Comparison of methods to quantify the number of bites in calves grazing winter oats with different sward heights

Laura B. Nadin a, b, *, Federico Sánchez Chopa a, b, Malcolm J. Gibb c, Júlio Kuhn da Trindade d, Glauco Azevedo do Amaral d, Paulo C. de Faccio Carvalho d, Horacio L. Gonda a

a Departamento de Producción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Paraje Arroyo Seco s/n., 7000, Tandil, Argentina
b Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
c Formerly at the Institute of Grassland and Environmental Research, North Wyke, Okehampton, Devon EX20 2SB, UK
d Departamento de Plantas Forrageiras e Agrometeorologia, Faculdade de Agronomia, Universidade Federal do Rio Grande do Sul. Av. Bento Gonçalves 7712, Agronomia, CEP 91501-970 – Caixa Postal: 776, Porto Alegre, RS, Brazil

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A B S T R A C T

The requirement to measure the constituents of ingestive behaviour in grazing ruminants, such as the number and type of jaw movements, is essential for understanding the herbage intake process. In this experiment, three methods of recording grazing behaviour were compared: visual observation (VO; four trained observers), solid-state behaviour recorder (SSBR) and acoustic recorder (AR), in order to quantify the number of bites taken by Holstein–Friesian calves (138 ± 11 kg LW) grazing winter oat (Avena sativa) pastures with different sward surface heights (SSH): tall (T; 52.4 ± 9.9 cm), medium (M; 26.5 ± 4.9 cm) and short (S; 14.6 ± 3.4 cm). The number of bites was recorded on nine calves with VO, SSBR and AR during 5-min grazing sessions on each sward height. In addition, the total grazing jaw movements (GJM) and bites per GJM (PB) were calculated from the SSBR and the AR methods. The experiment was conducted on 3 days, and on each day three animals grazed on each of the three sward heights in a randomised sequence. Sound files from AR were analysed visually and aurally (Sound Forge 9.0) and recordings by SSBR were analysed using the dedicated software ‘Graze 8.0’. Data were analysed by three-way ANOVA. There were no statistical differences (P > 0.05) in the number of bites counted by the observers. The number of bites did not differ significantly between AR and VO, but were lower for SSBR (P < 0.001). Mean values of number of bites were: 191, 153 and 192 for VO, SSBR and AR, respectively. Comparison of recording analyses from AR and SSBR showed a small difference (<5%; P < 0.05) in total GJM (388 and 407 for SSBR and AR, respectively) and a larger difference (19%; P < 0.01) in PB (0.395 and 0.472 for SSBR and AR, respectively). The main problem appeared to be in the identification of bites using SSBR, which led to an underestimation of the number of bites and bites per GJM using that method under the present grazing conditions. The AR method proved to be accurate for identifying biting jaw movements.

1. Introduction

The requirement to measure the constituents of ingestive behaviour by grazing ruminants, to either estimate intake or explain the observed intake, has led to the development of various automated devices (Beauchemin

Abbreviations: SSBR, solid state behaviour recorder; VO, visual observation; AR, acoustic recorder; SSH, sward surface height; GJM, total grazing jaw movements; PB, bites per GJM.

* Corresponding author at: Departamento de Producción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional del Centro de la Provincia de Buenos Aires, Paraje Arroyo Seco s/n., 7000, Tandil, Argentina.
Tel.: +54 0249 4439850; fax: +54 0249 4439850.
E-mail address: lnadin@vet.unicen.edu.ar (L.B. Nadin).

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et al., 1989; Boland, 2005; Delagarde et al., 1998, 1999; Desnoyers et al., 2009; Galli et al., 2006; Laca et al., 1992; Rutter et al., 1997; Ungar and Rutter, 2006). Among these automated devices, the IGER solid state behaviour recorder (SSBR) has been widely used in many studies (Gibb et al., 1998, 1999, 2002; Orr et al., 2005; Rutter et al., 2002). This method allows the continuous recording of jaw movements over a 24-h period. Its associated software ‘Graze 8.0’ (Rutter, 2000), utilises algorithms formulated to analyse and discriminate different jaw movements, namely bites and non-bites during grazing, and rumination. However, these algorithms were developed and validated for interpreting recordings made with cattle and sheep grazing temperate pastures (perennial ryegrass and/or white clover) in which sward surface height (SSH) varied between approximately 3 and 25 cm.

In the Province of Buenos Aires, Argentina, swards of Italian ryegrass ( Lolium multiflorum ) or oats ( Avena sativa ) are frequently used to provide grazing for beef and dairy cattle during the late autumn, winter and early spring. In many cases, due to the high rate of growth, by the time these pastures are grazed, sward height can achieve 30 or 50 cm, resulting in a very different plant morphology compared with the swards used in the development of ‘Graze’. As a consequence, a wider variety of jaw movements may be performed in these taller swards. In addition, these swards are generally utilised under a rotational stocking system, with animals occupying a paddock for a period of 1–7 days, resulting in a rapid modification of the sward height and lamina:pseudostem ratio by repeated defoliations over a short period and, as a consequence, modification of grazing behaviour. In previous studies with calves grazing on tall swards, we have faced considerable difficulty trying to identify biting jaw movements using the SSBR and its associated ‘Graze’ software.

More recently, methods of acoustic recording have shown great potential for the identification of and differentiation between different grazing jaw movements (Galli et al., 2006; Laca and Wallis De Vries, 2000) and provide an alternative to the SSBR.

The present experiment was conducted with the objective of comparing methods (visual observation, SSBR and acoustic recording) to quantify the number of bites in Holstein Friesian calves grazing winter oat ( A. sativa ) pastures with three different sward surface heights (SSH). In addition, the effect of SSH on number of bites per grazing session was also analysed.

2. Materials and methods

The experiment was conducted between 2 and 16 July 2008, at the campus of the Universidad Nacional del Centro de la Provincia de Buenos Aires (37°19′S, 59°07′W), Tandil, Argentina.

All procedures regarding the use of animals complied with the requirements of the ethical guidelines published by the International Society for Applied Ethology.

2.1. Animals

Nine, 6-month-old Holstein–Friesian calves (138 ± 11 kg LW) were used. Two months prior to the experiment the calves were provided with experience of grazing oat swards, the close proximity of the observers and the wearing of head collars. When not being used for measurement, the calves were stocked on a common paddock of winter oats with a daily forage allowance (kg DM animal−1) equivalent to 0.05 of LW.

2.2. Sward preparation

The winter oats ( A. sativa cv. Calén ) were sown on 16 March 2008 at a seed rate of 120 kg ha−1 in an area treated with glyphosate herbicide 4 weeks previously. The area of the sward intended for use for the present study was kept ungrazed until three different sward surface heights (SSH) were created by cutting the sward. The SSHs were named as: tall (T), medium (M) and short (S). The T sward was left uncut and the M and S swards were cut to approximately 0.50 and 0.25 of SSH of the T sward, respectively, using a reciprocating-cutter-bar mower, the day prior to each recording day. After cutting, the cut plant material was immediately removed. The required height of the cutter bar above ground level was maintained by using a taut wire adjusted to the required height. The area of each treatment sward for each day of observation, measured approximately 6 m × 0.75 m. Before cutting, the SSH of the whole area intended for use the next day was assessed by making 60 height measurements using a sward stick similar to the design of the HFRO sward stick ( Hill Farming Research Organization, 1986 ), but in which the ‘contact’ window measured 15 mm × 35 mm.

Cutting the swards established a significant difference in SSH between the three sward heights ( F 2,147 = 1187.6, P < 0.001), and significantly affected the total herbage masses ( F 2,18 = 155.1, P < 0.001), lamina ( F 2,18 = 202.4, P < 0.001), pseudostem ( F 2,18 = 22.6, P < 0.001), dead ( F 2,18 = 25.2, P < 0.001) and the L:P mass ratio ( F 2,18 = 112.0, P < 0.001) (Table 1).

Following preparation of the three sward heights, the plots corresponding to each SSH were subdivided into three individual sub-plots with electric fencing, to provide grazing for one calf. Each sub-plot measured 2 m × 2.75 m

<table>
<thead>
<tr>
<th>SSH</th>
<th>Height</th>
<th>Herbage DM mass</th>
<th>L:P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Lamina</td>
<td>P</td>
</tr>
<tr>
<td>T</td>
<td>52.4 a</td>
<td>600.9 a</td>
<td>358.8 a</td>
</tr>
<tr>
<td>M</td>
<td>26.5 b</td>
<td>303.5 b</td>
<td>132.3 b</td>
</tr>
<tr>
<td>S</td>
<td>14.6 c</td>
<td>143.8 c</td>
<td>24.8 c</td>
</tr>
<tr>
<td>SEM*</td>
<td>0.56</td>
<td>18.62</td>
<td>11.98</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Means not sharing common letters (a,b) within a column differ by P < 0.05.

* SEM: standard error of the mean.
encompassing a 2 m × 0.75 m area of sward and a 2 m × 2 m area of bare soil.

2.3. Experimental procedure

The experiment comprised 3 days of recordings in which the number of bites taken by calves while grazing on winter oats with different SSHs was registered during 5-min grazing sessions. Three methodologies were used to measure the number of bites taken by the calves, as follows: visual and aural recognition of bites counted by four trained observers (visual observation, VO), recording of jaw movements using the IGER behaviour recorder (SSBR: Rutter et al., 1997) and acoustic recording (AR: Galli et al., 2006; Laca and Wallis De Vries, 2000).

Each recording day, three of the nine calves to be used for study were removed from the common grazing paddock to a handling area at 11:00 h. Beginning at 15:00 h, one calf at a time was fitted with a head collar to which the SSBR and the AR devices were attached. The AR apparatus incorporated a microphone held in place on the forehead of the calf and connected to a digital recorder. The calf was then walked to the experimental subplots and allowed to graze for 5 min on each of the three sward SHHs in turn, following a random sequence. During the grazing sessions, the number of bites was counted simultaneously by the observers (VO) and recorded by the SSBR and AR methods. After completing the observations and recordings on the three sward heights, the calf was returned to the handling area, where both devices were removed. The same procedure was then carried out with the other two calves in turn.

The allocation of the animals to the recording days, the order in which the animals were studied within the recording days, as well as the order in which each animal grazed the different sward heights were randomised.

To ensure that the time windows over which the results of the three methodologies were compared were the same, the following procedure was adopted. When a calf entered an individual recording plot as soon as it started to graze, the precise time was noted according to a digital watch, an audible signal (a shout) was made and the visual observations (VO) commenced immediately. After 5 min the procedure was terminated with a second audible signal. Recordings were subsequently downloaded to a computer.

The audible signal picked up and recorded by the microphone-digital recorder (AR) on the calf was used
to identify the 5-min recording window on the acoustic recording. The internal clock of the SSBR had previously been synchronized with the digital watch used by the visual observer who made the audible signal. However, to ensure that the 5-min time windows, over which jaw movements recorded by the SSBR and AR were precisely the same, the wave patterns recorded by the two methods were aligned on a screen and the time window identified from the AR recording was then marked on the SSBR recording using the facility in Graze. An example of the plots of AR and SSBR is shown in Fig. 1.

2.4. Swards measurements

Before being grazed, 20 SSH measurements were made across each of the T, M and S sub-plots. In addition, three samples of herbage were cut to ground level within three quadrats, measuring of 20 cm × 30 cm on each sub-plot, using scissors. The herbage cut from each quadrat was separated manually into lamina, pseudostem and dead fractions, before being dried at 65 °C until reaching a constant weight. The weights were used to calculate the total herbage mass (g m⁻²), as well as the masses of the different morphological components, lamina, pseudostem and dead material.

2.5. Measurement of number of bites

2.5.1. Visual observations

Four trained observers, standing in close proximity to the animals (within 3 m), counted the numbers of bites, based on visual and aural cues, during all the grazing sessions. In addition, a video recording was made (SP mode, real time, 25 frames s⁻¹) by a fifth operator using a handheld digital camera (Sony, digital video camera recorder, Digital 8, DCR-TRV530, Japan), to provide a record, if required, for the interpretation of any of the methods used to determine the number of bites.

2.5.2. Analysis of SSBR

The recordings from the three calves used on each day on each of the three different SSHs were made using a single SSBR. After fitting the recorder to a calf, three recordings were made, one on each of the three sward structures. The recorder was then fitted to the next calf and the procedure repeated. After the final period of measurement was completed with the third calf, the recorder was removed and switched off. The memory card was then removed and the single file containing the jaw movement data from the three animals was analysed using ‘Graze 8.0’. The nine individual, 5-min recordings corresponding to the precise periods of visual observation were marked using the facility in ‘Graze 8.0’, as described previously. The total grazing jaw movements (GJM) and bites were identified by an operator experienced in using the ‘Graze 8.0’ software. This software allowed the operator to set the parameters in order to obtain what were considered to be the most reliable results regarding identification of individual jaw movements and discrimination between biting and non-biting grazing jaw movements. This examination and analysis was conducted without the operator knowing the number of bites counted by the observers.

2.5.3. Analysis of AR

The sounds produced by the range of different jaw movements were recorded using a microphone (Omni directional microphone, ECM-F8, Sony, Japan) connected to a digital recorder (IC recorder, ICD P320, Sony, Japan). After completing the observations, the sound files were downloaded to a computer in a MP3 format. This allowed the sound recordings to be interpreted by an operator examining both the digitised sound-wave patterns and aurally by listening to the recordings (Miloc et al., 2009; Sound Forge 9.0, Sony Creative Software Inc., USA). The numbers of bites and the total GJM were both registered. As for the SSBR analysis, this examination was conducted without the operator knowing the number of bites counted by the observers.

2.6. Statistical analyses

Variables of the sward and the number of bites counted by the observers were analysed by ANOVA using the mixed model procedure (SAS Institute Inc., 2009). Classes included into the model were sward height (fixed effect) and day of measurement (random effect), and sward height, day and observer (fixed effect); for sward variables and bites counted by the observers, respectively. Because the variable lamina:pseudostem mass ratio was not normally distributed, data were transformed to root square before statistical analysis.

Before analysis, normal distribution of number of bites (P = 0.38) and total grazing jaw movements (GJM; P = 0.31) was tested by the Shapiro–Wilks test. The numbers of bites, total GJM and bites per GJM data were analysed according to a three-way ANOVA. Classes included into the model were method and sward height (fixed effects) and the day of measurement (random effect) (mixed model procedure, SAS Institute Inc., 2009). Animals (n = 9) were treated as independent replicates. Comparison of individual means was performed by the lsmeans/DIFF statement of SAS (SAS Institute Inc., 2009).

3. Results

3.1. Number of bites

Prior to the start of the experiment, care was taken to accustom the animals to the close proximity of operators in order to avoid disruption of their grazing activity. Thus, during the 5-min recordings, despite the subject calf being enclosed within a small sub-plot of 5.5 m² and closely surrounded by four observers and a fifth operator with a video recording camera, there were no signs of disturbance and animals grazed continuously throughout observation sessions.

There were no statistical differences among the numbers of bites counted by the four observers neither across (F₃,₇₂ = 0.59, P = 0.51) nor within (F₃,₂₄ = 0.07, P = 0.97; F₃,₂₄ = 0.40, P = 0.75; F₃,₂₄ = 0.30, P = 0.82, for T, M and S, respectively) the SSHs (Table 2). The
Interactions observer × SSH, observer × day, SSH × day and SSH × observer × day, were not significant (F_{6, 72} = 0.02, P > 0.99; F_{6, 72} = 0.26, P = 0.95; F_{4, 72} = 0.63, P = 0.64; F_{12, 72} = 0.09, P > 0.99, respectively).

While there were no differences between the number of bites registered by the VO and the AR methods, bites counted with the SSBR method were lower than those from VO and AR (F_{12, 64} = 23.1, P < 0.001) (Table 3). The interaction SSH × method was not significant (F_{6, 64} = 1.64, P = 0.176, Table 3).

Wave patterns for the jaw movements recorded by the SSBR method showed an evident lack of clear sub-peaks associated with the main peaks of biting jaw movements (Fig. 2A).

### 3.2. Total grazing jaw movements and number of bites per GJM

Total GJM and number of bites per GJM (PB) from the two automatic methods, SSBR and AR, are presented in Table 4. Analysis of variance showed a significant effect due to recording methods on the total number of GJM (F_{1, 40} = 4.2, P = 0.048), as well as on PB (F_{1, 40} = 12.9, P = 0.001). For total GJM and PB, the interactions between method and SSH were not significant (Table 4; F_{2, 40} = 1.45, P = 0.246 and F_{2, 40} = 3.12, P = 0.055; for total GJM and PB, respectively).

The total GJM were higher on M than on T, and on T than on S swards (F_{2, 40} = 8.7, P = 0.001). The PB ratios did not differ among SSSHs (F_{2, 40} = 2.9, P = 0.067, Table 4).

### 4. Discussion

Periods of fasting have been shown to significantly increase bite rate (Gregorini et al., 2007; Patterson et al., 1998). Nevertheless, in the present study the decision was taken to fast the animals prior to undertaking the measurements in order to ensure a 15-min period of intensive grazing for comparison of the different methodologies on different sward heights.

The VO method has been useful in demonstrating the effect of sward height on bite rate (Chilibroste et al., 2000; Erlinger et al., 1990; McGilloway et al., 1999; Mezzalir, 2009; Realini et al., 1999), as well as a method of reference in the development of automatic devices (Delagarde et al., 1999; Rutter et al., 1997) and software for data analysis (Clapham et al., 2011; Rutter, 2000). Although it could not be assumed that VO gave an exact count of the number of bites, in the conditions under which the VO method was used in the present study, we are confident that it represented a reliable estimation of the actual number of bites against which the automated methods could be compared.

The close agreement between the number of bites registered by VO and AR methods (Table 3), showed that the AR method can be a reliable technique for counting the number of bites. The advantage of both the VO and AR methods over the SSBR method is the ability of the operator to utilize the diagnostic aural cue for a bite as well as a simply visual one.

In contrast, the number of jaw movements identified as bites from the SSBR was significantly lower compared with the VO method. Also, the waveforms of the SSBR recordings were found to differ from those previously recorded with dairy cows and heifers grazing perennial ryegrass swards. The SSBR and its associated software ‘Graze’ were developed and validated for interpreting recordings made with cattle and sheep grazing temperate pastures (perennial ryegrass and/or white clover) in which sward heights varied between approximately 3 and 25 cm. As shown in Fig. 2, the wave patterns for the jaw movements recorded on the temperate perennial ryegrass swards (Fig. 2A; from a study described by Forbes et al., 2007, M.J. Gibb, pers. comm.) differ slightly in shape from those of the present study (Fig. 2B), but particularly in the presence of clear secondary sub-peaks associated with a main peak (one of the criteria used by the algorithms of the ‘Graze’ software to identify a jaw movement as a bite). The lack of such clear sub-peaks in recordings from the present experiment made distinction of bites from amongst the various jaw movements extremely difficult. The differences in waveforms could have been due to the sward height and structure, pasture species morphology, the age and grazing experience of the calves, and therefore, the way the animals behaved when confronted with these swards.
Comparing the two automated recording systems, in contrast with previous studies by Champion et al. (1998) and Ungar and Rutter (2006), numbers of bites determined by SSBR were consistently lower than those determined by AR. However, as stated previously, those studies were conducted with dairy cows grazing relatively short pastures. Some explanation for the relatively large discrepancies between the number of bites determined by SSBR and AR methods may be found by examination of the total GJM and PB values (Table 4). While a small difference (<5%) in total GJM was observed between SSBR and AR, the difference in PB values between the automatic methods was 19%. The difficulty, therefore, appears not to lie in the ability of the operator using ‘Graze’ to identify GJM, but to distinguish between biting and non-biting GJM (movements that are not bites and therefore include movements which are masticatory or manipulative in function (Gibb et al., 1999)).

Though well proven under temperate pasture conditions, the SSBR was clearly inferior to the AR system for determining the number of bites taken by calves grazing the winter oat swards offered in this experiment. Further potential advantages of the AR over SSBR are the accurate discrimination between compound jaw movements associated with the gathering or biting the herbage and chewing the material already in the mouth (Laca et al., 1994); manipulative jaw movements and bites (Laca and Wallis De Vries, 2000); the determination of plant parts or species being eaten; as well as the possible direct determination of dry matter intake (Galli et al., 2006).

Table 4
Total number of grazing jaw movements (GJM) and the number of bites per GJM (PB) identified from recordings made using solid-state behaviour recorders (SSBR) or acoustic recorder (AR) in calves grazing an Avena sativa sward with three different sward surface heights (SSH: T, tall; M, medium; S, short) for periods of 5 min.

<table>
<thead>
<tr>
<th>SSH</th>
<th>Total GJM</th>
<th>Mean</th>
<th>PB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SSBR</td>
<td>AR</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>392</td>
<td>402</td>
<td>397 a</td>
</tr>
<tr>
<td>M</td>
<td>418</td>
<td>424</td>
<td>421 c</td>
</tr>
<tr>
<td>S</td>
<td>354</td>
<td>395</td>
<td>374 b</td>
</tr>
<tr>
<td>Mean</td>
<td>388</td>
<td>407</td>
<td>395</td>
</tr>
</tbody>
</table>

| SEM a | 9.05 | 0.016 |
| Significance of effects (P) | Method | 0.048 | Method | 0.001 |
|                              | SSH   | 0.001 | Method | 0.067 |
|                              |       | 0.055 |

Means not sharing common letters (a, b, c) within a column differ by P < 0.05.

* SEM: standard error of the mean.
With regard to the effect of SSH, number of bites (Tables 2 and 3) and total GJM and PB ratio (AR method, Table 4) were higher in M than in T and S swards. This may have been due to the occurrence of a higher proportion of more manipulative, non-biting jaw movements during grazing in T and S than M, as suggested by the PB values from the AR method (Table 4). In the present experiment, where calves were offered a tall grass pasture, evidence from visual observation would suggest that animals in the T swards performed more head movements while trying to sever a bite. This could have led to an increase in handling time (Laca et al., 1994). Alternatively, in the S swards characterised by having the lowest lamina:pseudostam ratio (Table 1), evidence from visual observation would suggest that pseudostems could have represented a physical barrier making the act of severance of a bite more difficult to perform (Benvenuti et al., 2009).

5. Conclusions

Under the experimental conditions of the present study, the close agreement found between the number of bites estimated by VO and AR methods supports for the use of the AR method as an accurate method for identifying the biting jaw movements in grazing animals. A recent development of software for identifying and quantifying ingestive events – biting and non-biting jaw movements – from sound files (Clapham et al., 2011) appears to have overruled the limitation of the AR method. When used in young calves offered tall grass swards, as presented in this study, the SSBR failed to allow reliable discrimination between biting and non-biting grazing jaw movements.

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