

Prediction of bite number and herbage intake by an accelerometer-based system in dairy sheep exposed to different forages during short-term grazing tests

Questa è la versione Post print del seguente articolo:

Original

Prediction of bite number and herbage intake by an accelerometer-based system in dairy sheep exposed to different forages during short-term grazing tests / Giovanetti, V., Cossu, R., Molle, G., Acciaro, M., Mameli, M., Cabiddu, A., Serra, M.G., Manca, C., Rassu, S.P.G., Decandia, M., Dimauro, C.. - In: COMPUTERS AND ELECTRONICS IN AGRICULTURE. - ISSN 0168-1699. - 175:(2020), p. 105582. [10.1016/j.compag.2020.105582]

Availability:

This version is available at: 11388/236024 since: 2021-02-05T10:15:39Z

Publisher:

Published

DOI:10.1016/j.compag.2020.105582

Terms of use:

Chiunque può accedere liberamente al full text dei lavori resi disponibili come "Open Access".

Publisher copyright

note finali coverpage

(Article begins on next page)

This is the Author's accepted manuscript version of the following contribution:

Prediction of bite number and herbage intake by an accelerometer-based system in dairy sheep exposed to different forages during short-term grazing tests / Giovanetti, V.; Cossu, R.; Molle, G.; Acciaro, M.; Mameli, M.; Cabiddu, A.; Serra, M. G.; Manca, C.; Rassu, S. P. G.; Decandia, M.; Dimauro, C.. - In: COMPUTERS AND ELECTRONICS IN AGRICULTURE. - ISSN 0168-1699. - 175:(2020), p. 105582. [10.1016/j.compag.2020.105582]

The publisher's version is available at:

<https://dx.doi.org/10.1016/j.compag.2020.105582>

When citing, please refer to the published version.

1 **Highlights**

- 2
- Tri-axial accelerometer sensors are widespread to monitor behaviour of animals.
- 3
- Micro-swards were used to test the BEHARUM sensor to predict feeding behaviour of sheep.
- 4
- Partial least squares regression analysis predicted bite number and herbage intake in sheep.
- 5
- Reasonable accuracy and precision of the prediction models were found by the model evaluation
- 6 system.

7

8 Prediction of bite number and herbage intake by an accelerometer-based system in dairy sheep
9 exposed to different forages during short-term grazing tests

10

11 V. Giovanetti^a, R. Cossu^b, G. Molle^a, M. Acciario^a, M. Mameli^c, A. Cabiddu^a, M.G. Serra^a, C.
12 Manca^a, S.P.G. Rassu^b, M. Decandia^{a*}, C. Dimauro^b.

13 ^a AGRIS Sardegna, 07040 Olmedo, Italy

14 ^b Dipartimento di Agraria, Università di Sassari, viale Italia 39, 07100 Sassari, Italy

15 ^c Electronic Systems, Via Galileo Galilei 16, 07041 Alghero, Italy

16

17 * corresponding author

18 E-mail addresses: vgiovanetti@agrisricerca.it (V. Giovanetti), rossellacossu@uniss.it (R. Cossu),
19 gmolle@agrisricerca.it (G. Molle), macciario@agrisricerca.it (M. Acciario), ma.mameli@libero.it
20 (M. Mameli), acabiddu@agrisricerca.it (A. Cabiddu), gserra@agrisricerca.it (M.G. Serra),
21 cmanca@agrisricerca.it (C. Manca), pgrassu@unss.it (S.P.G. Rassu), mdecandia@agrisricerca.it
22 (M. Decandia), dimauro@uniss.it (C. Dimauro)

23

24 ABSTRACT

25 The accurate estimation of herbage intake is a key to adequately feed grazing ruminants. Ten dairy
26 Sarda sheep fitted with a halter equipped with an accelerometer (BEHARUM device) were allowed
27 to graze micro-swards of Italian ryegrass (*Lolium multiflorum* L.), alfalfa (*Medicago sativa* L.), oat
28 (*Avena sativa* L.), chicory (*Cichorium intibus* L.) and a mixture (Italian ryegrass and alfalfa) for six
29 minutes. Accelerometer data and video recordings of behaviour were collected simultaneously. The
30 raw acceleration data were processed to calculate 15 variables: sum, mean, variance and inverse

31 coefficient of variation (ICV, mean/standard deviation) for the X, Y and Z axes and the resultant. A
32 database was created that included the acceleration variables and herbage intake (FMI, DMI, g),
33 intake rate (FMIR, DMIR, g/minute), bite mass (FMBM, DMBM, g) either on fresh (FM) or dry
34 matter basis (DM) and the logarithm of number of bites (LB) and bite rate (LBR) measured during
35 the tests. Partial least square regression analysis (PLSR) was used to verify if acceleration variables
36 could be used as predictors of behavioural traits. The precision and accuracy of PLSR were
37 evaluated implementing the Model Evaluation System, in which predicted values were regressed
38 against observed ones, based on r^2 and Dent & Blackie test. The PLSR showed an overall good
39 accuracy (Dent & Blackie test $P=ns$) and was proven precise for the estimation of LB ($r^2=0.87$),
40 LBR ($r^2=0.86$), DMI and DMIR ($r^2=0.71$). To conclude, BEHARUM can accurately estimate with
41 high to moderate precision number of bites and herbage intake of sheep short term grazing
42 Mediterranean forages.

43

44 Keywords: feeding behaviour, accelerometer, dairy sheep

45

46 1. INTRODUCTION

47 Studying the feeding behaviour of ruminants and monitoring the energy intake during grazing is of
48 fundamental importance to improve feeding efficiency, animal productivity and pastures
49 management, respecting environment and animal welfare (Oudshoorn et al., 2013; Swain and
50 Friend, 2013). The productivity of grazing animals depends indeed on feed intake, which is a
51 difficult parameter to measure, especially for long periods of time (Milone et al., 2012). Intake can
52 be defined as the product of bite mass, bite rate and grazing time (Hodgson 1985), but its prediction
53 is very difficult especially because bite mass is the most variable component of grazing behaviour,

54 being influenced by a **number of factors** such as pasture characteristics, animal requirements, access
55 time to pasture, diet supplementation level and supplement composition.

56 Various methods to estimate daily intake at pasture exist but they are often invasive for the animals
57 and difficult to apply on rangeland (Bonnet et al., 2015). The grazing process involves selecting,
58 severing, chewing and swallowing the herbage. Therefore, it can be defined as a sequence of bite
59 and chew jaw movements that could have important implications for the estimation of intake
60 (Milone et al., 2012; Navon et al., 2013). The “chew-bite” complicates the monitoring of grazing
61 behavior. It consists of chewing herbage already in the mouth while the animal continues to graze
62 and new fresh herbage is severed with the same jaw movement (Galli et al., 2011; Milone et al.,
63 2012).

64 Several technologies have been developed to monitor animal jaw movements such as automatic
65 recording of electrical impulses (Rutter et al., 1997) or sounds (Ungar and Rutter, 2006). Acoustic
66 devices, for example, allow the evaluation of bite rate and can distinguish among different jaw
67 movements of ruminants, such as biting, chewing and even chew-biting. However, these methods
68 are still limited to simplified and controlled grazing conditions (Bonnet et al., 2015).

69 Outdoor application using microphones can be confused because of environmental noises.

70 Therefore, automatic recording and analyses of sounds still requires significant development.

71 Accurate direct measurement of biting and chewing activity was obtained recently by (Campos et
72 al., 2019) with goats using a surface electromyography based sensor system which was able to
73 estimate short-term fibre intake. Also, accelerometer sensors have been tested to automatically
74 count jaw movements (Umemura et al., 2009; Oudshoorn et al., 2013; Rombach et al., 2018).

75 However, as for acoustic sensors, the sensitivity of accelerometers could result in interferences and
76 undesirable signals during recording sessions. Therefore, significant developments are required in
77 order to isolate the signal relative to the jaw movements of grazing animals. Recent studies from
78 our laboratories have shown that a device based on a tri-axial accelerometer (BEHARUM) can

79 measure accurately the times devoted to grazing and to lesser extent, ruminating activities in sheep
80 grazing Mediterranean forages during short grazing sessions (Giovanetti et al., 2017; Decandia et
81 al., 2018). BEHARUM has been shown to perform well for estimating sheep number of bites
82 (Giovanetti et al., 2017) but has never been tested to estimate herbage intake rate and herbage intake
83 of grazing ruminants. This is regarded as a rather ambitious objective by some authors (Rombach et
84 al., 2019; Timmer et al., 2016) who state that the use of eating, chewing or prehension bites to
85 predict herbage dry matter intake provides accurate estimates only when combined with data on
86 animal and feed characteristics. With the above in mind, an experiment was undertaken to test the
87 hypothesis that automatic monitoring of foraging by an accelerometer-based device (BEHARUM)
88 can provide adequate estimation of short-term feeding behavior traits and herbage intake in sheep,
89 irrespective of further measurements on herbage and animal characteristics.

90 The particular objective of this current study was to derive a model to predict sheep behavioural
91 traits as number of bites, bite rate, bite mass, intake and intake rate, on the basis of variables
92 calculated from acceleration data recorded by BEHARUM. To challenge the ability of BEHARUM
93 to cope with the diversity of pasture characteristics (botanical composition, forage mass and plant
94 structure) typical of Mediterranean pastoral areas, the experiment included a wide range of forage
95 species offered either as monocultures or a binary mixture.

96

97 2. MATERIALS AND METHODS

98

99

100 2.1 Experimental design and location

101 Five treatments, four monocultures and one mixture, were established by sowing the forage species
102 in boxes to create micro-swards (Orr et al., 2005). The boxes were paired in individual racks and

103 offered to ten milking Sarda dairy sheep according to a 5x5 Latin-Square design with two
104 replications. The monocultures were: Italian ryegrass (*Lolium multiflorum* L., LL), alfalfa
105 (*Medicago sativa* L., AA), oat (*Avena Sativa* L., OO) and chicory (*Cichorium intibus* L., CC); the
106 mixture consisted of Italian ryegrass and alfalfa (LA). Within each replicate, the five experimental
107 animals were subjected to the treatments in succession. The order in which the tests, each of six min
108 duration for each treatment, were conducted within any day was randomized. The study was
109 conducted at Bonassai experimental farm of the agricultural research agency of Sardinia (AGRIS
110 Sardegna), located in the NW of Sardinia, Italy (40° 40' 16.215" N, 8° 22' 0.392" E, 32m a.s.l).

111

112 2.2 Micro-sward establishment and management

113 The four experimental monocultures and the mixture were sown in polystyrene boxes (28.5 cm x
114 46.5 cm x 16 cm) weighing 166 g when empty, at the Agriplant garden located at about 10 km from
115 AGRIS experimental farm. The forage species were chosen because they are widespread in
116 Mediterranean sheep production systems and stimulate a wide range of behavioural responses,
117 which have been already explored in a previous micro-sward study (Giovanetti et al., 2011). An
118 additional species, barley (*Hordeum vulgare* L.), was used to adapt the animals to the experimental
119 routine.

120 The polystyrene boxes had 18 mm drainage holes drilled in the base spaced at 10 cm (10 holes in
121 total). They were filled with a compost mix (CompaQstrat, N, P, K: 140, 160, 180 mg/l) and then
122 sown in February 2016 (on the 8th of February for LL, AA, CC, LA; on the 29th of February for
123 OO and barley) by an automatic planter at a rate of 600 seeds/m² in order to establish a plant
124 density similar to that reported in pastures grazed by dairy sheep. Before sowing alfalfa seeds were
125 inoculated with their specific rhizobium (*Ensifer meliloti* strains) and the seeds of the mixture (LA)
126 were thoroughly mixed. All boxes were subsequently covered with a layer of silica and immediately

127 irrigated. After sowing micro-swards were kept in a dark room at 18°C for three days to promote
128 seed germination.

129 Overall 275 boxes were sown (35 oats, 35 ryegrass, 35 chicory, 35 alfalfa, 35 mixture alfalfa +
130 ryegrass, 100 barley). The micro-swards were kept in a polytunnel from seed germination until
131 utilization. Each box was hand-watered when soil surface was deemed dry and then transported to
132 AGRIS experimental farm two days before the experiment started.

133

134 2.3 Animal management and training

135 The animal protocol implemented was in compliance with the EU Council Directive 98/58/EC
136 regulation on animal welfare and all measurements were taken by personnel previously trained and
137 authorized by the institutional authorities managing ethical issues both at Agris Sardegna and the
138 University of Sassari.

139 Two replicate groups of five Sarda lactating ewes, homogeneous for age (4 ± 0.2 yrs; means \pm SE),
140 body weight (43.7 ± 1.2 kg), body condition score (2.65 ± 0.06), stage of lactation (104 ± 2 DIM)
141 and milk production (1.220 ± 0.08 kg head⁻¹ day⁻¹) were used for the micro-swards test. Before the
142 beginning of the experiment, the selected ewes were fed at pasture and then accustomed to consume
143 only hay and concentrate in individual boxes, by gradually reducing the time spent at pasture (pre-
144 experimental period, 10 days). After this period, animal training began with an adaptation period of
145 4 days. Each animal was offered two micro-swards of barley in a rack for 6 minutes after morning
146 milking. The same protocol as during the experimental period was used. Animals were also trained
147 to wear the BEHARUM device (see section 2.4). All animals were machine milked once-a-day and
148 fed with a common basal diet consisting of alfalfa hay (0.4 kg head⁻¹ day⁻¹), ryegrass hay (1.2 head⁻¹
149 day⁻¹) and a commercial concentrate (0.5 head⁻¹ day⁻¹) split in two equal meals.

150

151 2.4 Description of BEHARUM device

152 The BEHARUM device consists of a halter equipped with a tri-axial accelerometer sensor
153 positioned under the lower jaw of the animal. This positioning allows to avoid the use of a
154 gyroscope or a counterweight for stabilization and, since the accelerometer measures accelerations
155 in the X (longitudinal), Y (horizontal) and Z (vertical) axes, it also enable to detect animal head and
156 jaw movements related to biting activity. The sensor is inserted in a micro-electromechanical
157 compact system (MEMS) with on-board peripherals, and samples raw accelerations at a frequency
158 of 62.5 Hz. The power supply of the system is guaranteed by two Lithium-Polymer (Li-Po) battery,
159 connected via on-board battery connector, with a duration of about 24 hours. The accelerations are
160 converted through an analogue-to digital converter with a resolution of 8 bits, in digital levels
161 ranging from 0 to 255. Three converted values per second per axis could be either sent (using a
162 LoRa wireless system) to a nearby computer receiver or recorded in a micro secure digital (SD)
163 card inserted in the MEMS. The BEHARUM device is activated or deactivated using software
164 (DAS Client, Electronic Systems, Italy) installed on a computer and manages data acquisition. In
165 this current experiment, we adopted the recording of acceleration on micro SD card (Giovanetti et
166 al., 2017; Decandia et al., 2018).

167

168 2.5 Experimental routine and measurements

169 During the five-day experimental period, 10 behavioural tests per day were conducted in
170 succession. Before the tests, 10 sward surface height (SSH) measurements were taken on each
171 micro-sward using a sward stick (Bircham, 1981) and the exterior surface of each box was cleaned
172 from soil, water or any other extraneous material in order to ensure that this material did not
173 become detached during the tests, biasing their results. After the morning milking animals were
174 fitted with the BEHARUM device and the two micro-swards of each treatment were offered to each
175 subject in a rack for six minutes (test time; Figure 1). After the test, the BEHARUM device was

176 removed and the animals had *ad libitum* access to hay in order to standardize post-ingestive effects
177 of the herbage meal. The behaviour of each experimental animal was video-recorded during the test
178 by a fixed camera (Sanyo Xacti VPC-TH1, Sanyo Electric Co., Ltd. OSAKA, Japan). During the
179 test, the observed animal could see and be seen by the other experimental animals. The micro-sward
180 boxes were weighed before and after each test to an accuracy of 0.5 g in order to determine the
181 biomass removed. Ungrazed boxes of the same forage species as that being tested were placed in
182 the same micro-environment as the grazed box and weighed directly before and after each test in
183 order to correct for evapotranspiration losses during the test period.

184 On two occasions during the experimental period, two micro-swards of the sown species, not
185 offered to the animals, were cut at the root-shoot interface after SSH measurement. The herbage
186 was then weighed to determine the biomass on offer. A fresh sub-sample of about 50 g was taken
187 and divided in laminae, stems and petiole to determine the sward structure. The remainder of the
188 sample was oven dried at 65°C for 72 hours and ground to pass a 1-mm screen to determine the
189 content of dry matter (DM), crude protein (CP), and neutral detergent fibre (NDF) by near infra-red
190 reflectance spectrometry (NIRS).

191 Hay and concentrate intakes were measured individually every day by weighing before the meals
192 were offered and the orts after (concentrate) or after 24 h (hay*). Video recordings were coded
193 manually, visually counting the number of prehension bites (including chew-bites) for each minute
194 (bite rate, BR) and then summing them to obtain the total number of bites made during the whole
195 eating time by each animal during the test. Total number of bites (NB) as well as the bite rate (BR)
196 were then log-transformed (LB and LBR, respectively), in order to obtain a normal distribution for
197 these variables. On the basis of weight, chemical measurements and video analysis the following
198 traits were calculated, with reference to each six minute test, as fresh (FM) and dry matter (DM):
199 herbage intake (FMI, DMI, g); intake rate (FMIR, DMIR, g min⁻¹); and bite mass (FMBM, DMBM,
200 g), calculated by dividing the herbage intake by the number of bites.

201

202 2.6 Preliminary data processing

203 Sum (SX , SY , SZ), mean (MX , MY , MZ), variance (VX , VY , VZ), inverse coefficient of variation
204 (i.e. mean/standard deviation, $ICVX$, $ICVY$, $ICVZ$) of acceleration data for each axis, as well as the
205 resultant mean ($MRES$), variance ($VRES$) and ICV ($ICVRES$) values of the three axes (Watanabe
206 *et al.*, 2008), were calculated for each six minute test. A dataset was then created including the
207 behavioural traits (LB , LBR , FMI , $FMIR$, DMI , $DMIR$, $FMBM$, $DMBM$) and the already
208 mentioned computed acceleration variables, giving a total of 23 variables and 50 records.

209

210 2.7 Statistical analyses

211 All behavioural variables (NB , LB , BR , LBR , FMI , $FMIR$, DMI , $DMIR$, $FMBM$ and $DMBM$) were
212 analyzed using the proc mixed for repeated measurement analysis (SAS Institute Inc, 2014) with
213 treatment as fixed effect and sheep, replicate and date as random effects based on the following
214 model:

$$215 Y_{ijkw} = \mu + \alpha_i + \beta_j + \lambda_k + \xi_w + \epsilon_{ijkw}$$

216 Where:

217 μ = overall mean

218 α_i = fixed effect of treatment

219 β_j = random effect of animal

220 λ_k = random effect of replicate

221 ξ_w = random effect of date

222 ϵ_{ijkw} = random error

223 Tukey's multiple comparison test was used to separate treatment estimated means ($P < 0.05$).

224 Regression analyses were performed to determine if the acceleration variables (SX, SY, SZ, MX,
225 MY, MZ, VX, VY, VZ, ICVX, ICVY, ICVZ, MRES, VRES, ICVRES) could be used as
226 explanatory variables of the response variables (FMI, FMIR, DMI, DMIR, FMBM, DMBM, LBR
227 and LB).

228 For this purpose, the partial least square regression (PLSR) was used because of its ability to handle
229 multivariate regression models with high collinearity among predictors and to make more efficient
230 prediction compared to ordinary multivariate regression or principal component regression
231 (Dimauro et al., 2011).

232 The general structure of the model was:

$$233 Y = XB + E$$

234 where Y is an $n \times m$ response matrix, X is an $n \times p$ design matrix, B is an $n \times m$ regression
235 coefficient matrix, and E is an $n \times m$ error term.

236 PLSR extracts a set of orthogonal new variables called latent factors, which are linear combinations
237 of the explanatory variables X, that best model the dependent variable Y. The maximum number of
238 latent factors depends on the size of X, which has a lower number of columns than Y (Dimauro et
239 al., 2013).

240 To validate the model a leave-one-out cross-validation method was used. The PLSR was carried out
241 with the PLS procedure (SAS Inst. Inc., Cary, NC). Finally, the precision and accuracy of the model
242 were assessed implementing the Model Evaluation System (MES, release 3.1.16, Tedeschi, 2006) in
243 which the predicted values were regressed against the observed ones. The evaluation of model
244 precision was based on the coefficient of determination (r^2) whereas that of model accuracy was
245 based on the Dent and Blackie test (Mayer et al., 1994), which simultaneously evaluates if the slope

246 of the regression of predicted upon observed values differs from 1 and if the regression intercept
247 differs from zero.

248

249 3. RESULTS

250

251 3.1 Forage characteristics and ingestive behaviour

252 Chemical and structural characteristics of offered forage treatments are presented in Table 1.

253 Chemical composition of forages was, as expected, characterized by low DM and NDF contents for
254 chicory, and a high CP value for alfalfa. Herbage mass on offer was comparable, even if a bit
255 higher, to that present in a pasture during the vegetative growth (Molle et al., 2004;) and was
256 characterized by a high percentage of leaves and a low percentage of stems in all forage treatments.

257 Forage species significantly ($P < 0.01$) affected all behavioural traits apart from FMI and FMIR
258 (Table 2). In particular, the number of bites and bite rate (NB and BR or expressed as logarithm, LB
259 and LBR) were significantly ($P < 0.01$) lower in CC than OO and LA. Although CC had the highest
260 fresh matter bite mass (FMBM), it also had lower dry matter bite mass values (DMBM) than AA.
261 This caused the lowest dry matter intake (DMI) for this forage species compared to the other
262 treatments. The dry matter intake rate (DMIR) was also lower in CC although it did not differ from
263 LA.

264

265 3.2 Prediction models

266 The predicted and observed values and results of the adequacy of predictions of the PLSR
267 procedure are shown in Figure 2. The PLSR was able to provide accurate estimates of the predicted
268 values for all dependent variables (Dent and Blackie Test P -value > 0.05). This meant that the

269 intercepts and slopes were not significantly different from 0 and 1, respectively, indicating that all
270 the regression lines passed through the origin and bisected the first quadrant angle.

271 The degree of precision of the models (r^2) was satisfactory, although it varied according to the
272 variable considered. Prediction of the number of bites as well as the bite rate, expressed as
273 logarithm (LB, LBR), reported the highest adjusted r^2 (0.87 and 0.86, respectively) followed by
274 DMI and DMIR variables ($r^2=0.71$) while the other traits (DMBM, FMBM, FMI and FMIR)
275 showed r^2 values ranging between 0.42 and 0.67.

276

277 4. DISCUSSION

278 4.1 Forage characteristics and ingestive behaviour

279 As expected, the different forage treatments significantly influenced almost all behavioural
280 variables (Table 2). This agreed with a previous micro-sward study (Giovanetti et al., 2011).
281 Chicory presented major differences with the other forages showing a significantly lower number of
282 bites (LB) compared to OO, LL and LA. This could be due to the high fresh matter bite mass
283 (FMBM) of CC which increased the time required for sheep to chew each bite. As reported by
284 Gregorini et al., (2013) in a study with dairy cows, the high leaf percentage and elevated sward
285 surface height of chicory (Table 1) allows greater accessibility of the forage. Therefore, sheep are
286 required to take the highest FMBM resulting in more mouth-handling and mastication before
287 swallowing. This process slows BR and since CC in the current study had very low dry matter
288 content compared to the other treatments, especially AA, dry matter bite mass (DMBM) was
289 reduced. These combined factors caused a low DMIR for chicory and the lowest herbage dry matter
290 intake (DMI).

291

292 4.2 Prediction models

Commentato [G1]: nella proof c'è scritto 2 volte

Commentato [G2]: nell proof c'è un ";", sostituire con ",,"

293 The aim of this work was to derive a model to predict sheep behavioural traits related to herbage
294 intake based on accelerometer variables. The literature is limited on the use of acceleration sensors
295 for the identification and classification of jaw movements, bite mass and intake. A modified
296 pedometer as a pendulum attached to a collar was used by Umemura et al., (2009) to count cattle
297 jaw movements. The system could not distinguish between prehension and mastication bites with
298 an accuracy of 90% compared to manual counts over 10 min segments. They also found that feed
299 intake when estimated indirectly as pasture disappearance by a rising plate meter could be predicted
300 by applying linear regressions to the number of bite counts although the slopes of the regression
301 lines based on pasture data were dependent on the level of available pasture mass. Correlation
302 coefficients greater than 0.90 were obtained with a subsequent experiment (Umemura, 2013) and it
303 was possible to distinguish grazing bites using a pedometer recording back-forth movements
304 installed on neck collars of cows.

305 In the current study, conducted under controlled conditions, PLSR was able to provide accurate and
306 precise estimates of the number of bites (LB) and bite rate (LBR) expressed as logarithm with
307 adjusted r^2 of 0.87 and 0.86, respectively (Figure 2). The BEHARUM system was unable to
308 distinguish bites from chew-bites but in agreement with Umemura et al., (2009), it is not strictly
309 necessary to distinguish between bite types to estimate feed intake. The detection of head
310 movements associated with herbage prehension could be sufficient.

311 A 1-axis accelerometer coupled to a microphone was used by Tani et al., (2013) to distinguish cattle
312 chewing activities. The device was able to achieve 90% accuracy, reaching 99% when the sensor
313 was attached to the cow's horn. Using a 3-axis accelerometer Oudshoorn et al., (2013) were able to
314 record cow bites at pasture. A number of threshold values were tested to determine the peak with
315 the best correlation to the observations. Their average correlation coefficient was only 0.65, lower
316 than the partial agreement with visual observation ($r^2=0.65$) obtained in our previous experiment
317 (Giovanetti et al., 2017) with grazing sheep. Andriamandroso et al., (2015) used a smartphone

Commentato [G3]: ripetuto 2 volte nella proof

Commentato [G4]: se lasciamo could not...la frase non ha molto senso. Io toglierei "not". Se hai la pubblicazione verifica un attimo.

Commentato [G5]: manca una parentesi....e segue un ; che va sostituito con la ,

Commentato [G6]: nella proof c'è il " ;"

Commentato [G7]: verificare le ()

318 inertial measurement unit (IMU) which combined accelerometers, gyroscope, magnetometer and
319 location sensors, to count the number of bites through frequency pattern of 1-axis acceleration data,
320 achieving a mean error of 4 to 5% when compared with visual observations.

321 The above results confirm the difficulty to count bites using an accelerometer in free ranging
322 animals. More recently (Rombach et al., 2018) tried to validate the RumiWatch System (RWS; Itin
323 and Hoch GmbH, Liestal, Switzerland) for the determination of ingestive and rumination
324 behaviours of dairy cows during grazing and supplementation in the barn. The algorithms tested in
325 the evaluation software were not able to differentiate between mastication and true prehension bites
326 while eating. Indeed, the number of prehension bites was overestimated both for grazing and
327 supplemented cows. They achieved a low relative prediction error (≤ 0.10) for the number of
328 rumination boluses, rumination chews, and total eating chews. However, a high relative prediction
329 error (> 0.10) for the number of prehension bites and time spent in prehension and eating was found.

330 As the estimate of free-grazing animals intake is arduous because of the difficulty of accurately
331 establishing the weight of each bite (BM), the current study used sown micro-sward boxes (Orr et
332 al., 2005). The average BM was determined by weighing the micro-swards before and after the
333 animal fed. The estimation of bite mass through accelerometer data, both expressed on fresh
334 (FMBM) or dry matter basis (DMBM) was found to be accurate (Dent & Blacky test non-

335 significant), but not precise ($r^2=0.42$). Better results were obtained by Bonnet *et al.* (2015), who
336 combined the hand-plucking method with acoustic sensors coupled with continuous bite
337 monitoring, that achieved an accuracy ranging between 80% and 94% in a short-term intake study.

338 The prediction of intake and intake rate based on the continuous automated recording and analysis
339 of acceleration variables was accurate and overall precise, being slightly more precise when
340 expressed on dry (DMI and DMIR, $r^2=0.71$) than fresh matter basis (FMI and FMIR, $r^2=0.67$ and
341 0.68, respectively), regardless the type of forage. This important result was however obtained in a
342 situation in which the animal was exposed for a short time to a familiar, palatable sward and for that

Commentato [G8]: verificare ()

Commentato [G9]: nella proof c'è il ";"...sostituire

343 reason an intense grazing pressure was exerted on it. Galli et al., (2011) using acoustic method in
344 sheep exposed to micro-swards derived a model which predicted herbage DMI on the basis of
345 energy in the chewing sound with a precision slightly higher than for our model ($r^2=0.79$). Similar
346 to the BEHARUM model, the acoustic model did not consider either the pasture depletion level or
347 the forage type.

348

349 5. CONCLUSIONS

350 The BEHARUM system appears very promising to accurately and precisely estimate the number of
351 bites for sheep grazing short sessions of diverse Mediterranean forages. Moreover, the system
352 provides an accurate estimate of herbage DMI and herbage DMIR, even if at a lower level of
353 precision. This is despite the rather poor performance of BEHARUM for the estimation of the bite
354 mass. Overall, caution is advised when using BEHARUM or other accelerometer-based devices to
355 estimate herbage intake in long grazing sessions when a wider variety of behavioural activities can
356 be expressed by the grazing animal and bite mass can markedly change during the grazing session.

357 A combination of information provided by different sensors and the external validation of the
358 derived models will probably allow better estimates of herbage intake in the future. Further research
359 and development efforts should be devoted to the automation of data analysis, a bottle-neck for the
360 transfer of these technologies to the commercial farm level.

361

362 ACKNOWLEDGEMENTS

363 The financial support (Projects CRP-17287 PO Sardegna, FSE 2007–2013 LR 7/2007) of the
364 Government of Sardinia (Italy) is gratefully acknowledged. L. Bertuolo for help in micro-sward
365 establishment. S. Picconi, S. Pintus, G. Scanu, M. Niolu, M. Delrio, and M.A. Sanna for their
366 technical support.

Commentato [G10]: verifica parentesi

367

368 6. REFERENCES

369 Andriamandroso, A.L.H., Lebeau, Frédéric; Bindelle, J., 2015. Changes in biting characteristics
370 recorded using the inertial measurement unit of a smartphone reflect differences in sward
371 attributes, in: 7th Conference on Precision Livestock Farming. pp. 283–289.

372 Bircham, J. S. 1981. Herbage growth and utilization under continuous stocking management. PhD
373 Thesis. University of Edinburgh, Edinburgh, UK.

374 Bonnet, O.J.F., Meuret, M., Tischler, M.R., Cezimbra, I.M., Azambuja, J.C.R., Carvalho, P.C.F.,
375 2015. Continuous bite monitoring: A method to assess the foraging dynamics of herbivores in
376 natural grazing conditions. *Animal Production Science* 55, 339–349. doi:10.1071/AN14540

377 Campos, D.P., Abatti, P.J., Bertotti, F.L., De Paula Vieira, A., Hill, J.A.G., da Silveira, A.L.F.,
378 2019. Short-term fibre intake estimation in goats using surface electromyography of the
379 masseter muscle. *Biosystems Engineering* 183, 209–220.
380 doi:10.1016/j.biosystemseng.2019.04.021

381 Decandia, M., Giovanetti, V., Molle, G., Acciaro, M., Mameli, M., Cabiddu, A., Cossu, R., Serra,
382 M.G., Manca, C., Rassu, S.P.G., Dimauro, C., 2018. The effect of different time epoch settings
383 on the classification of sheep behaviour using tri-axial accelerometry. *Computers and*
384 *Electronics in Agriculture* 154, 112–119. doi:10.1016/j.compag.2018.09.002

385 Dimauro, C., Cellesi, M., Gaspa, G., Ajmone-Marsan, P., Steri, R., Marras, G., Macciotta, N.P.P.,
386 2013. Use of partial least squares regression to impute SNP genotypes in Italian Cattle breeds.
387 *Genetics Selection Evolution* 45, 1–8. doi:10.1186/1297-9686-45-15

388 Dimauro, C., Steri, R., Pintus, M.A., Gaspa, G., MacCiotta, N.P.P., 2011. Use of partial least
389 squares regression to predict single nucleotide polymorphism marker genotypes when some
390 animals are genotyped with a low-density panel. *Animal* 5, 833–837.

391 doi:10.1017/S1751731110002600

392 Galli, J.R., Cangiano, C.A., Milone, D.H., Laca, E.A., 2011. Acoustic monitoring of short-term
393 ingestive behavior and intake in grazing sheep. *Livestock Science* 140, 32–41.
394 doi:10.1016/j.livsci.2011.02.007

395 Giovanetti, V., M. Decandia, M. Acciaro, A. Cabiddu, M. Sitzia, S. Picconi, and G. Molle. 2011. A
396 short-term test to assess sheep propensity towards Mediterranean forages offered as micro-
397 swards. *Proc. of 8th International symposium on the nutrition of herbivores. Advances in*
398 *Animal Biosciences*. Pp 314.

399 Giovanetti, V., Decandia, M., Molle, G., Acciaro, M., Mameli, M., Cabiddu, A., Cossu, R., Serra,
400 M.G., Manca, C., Rasso, S.P.G., Dimauro, C., 2017b. Automatic classification system for
401 grazing, ruminating and resting behaviour of dairy sheep using a tri-axial accelerometer.
402 *Livestock Science* 196, 42–48. doi:10.1016/j.livsci.2016.12.011

403 Gregorini, P., Minnee, E.M.K., Griffiths, W., Lee, J.M., 2013. Dairy cows increase ingestive
404 mastication and reduce ruminative chewing when grazing chicory and plantain. *Journal of*
405 *Dairy Science* 96, 7798–7805. doi:10.3168/jds.2013-6953

406 Hodgson, J. 1985. The control of herbage intake in the grazing ruminant. *Proc. of the Nutrition*
407 *Society* 44, 339–346.

408 Mayer, D.G., Stuart, M.A., Swain, A.J., 1994. Regression of real-world data on model output: an
409 appropriate overall test of validity. *Agricultural Systems* 45, 93–104.

410 Milone, D.H., Galli, J.R., Cangiano, C.A., Rufiner, H.L., Laca, E.A., 2012. Automatic recognition
411 of ingestive sounds of cattle based on hidden Markov models. *Computers and Electronics in*
412 *Agriculture* 87, 51–55. doi:10.1016/j.compag.2012.05.004

413 Molle, G., Decandia, M., Ligios, S., Fois, N., Treacher, T.T., Sitzia, M., 2004. Grazing management
414 and stocking rate with particular reference to the Mediterranean environment. In: Pulina, G.
415 (Ed.), Dairy Sheep Nutrition. CAB International, pp. 191–211.

416 Navon, S., Mizrach, A., Hetzroni, A., Ungar, E.D., 2013. Automatic recognition of jaw movements
417 in free-ranging cattle, goats and sheep, using acoustic monitoring. *Biosystems Engineering*
418 114, 474–483. doi:10.1016/j.biosystemseng.2012.08.005

419 Orr, R.J., Young, K.L., Cook, J.E., Champion, R.A., 2005. Development of a micro-sward
420 technique for determining intake characteristics of perennial ryegrass varieties. *Euphytica* 141,
421 65–73. doi:10.1007/s10681-005-5365-y

422 Oudshoorn, F.W., Cornou, C., Hellwing, A.L.F., Hansen, H.H., Munksgaard, L., Lund, P.,
423 Kristensen, T., 2013. Estimation of grass intake on pasture for dairy cows using tightly and
424 loosely mounted di- and tri-axial accelerometers combined with bite count. *Computers and*
425 *Electronics in Agriculture* 99, 227–235. doi:10.1016/j.compag.2013.09.013

426 Rombach, M., Münger, A., Niederhauser, J., Südekum, K.H., Schori, F., 2018. Evaluation and
427 validation of an automatic jaw movement recorder (RumiWatch) for ingestive and rumination
428 behaviors of dairy cows during grazing and supplementation. *Journal of Dairy Science* 101,
429 2463–2475. doi:10.3168/jds.2016-12305

430 Rombach, M., Südekum, K.H., Münger, A., Schori, F., 2019. Herbage dry matter intake estimation
431 of grazing dairy cows based on animal, behavioral, environmental, and feed variables. *Journal*
432 *of Dairy Science* 102, 2985–2999. doi:10.3168/jds.2018-14834

433 Rutter, S.M., Champion, R.A., Penning, P.D., 1997. An automatic system to record foraging
434 behaviour in free-ranging ruminants. *Applied Animal Behaviour Science* 54, 185–195.
435 doi:10.1016/S0168-1591(96)01191-4

436 SAS Institute Inc, 2014. SAS Institute Inc., SAS Institute Inc. MarketLine Company Profile.

437 Swain, D.L., Friend, M.A., 2013. Opportunities for telemetry techniques in studies on the
438 nutritional ecology of free-ranging domesticated ruminants. *Animal* 7, 123–131.
439 doi:10.1017/S1751731112000870

440 Tani, Y., Yokota, Y., Yayota, M., Ohtani, S., 2013. Automatic recognition and classification of
441 cattle chewing activity by an acoustic monitoring method with a single-axis acceleration
442 sensor. *Computers and Electronics in Agriculture* 92, 54–65.
443 doi:10.1016/j.compag.2013.01.001

444 Tedeschi, L.O., 2006. Assessment of the adequacy of mathematical models. *Agricultural Systems*
445 89, 225–247. doi:10.1016/j.agsy.2005.11.004

446 Timmer, B., R. L. G. Zom, G. Holshof, M. Spithoven, and C. G. Van Reenen. 2016. The
447 application of behavior sensors and sward measurement to support grazing management. In:
448 M. Höglind, A. K. Bakken, K. A. Hovstad, E. Kallioniemi, H. Riley, H. Steinshamn, and L.
449 Østrem, ed. Wageningen Academic Publishers, Proceedings of the 26th General Meeting of
450 the European Grassland Federation Trondheim, Norway 4-8 September 2016. Pages 80–82

451 Umemura, K., Wanaka, T., Ueno, T., 2009. Technical note: Estimation of feed intake while grazing
452 using a wireless system requiring no halter. *Journal of dairy science* 92, 996–1000.
453 doi:10.3168/jds.2008-1073

454 Umemura, K., 2013. Technical note: Monitoring grazing bites and walking activity with
455 pedometers. *Journal of dairy science* 96, 1090–1093. doi: 10.3168/jds.2012-5872

456 Ungar, E.D., Rutter, S.M., 2006. Classifying cattle jaw movements: Comparing IGER Behaviour
457 Recorder and acoustic techniques. *Applied Animal Behaviour Science* 98, 11–27.
458 doi:10.1016/j.applanim.2005.08.011

459 Watanabe, N., Sakanoue, S., Kawamura, K., Kozakai, T., 2008. Development of an automatic
460 classification system for eating, ruminating and resting behaviour of cattle using an

461 accelerometer. Japanese Society of Grassland Science 54, 231–237.

462 <http://dx.doi.org/10.1111/j.1744-697X.2008.00126.x>.

463

464 **Table 1.** Chemical composition (on DM basis) and sward structure of forage species offered as
 465 micro-swards during the behavioural test.

	OO	LL	CC	AA	LA
Chemical composition					
DM (%)	12.27	12.92	7.64	13.68	11.40
CP (% DM)	23.24	19.48	20.54	25.46	24.10
NDF (% DM)	46.80	49.74	33.52	36.76	45.53
Sward structure					
Herbage mass (g DM m ⁻²)	767	742	748	715	765
SSH (cm)	30.5	20.0	26.6	26.2	19.5
Leaves (% FM)	73.9	82.0	90.1	61.0	74.5
Stems (% FM)	25.6	17.8	9.5	38.9	25.5
Leaves (% DM)	85.1	84.5	100.0	66.3	54.4
Stems (% DM)	14.9	15.5	0.0	33.7	45.6

466 OO=oats; LL=Italian ryegrass; CC=chicory; AA=alfalfa; LA=mixture of Italian ryegrass and
 467 alfalfa. SSH=sward surface height; FM=fresh matter; DM=dry matter.

468

469 **Table 2.** Behavioural traits of dairy sheep fed different forage micro-sward treatments (least square mean \pm SE)

	OO	LL	CC	AA	LA	P<
NB (n)	280 \pm 29 ^a	255 \pm 28 ^a	168 \pm 28 ^b	205 \pm 28 ^{ab}	266 \pm 28 ^a	0.01
LB (n)	2.44 \pm 0.06 ^a	2.36 \pm 0.06 ^{ab}	2.17 \pm 0.06 ^b	2.29 \pm 0.06 ^{ab}	2.39 \pm 0.06 ^a	0.01
FMI (g)	316 \pm 37	299 \pm 37	302 \pm 37	287 \pm 37	294 \pm 37	ns
DMI (g)	38 \pm 4.6 ^a	39 \pm 4.6 ^a	23 \pm 4.6 ^b	40 \pm 4.6 ^a	33 \pm 4.6 ^a	0.001
FMBM (g)	1.13 \pm 0.1 ^b	1.24 \pm 0.1 ^b	1.85 \pm 0.1 ^a	1.46 \pm 0.1 ^b	1.10 \pm 0.1 ^b	0.001
DMBM (g)	0.14 \pm 0.01 ^b	0.16 \pm 0.01 ^{ab}	0.14 \pm 0.01 ^b	0.20 \pm 0.01 ^a	0.12 \pm 0.01 ^b	0.001
BR (n min ⁻¹)	43.3 \pm 4.5 ^a	39.2 \pm 4.4 ^a	25.9 \pm 4.4 ^b	31.7 \pm 4.4 ^{ab}	41.0 \pm 4.4 ^a	0.01
LBR (n min ⁻¹)	1.63 \pm 0.07 ^a	1.55 \pm 0.07 ^{ab}	1.36 \pm 0.07 ^b	1.48 \pm 0.07 ^{ab}	1.58 \pm 0.07 ^a	0.01
FMIR (g min ⁻¹)	48.59 \pm 5.7	45.97 \pm 5.7	46.64 \pm 5.7	44.44 \pm 5.7	45.34 \pm 5.7	ns
DMIR (g min ⁻¹)	5.93 \pm 0.7a	6.03 \pm 0.7a	3.56 \pm 0.7b	6.12 \pm 0.7a	5.16 \pm 0.7ab	0.001

470 OO= oats; LL= Italian ryegrass; CC= chicory; AA= alfalfa; LA= mixture of Italian ryegrass and alfalfa; NB=
471 number of bites; LB= logarithm of number of bites; FMI= fresh matter intake; DMI= dry matter intake; FMBM=
472 fresh matter bite mass; DMBM= dry matter bite mass; BR= bite rate; LBR= logarithm of bite rate; FMIR= fresh
473 matter intake rate; DMIR= dry matter intake rate; values in the same row with different letters differ significantly
474 (P<0.05).



475

476 **Figure 1.** Layout of micro-sward test for sheep fitted with the BEHARUM device. While the animal face the
477 two micro-swards of the forage under study, its behaviour is video recorded by a fixed camera. Ungrazed box
478 of the same forage species as that being tested is placed in the same micro-environment as the grazed boxes.

479

480

481

482

483

484

485

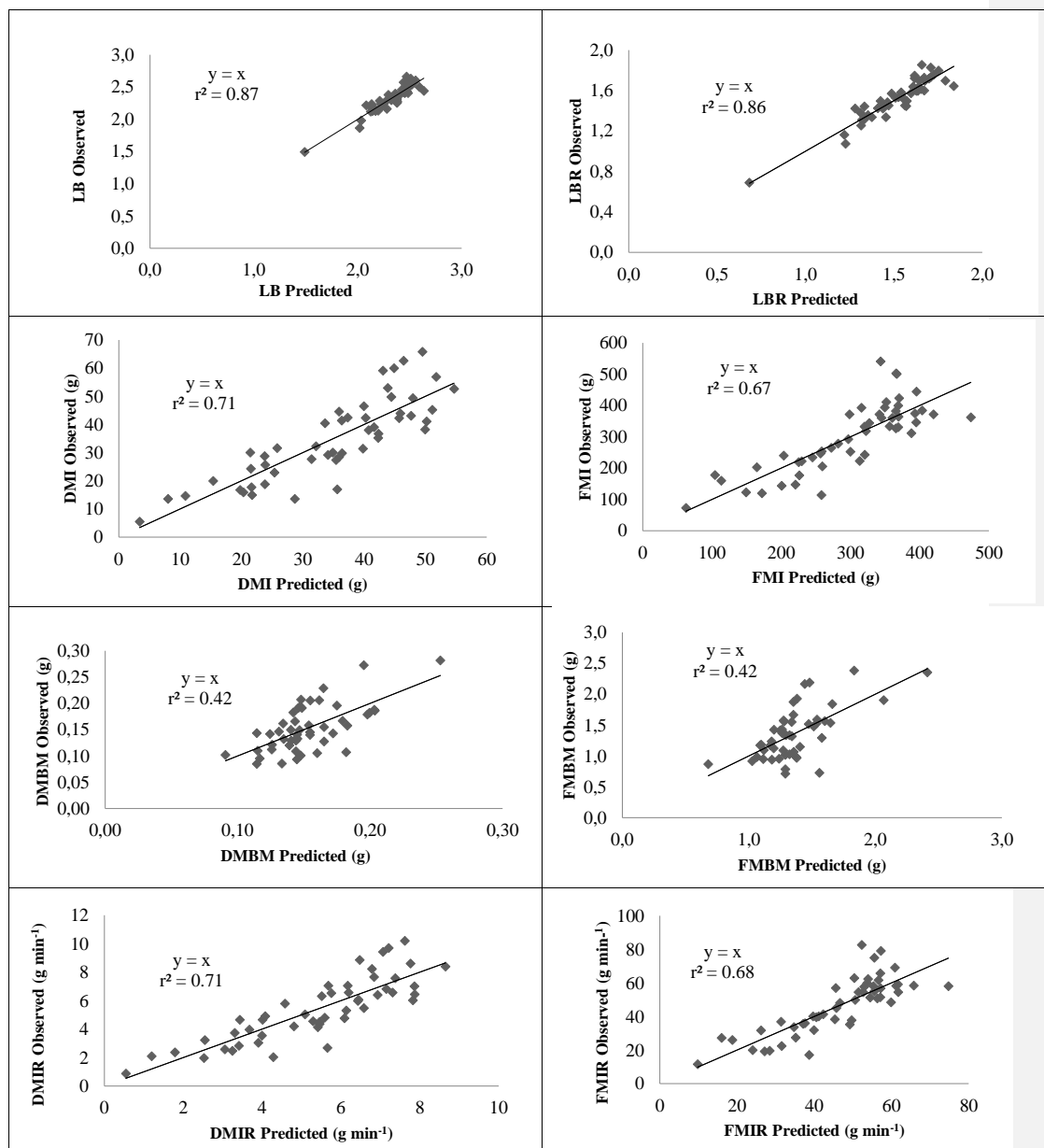
486

487

488

489

490



491 **Figure 2.** Observed versus predicted plots for behavioural variables determined from sheep grazing micro-
 492 sward boxes of different pasture species. LB=logarithm of number of bites; LBR =logarithm of bite rate;
 493 DMI=dry matter intake; FMI=fresh matter intake; DMBM=dry matter bite mass; FMBM=fresh matter bite
 494 mass; DMIR=dry matter intake rate; FMIR=fresh matter intake rate.