
331 variables. Dairy cows were offered various micro-swards differing in amount and height of
332 alfalfa or fescue herbage. Such treatments generated a wide range of DMI both within
333 and between sward structures, as well as different relationships between plant structure,
334 plant tissue chemistry, biting and chewing requirements and intake rate. Therefore, we
335 were able to test whether behavioral and acoustic measurements can predict DMI when
336 DMI differences are driven by both grazing time and bite mass.

337 Overall, results clearly show that the acoustic methods can account for changes in
338 DMI caused both by changes in grazing time and by changes in intake rate. Cows were

339able to maintain a relatively high intake rate across a wide range of herbage mass and
340sward structure by exhibiting different biting and chewing behavior when grazing alfalfa or
341fescue. Alfalfa and fescue did not differ in average intake rate and bite mass, but greater
342biting rate was observed in alfalfa over fescue (Table 1). Moreover, the greater biting rate
343in alfalfa was associated with less time per bite because cows spent less time chewing
344per bite and had a greater proportion of jaw movements to compound chew-bites than
345when grazing fescue. Taller swards resulted in greater bite mass and greater intake rate
346(Table 1), because bite rate and time per bite were about the same when tall or short
347swards were offered. Ultimately, results suggest that intake rate may be controlled by a
348constant rate of jaw movements that are allocated to biting, chewing or simultaneous
349chewing and biting as animals encounter forages with different structural properties that
350affect ease of prehension, fracture and swallowing. Consequently, different relationships
351between sward structure, bite mass, biting rate and intake rate can be generated (Table
3521).

353 As expected, the relationship between overall chewing sound energy and DMI was
354linear (Figure 3), in spite of the clear differences in NDF content and chewing
355requirements between alfalfa and fescue. Alfalfa had lower NDF but the same ingestive
356chewing per unit of NDF intake as fescue (Table 1). Consequently, more diluted NDF
357content resulted in lower ingestive chewing per unit of DMI in alfalfa over fescue (Table 1).
358Interestingly, less chewing per bite and per unit of mass in alfalfa were associated with
359less chewing sound energy per bite, and with a similar chewing sound energy per unit of
360DMI in alfalfa and fescue (Table 2), which is consistent with previous comparisons of
361chewing sounds between orchardgrass and alfalfa in grazing sheep (Galli *et al.*, 2011).
362Based on these results, estimations of DMI by the acoustic method would be possible

363when ruminants (cows or sheep) are grazing a variety of pastures, even if different plant
364species are present.

365 Partly, chewing sound is produced by rupture of cells and extrusion of water (Galli
366*et al.*, 2006). Therefore, the relationship between DMI and sound may depend on plant
367water content. In practice, this could be overcome by recalibrating the equations for
368forages with widely different water content, such as standing dry annual grass in summer.
369Certainly, changes in forage characteristics such as water content, anatomy of tissues,
370and fiber content, and animal characteristics such as dentition, head size and anatomy
371will tend to affect the relationship between intake and sound produced by the ingestion of
372forage. Sound is produced as a result of waves created in the air and in the bones of the
373head as plant structures are comminuted by biting and chewing. The waves are
374transmitted, filtered and modified by the bones, cavities and soft tissues of the animal's
375head. However, this work shows that for cows of similar size and breed, one equation that
376includes a term for species was sufficient to predict intake with relatively high precision.

377 Chewing rate and efficiency per unit of mass can also decrease when bites are
378small (Laca and WalliesDeVries, 2000), and particularly when fiber content is low
379(McLeod *et al.*, 1990). In short swards, smaller bites require more chews per unit mass,
380particularly in alfalfa. Moreover, when factored alone, bite mass was able to explain about
38141 % of the variation in chews per unit of mass, but it only accounted for 16 % of the
382observed variability in chewing EFD per unit of mass. This suggests that chewing sound
383data is more consistent and carries a more precise and robust measure of intake rate
384than biting and count of chewing events alone. Chewing EFD contains direct information
385about amount and quality (i.e. NDF) of the forage processed at each single chewing
386event. In other words, the sound of chewing should be a better predictor of DMI than

387biting and chewing behavior, which is supported by the fact that chewing sound EFD and
388not biting or chewing appeared in the best predictive models for DMI (Table 3).

389 Path analysis of chewing sounds confirmed several meaningful relationships
390between plant characteristics, components of chewing sounds, and chewing EFD per DMI
391previously reported for grazing sheep (Galli *et al.*, 2011). When cows allocated more
392chews per g of DMI in direct response to plant treatments, chews had lower intensity
393(indirect effect) and shorter duration (indirect effect). Conversely, when cows invested
394fewer chews per g of DMI in direct response to plant treatments, chews were more
395intense (indirect effect) and of longer duration (indirect effect), which indicates a high
396degree of compensation between overall chewing efforts and properties of chewing
397sounds. This compensatory chewing mechanism may explain why significant differences
398in chewing requirements (i.e. alfalfa vs. fescue) can result in similar chewing EFD per
399DMI, even when chew duration and intensity are not responsive to plant differences.
400Hypothetically, when cows reach a "full mouth" of forage, the number of chews per DMI is
401inevitably reduced, although it is possible that the greater amount of food present in the
402mouth would result in longer and more intensive chews that would stabilize chewing EFD
403per unit of DMI against the effects of varying bite mass.

404 Energy of chewing sounds measured as overall chewing sound EFD was the
405strongest predictor of DMI, as previously noted in studies with steers (Laca and
406WallisDevries, 2000; Galli *et al.*, 2006) and sheep (Galli *et al.*, 2011). As a single
407predictor, the total chewing EFD ($R^2= 72\%$, $CV= 28\%$) was more accurate than grazing
408time ($R^2= 67\%$, $CV= 30\%$) or the number of total chews ($R^2= 64\%$, $CV= 32\%$). A plausible
409explanation is that total chewing EFD captures information from both eating time and
410intake rate. Therefore, for any given eating time an increase in chewing EFD will indicate
411greater intake rate and vice versa.

412 The results of the present study therefore confirm the potential to accurately
413 estimate DMI of grazing animals by means of ingestive sounds. Furthermore, sound-
414 based estimation of DMI could be successfully scaled across different sward types, and
415 plant-specific models could be developed to further improve predictions, in particular by
416 adding factors to adjust for differences in sward height or plant species (Table 3). The
417 best model combining total chewing EFD, number of chew-bites, and categorical factors
418 for plant species and plant height accounted for most of the variability in DMI ($R^2= 0.91$),
419 while rendering a CV equal to 17%, which is in the order of the 18% CV estimated for
420 sound-based predictions of DMI in sheep (Galli *et al.*, 2011). Furthermore, in both dairy
421 cows and sheep, the number of chew-bites was the only ingestive behavior variable that
422 added relevant information to DMI predictions, reinforcing the value of acoustic
423 methodologies to accurately discriminate compound events of chewing and biting, which
424 are ignored by most of the alternative jaw recording techniques.

425 The acoustic method could bring accurate estimations of DMI when cows are
426 grazing pastures, even if many forage species are present. Based on the cross-validation,
427 the best predictive model had a square root of the mean squared prediction error equal to
428 3.8 g ($R^2_{K\text{-fold}}= 0.88$). This is a good estimate of the standard error for predictions of
429 expected DM intake for observations not included in the training data set. As DMI was
430 22.4 gDM, the CV was 17%.

431 This research brings new insights into the ingestive process of grazing ruminants.
432 The combined manipulation of grazing and micro-sward treatments, and acoustic
433 recording of biting and chewing sounds, allowed testing of sound-based predictions of
434 DMI while bringing insights into the regulation of herbage intake rate. Future research is
435 necessary to extend acoustic measurements of forage intake over longer time periods
436 (i.e. complete grazing bouts or daily measurements) and to assess the feasibility of
36

437scalable sound-based predictions of DMI. Ingestive sounds integrate valuable information
438to predict intake, while offering an unprecedented opportunity to remotely monitor
439sensible differences of feeding behavior in free ranging animals. Further work is also
440necessary to strengthen progress on the automation of sound signal analysis to develop
441recording and processing systems for direct estimation of grazing intake under on-farm
442conditions.

443**Conclusions**

444 Findings support the hypothesis that herbage intake rate is controlled by a
445constant (maximum) jaw movement rate, and by the ability of cows to differentially
446allocate jaw movements to biting, chewing or simultaneous chewing and biting as they
447encounter forages with different structural, physical and chemical properties that affect
448ease of apprehension, fracture and swallowing. In this study, different intertwined
449relationships between sward structure, bite mass, biting rate and intake rate were
450encountered between plant treatments. Chewing sound energy was the single best
451predictor of DMI and low variability of chewing sound energy was seen in response to
452plant tissue characteristics and feeding behavior. Therefore, findings of the present study
453reinforce the idea of applying generalized sound-based predictions of DMI, using chewing
454sound energy as the main predictor.

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532

533**Tables**534**Table 1.** *Effect of forages on ingestive behavior of cattle*

		Alfalfa	Fescue	Mean	RMSE	P value
Intake rate (g DM / min) n= 48	Tall	32 ^a	22 ^b	27	4.92	<0.001
	Short	18 ^b	19 ^b	19		
	Mean	25	21			
Bite mass (g DM) n= 48	Tall	1.0 ^a	1.1 ^a	1.1	0.11	<0.001
	Short	0.5 ^c	0.8 ^b	0.7		
	Mean	0.8	1.0			
Bite rate (min ⁻¹) n= 47	Tall	30	20	25	5.37	<0.001
	Short	31	23	27		
	Mean	31 ^a	21 ^b			
Chews per g DM n= 47	Tall	2.4	3.3	2.9	0.58	<0.001
	Short	1.6	3.1	2.4		
	Mean	2.0 ^b	3.2 ^a			
Chews per g NDF n= 47	Tall	5.70	6.19	5.94	1.54	0.636
	Short	5.09	5.33	5.21		
	Mean	5.39	5.76			

535Means followed by different letters differ significantly (Tukey-Kramer HSD, $P < 0.05$), RMSE= root of the
536mean squared error
537

538**Table 2.** *Effect of forage species and plant height on acoustic variables in cattle.*

Variable ¹		Alfalfa	Fescue	Mean	RMSE	P value
Chewing EFD (fJ/m ²) per g DMI	Tall	1.7	2.5	2.1 ^b	0.75	0.013
	Short	2.8	2.9	2.8 ^a		
	Mean	2.2	2.7			
Chewing EFD (fJ/m ²) per bite	Tall	1.6	2.6	2.1	0.55	<0.001
	Short	1.3	2.3	1.8		
	Mean	1.5 ^b	2.5 ^a			
Biting intensity (fW/m ²)	Tall	24	34	28	1.51	<0.001
	Short	17	38	28		
	Mean	21 ^b	36 ^a			
Biting duration (ms)	Tall	177	184	180	12.9	<0.001
	Short	166	118	178		
	Mean	166 ^b	192 ^a			

539¹ EFD= average energy flux density of sound; DMI= dry matter intake. Means followed by different letters
540differ significantly (Tukey-Kramer HSD, P<0.05). RMSE= root of the mean squared error.

541**Table 3.** Models to estimate dry matter intake of cattle based on acoustic (AMS) or behavior
542(BMS) measurements from sounds.

	Best overall models without species and biomass effects				Best model including species effect	Best model including species and biomass effects
	1 <i>p</i>	2 <i>p</i>	3 <i>p</i>	4 <i>p</i>		
AMS (<i>p</i>)						
Intercept	5.59	9.8	6.7	10.2	10.2	13.1
Chewing total EFD	0.33	0.38	0.31	0.29	0.38	0.30
Chewing EFD per bite		-3.35	-4.94	-5.16	-5.16	-6.19
Chewing EFD per unit eating time			12.3	27.9	27.8	21.1
Chewing EFD per chew				-15.0	-15.0	-11.1
Alfalfa vs. Fescue					0.27	-0.94
Tall vs. Short						-2.96
R ² adj.	0.73	0.76	0.80	0.84	0.84	0.88
R ² K-fold	0.71	0.73	0.75	0.78	0.78	0.88
AIC	174	170	164	156	158	144
RMSE (g DM)	6.5	6.1	5.2	5.2	5.2	4.4
CV (%)	28	26	25	23	23	19
BMS (<i>p</i>)						
Intercept	5.3	3.15			2.90	3.87
Number of chew-bites	1.02	0.72			0.69	0.65
Chewing time		0.57			0.63	0.59
Alfalfa vs. Fescue					0.46	0.18
Tall vs. Short						-2.64
R ² adj.	0.77	0.83			0.83	0.87
R ² K-fold	0.75	0.79			0.76	0.83
AIC	165	155			157	145
RMSE (g DM)	5.9	5.2			5.2	4.6
CV (%)	25	23			23	20
AMS and BMS (<i>p</i>)						
Intercept		2.88			2.81	3.73
Chewing total EFD		0.17			0.19	0.17
Number of chew-bites per bite		0.63			0.63	0.59
Alfalfa vs. fescue					0.19	-0.05
Tall vs. Short						-2.57
R ² adj.		0.86			0.86	0.91
R ² K-fold		0.85			0.84	0.88
AIC		144			146	131

RMSE (g DM)	4.6	4.6	3.8
CV (%)	20	20	17

543N= 46; EFD= average energy flux density of sound, $R^2_{adj.}$ = R^2 adjusted by p, R^2_{K-fold} = R^2 from K-fold
544cross-validation, AIC= Akaike's information criterion, RMSE= root of the mean squared error. Each column
545represents the best model with a given number of predictors (p). Coefficients for Tall vs. Short plants and
546Alfalfa vs. Fescue are the effects for tall plants and alfalfa plants, respectively.

547

548**Figure 1.** Schematic illustration of experimental micro-swards and acoustic device on dairy cow's
549forehead.

550

551**Figure 2.** Example of soundtrack showing a typical sequence of bites, chews and chew-bites,
552collected with a dairy cow grazing a micro-sward of tall alfalfa.

553

554**Figure 3.** Relationship between dry matter intake (DMI) and total energy flux density of chewing
555sounds (EC) in dairy cows, $EC = 3.2 + 2.13 \text{ DMI}$, $P < 0.0001$, $R^2 = 0.74$, $n = 47$. Solid line: overall
556linear regression, (\circ): Tall alfalfa, (\bullet): Short alfalfa, (\square): Tall fescue, (\blacksquare): Short fescue.

557

558**Figure 4.** Path diagram depicting direct and indirect effects of plant treatments and acoustic
559chewing variables on total chewing energy flux density (EC) per gram of dry matter intake (DMI) in
560dairy cows. Only significant ($P < 0.05$) paths are shown. Forage species x Plant biomass
561interaction and Number of bites were also considered in structural equations but the effects were
562not significant and are not shown in this diagram. Paths from categorical plant variables are given
563for "Alfalfa" and "Tall". For example, a change from fescue to alfalfa reduces chews per g DMI.

564

565**Figure 5.** Relationship between observed (x) and predicted (y) dry matter intake (DMI) of dairy
566cows grazing alfalfa or fescue, based on behavioral (BMS), acoustic (AMS) predictors and
567categorical effects for plant species and plant height ($P < 0.0001$, $R^2 = 0.91$, $RMSE = 3.8 \text{ g DM}$,
568 $CV = 17 \%$). Solid line: $y = x$. Predictive models were: $DMI = 33.73 + 0.17 \text{ Chewing total EFD} +$
569 $0.59 \text{ Number of chew-bites} - 0.05 \text{ Alfalfa vs. Fescue} - 2.57 \text{ Tall vs. Short}$

570