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ESTIMATION OF GRASS BIOMASS CONSUMED BY RABBITS HOUSED IN MOVABLE PADDOCKS

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Abstract: Biomass allowance is a key feature in pasture-based rabbit production systems. It conditions not only the stock density (rabbits/m^2) and/or the number of grazing days, it also influences the grazing behaviour of animals. When herbage restriction occurs, pelleted feed and/or cereal intake goes up. Inadequate pasture management may also impair the biomass quantity and quality if overgrazing occurs. To avoid the undesirable effects of overgrazing and better manage pellet and cereal intake, information on both biomass availability and rabbits' grazing capacity are needed. Here, we present an adaptation of the rising plate meter method (developed for biomass intake measures for ruminants) for use in rabbit. To this end, we designed an experiment where two groups of 12 rabbits each were kept in two different fields: under an apple orchard (AO) or on fallow land (FL). We followed the animals for 5 consecutive weeks (from 45 to 80 d old). Rabbits lived in 25 m^2 movable paddocks, and every week a new paddock location (called paddock-spot) was made available for them. At each new paddock-spot, we measured the herbage height inside the paddocks and performed samplings of the available biomass (*i.e*. herbage cut after herbage height measurement) outside the paddocks. From this data we estimated the available biomass inside each paddock-spot by fitting linear regression equations of biomass to herbage height. Overall, rabbits in the AO and FL had access to 1328±65.7 and 1386±58.6 kg of dry matter (DM) per ha, respectively. In every field and paddock-spot, the biomass available was lower than the rabbits' grazing capacity; overgrazing was the rule. Roughly, and under a restricted herbage allowance, rabbits in the AO ingested 45.2 g DM/d and rabbits in the FL 43.4 g DM/d. In the last week (64 to 80 d old), the biomass intake of rabbits in the AO and AL represented 26.4 and 23.5% of the total DM intake, respectively. These values, however, does not represent the real grazing capacity of growing rabbits. In this study, we provide some advice on the sampling method to obtain reliable biomass estimations and we mention two methods for handling influential observations in linear regression.

Key Words: *Oryctolagus cuniculus*, grazing, rising plate meter, biomass, cage-free housing, rabbit.

INTRODUCTION

Rabbit farming systems based on outdoor access to pasture are emerging (Roinsard *et al.*, 2016). In France, we count around 50 organic rabbit breeders (Gidenne *et al.*, 2022), raising about 30 females each (Huang *et al.*, 2021). Although organic (outdoor pasture-based) rabbit production is still a niche market, this production system may be boosted by the current societal demands (*i.e.* European Citizens' Initiative '*End the Cage Age*') and the legislation to come (European Commission, 2021). Moreover, pasture-based rabbit farming may contribute to the agroecological transition through the emergence of integrated crop-livestock (or crop-rabbit) systems - a way of food production that presents a series of ecological, societal and cultural benefits (Bonaudo *et al.*, 2014).

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Grass, and more generally, grasslands have many advantages (Bengtsson *et al.*, 2009). These ecosystems provide services such as water regulation (erosion prevention, flood regulation, etc.) and supply, carbon sequestration, biodiversity conservation and landscape maintenance. They are also a source of cultural ecosystem services like tourism or recreation. Nonetheless, the most studied benefit of grass and grassland is biomass (fodder) used for animal production.

Although the virtues of grasslands and the nutritional qualities of forage species are well documented for ruminants and horses (*c.f.* https://www.feedipedia.org/), few studies have attempted to determine the use of grass by farm rabbits (Martin *et al.*, 2016; Legendre *et al.*, 2019, Fetiveau *et al.*, 2021). Moreover, to date, we still do not know if it is possible to raise rabbits commercially on grass alone, without any input of compound feed pellets or cereals. In addition to this lack of information, the methods used to estimate rabbits grass intake capacity should be refined. For instance, Martin *et al.* (2016) and Legendre *et al.* (2019) used an indirect method for mobile-cages. They cut a small surface outside the rabbits' enclosure and used the biomass measured in this area to estimate the biomass available for rabbits, with no information on the grass composition or height inside the rabbits' enclosure. Another source of error relates to the intake estimation calculated from the complete sampling of the grass not ingested by rabbits inside the enclosure; in some cases, the biomass ingestion could be negative. To reduce the accumulation of sampling errors, Fetiveau *et al.* (2021) used an alternative method. They estimated the biomass inside the rabbits' paddock by cutting the herbage at several points before and after the introduction of rabbits. This practice, however, presents the disadvantage of reducing the herbage allowance for the animals.

Fortunately, other methods, such as the rising plate meter (Stockdale and Kelly, 1984), the n-alkanes (Mayes *et al.*, 1986) or net-energy intake estimation (Smit *et al.*, 2005), all developed in ruminants, may be also adapted for rabbits. Among these methods, the rising plate meter presents a series of advantages. This method is less time-consuming and cheaper than the n-alkanes method, with no need for special formulations containing the n-alkanes or complex laboratory analyses to estimate the residuals in faeces (Smit *et al.*, 2005; see practical considerations). Compared to the net-energy method, the rising plate meter is easier to implement. It also requires less information and fewer calculations. It also provides information on the real state of the pasture. Additionally, the rising plate meter is presented as robust, repeatable and easy to use, making this method feasible for farmers.

Based on the need for a better description of the rabbits' herbage intake capacity under pasture conditions, the aim of this study is to present the adaptation of the rising plate meter method for measuring the biomass availability for growing rabbits raised in movable paddocks.

MATERIAL AND METHODS

Animals

We followed 24 young crossbreed (¼ Fauve-de-Bourgogne×½ INRA-1777×¼ Belier) rabbits from 45 to 80 d of age. At 35 d of age, animals were vaccinated against rabbit haemorrhagic diseases (VHD 1 and VHD 2) and myxomatosis. At this date, we also identified and weaned the animals in wired cages (wide×long×height: 92×90×80 cm) with plastic flooring and platforms. Ten days after weaning (45 d old), we transferred the rabbits from the INRAE Occitanie-Toulouse Rabbit Experimental Station (43° 31' 51.69" N; 1° 29' 52.51" E), France, to the apple orchard of the INRAE PACA-Gotheron (44° 58' 27.12' N; 4° 55' 49.44" E), France. The experiment took place in spring 2022, between March 21st and April 25th.

Housing conditions

We housed half of the animals (6 males and 6 females) in a 25 m² paddock equipped with a wood shelter (Figure 1) placed in an apple orchard (AO). The AO was planted in 2005. There is a 5-metre space between tree lines and the trees are spaced 2 m apart. This results in a density of 1000 trees per ha. We housed the remaining rabbits (6 males and 6 females) in a plot of fallow land (FL), in paddocks with the same dimensions and features as those in the AO.

We recorded the temperature and relative moisture inside the wood shelters by placing data loggers (OM-CP-RHTEMP101A-Logger, Omega Engineering Inc., Norwalk, CT 06854, USA) in the rabbits' resting area,

Figure 1: Mobile paddock (25 m^2) and wood shelters (0.75 m^2; 125×60 cm) for a group of 12 rabbits. Left, Apple Orchard. Right, Fallow Land (paddock-spot 1; 12 d before the introduction of animals). Photos: ®INRAE/Savietto.

about 45 cm from the top floor and 5 cm from the roof. We obtained information on the outdoor relative humidity, temperature, rainfall and wind-speed from the weather station located at the INRAE PACA-Gotheron Centre.

Climatic conditions

Outdoor temperatures varied from 0 to 23.4°C (average: 11.5±5.4°C). The lowest and highest thermal amplitudes registered were 3.2 and 19.6°C (average: 11.4°C), respectively. Relative humidity and wind-speed values ranged from 18.0 to 99.0% and 1.0 to 14.0 m/s, for a respective average of 63.1±19.7% and 5.3±3.1 m/s. During the whole period, the cumulated precipitation height was 55 mm.

Average temperatures and relative humidity inside the AO shelter were 18.1±8.3°C and 67.4±21.9%. Values inside the FL shelter were 17.0±8.6°C and 67.1±23.9%.

On average, and for spring 2022, rabbits were exposed to mild temperatures throughout the experiment. Shelters seemed to provide an adequate temperature within the rabbit's comfort zone (from 15 to 25°C; Cervera and Fernández-Carmona, 2010). The average heat indices, calculated from the temperature and relative humidity inside the AO and FL shelters (http://www.wpc.ncep.noaa.gov/html/heatindex.shtml), were 18 and 17°C, respectively.

Experimental procedures

We introduced the animals to the different fields late in the afternoon, when rabbits were 45 d old. They had no access to the field on the first night, only to their shelter. In the next morning and before letting the animals graze, we performed the grass measurements inside the paddocks.

In an attempt to provide the maximum biomass throughout the experiment, we decide to move the paddocks to a new location (hereinafter called paddock-spot) approximately every week. We moved the paddocks on both AO and FL on the same day. On the moving day, we held the animals inside the shelter before moving the paddocks. When the transfer was complete, we opened the shelters (no more than 10 min apart from one field to another) to provide animals access to the new grazing area. In practice, the rabbits spent eight, seven, five, eight and six days in the paddock-spot 1, 2, 3, 4, and 5, respectively. We performed all grass measures before moving the paddocks to a new spot.

Rabbits had free access to water and pelleted feeds (11.3 MJ of digestible energy per kg of dry matter (DM), 17.8% of crude protein, 2.8% of fat, 40.1% neutral detergent fibre, 22.7% acid detergent fibre and 7.9% acid detergent lignine on a DM basis) at all times. Diets contained no antibiotics or coccidian treatment. We measured the pellet intake of rabbits at each paddock-spot (five repetitions, two groups), as well as their live weight gain (individually weighed) at 45, 54, 58, 61, 66, 70, 74 and 80 d of life.

We withdrew two rabbits (one female from the AO field and one female from the FL) on the day the paddocks were moved to the paddock-spot 2 (n=11 animals per group; 6 males and 5 females). These two animals showed clinical signs of coccidiosis. One additional male in the AO field died of coccidiosis the day we moved the paddocks to the paddock-spot 3 (FL: 6 males and 5 females; AO: 5 males and 5 females). All the remaining animals ended the study.

Rising plate meter

To estimate the available biomass inside the paddocks, we used an electronic plate-meter (HerboMETRE®, ARVALIS - Institut du Végétal, Paris, France). The whole apparatus is composed of a plastic square plate (300×300 mm, 314.5 g) attached to a plastic yellow tube (590 mm, 75.3 g). This plastic tube freely slid around a graded aluminium tube (970 mm) that is attached to an ultrasound transducer (50 kHz). The transducer is calibrated to measure the height of the plate (every millimetre). The transducer stores all the measurements and calculates simple statistics (number of measures, mean and standard deviation). The whole system (Figure 2) works with an external 12 volt battery.

The equipment is simple to assemble (see https://youtu.be/dQjcH1c5Maw) and use. To start, connect the battery and the equipment will turn on. To turn it off, just disconnect the battery. No worries, data are automatically stored. Before starting the measurements, calibrate the equipment on a flat surface. Hit the calibrate button, place the rising plate in the lowest position and then hit the measure button. Place the rising plate in the highest position and perform the second measure required in this step. The HerboMETRE® is then calibrated and ready to use. The measurement range goes from 5 to 373 mm. The sensitivity is 1.0 mm, and the repetition of measurements is very good (CV around 2.4%). Importantly, ultrasounds are sensitive to variations in the temperature and relative humidity, as well as the plants. To reduce the accumulation of errors, best practice is to perform all measurements on the same day and period (all measures in the morning or all measures in the afternoon). It is also good practice to pay attention to the weather conditions before performing the measurements; it is preferable to avoid measurements after rainfall or late in the afternoon on hot days.

Figure 2: (A) HerboMETRE® and its elements. (B) Rising plate mounted on the yellow plastic tube around the aluminium axis tube. (C) Electronic ultrasound transducer. (D) Twelve volt battery. Photos: https://farm-store.eu/produit/herbo-metre/

Additional material needed to estimate the available biomass

Shear to cut the herbage (we used a Bosch ISIO 3.6 volt shear). A pair of scissors (adapted to cut the grass). Square frame (internal dimension 300×300 mm). Kraft paper bags (310×450 mm, 70 g/m^2). Photographic camera (optional; for research purposes only). Ruler (optional; for research purposes only).

Herbage measurements and sampling method to estimate biomass availability

At each paddock-spot (five per field; n=10 in total) and before letting the animals graze, we entered the paddock area and measured the height (mm) of the herbage. To cover the maximum surface possible, we followed an M-shape walking pattern (see Figure 3) and performed at least 24 measures inside each paddock-spot. While taking the measurements, we registered the proportion of different plant families by visually recording the number of areas (from 1 to 6) with a majority of grass, leguminous or other plants inside a 30×30 cm quadrant. We then subdivided the height measurements obtained into five classes (very low, low, medium, high and very high). Based on the information gathered, we chose the locations (outside the paddocks-spot) to sample the biomass to be the most representative possible of the herbage heights (points in all five classes) and composition (similar proportion of grass, leguminous and other plants) observed inside each paddock-spot.

The biomass sampling involves a series of sequential steps. First, we measure the biomass height (mm) with the HerboMETRE®. We then place the square frame around the HerboMETRE® measurement plate (see Figure 2.B) and cut the biomass (only plants with roots inside the wood frame) as close as possible to the ground (see Figure 4). We then store the biomass in paper bags (pre-weighed) for DM determination. We performed no chemical composition analysis.

For dry matter determination, we heat dried (60°C for 48 h) each biomass sample in a pre-heated oven. We then estimated the biomass availability (kg DM/ha) using the following formula:

$$\text{Biomass (kg DM/ha)} = \frac{\text{Dry matter (g)}}{0.09\ \text{m}^2} \times 10000$$

Statistical analysis and regression for biomass estimation

We used R software (version 4.2.1, R Core Team, 2022) to perform all the statistical analysis and graphics.

To analyse the live weight data, we used a mixed model. The field and age were included in the model as fixed effects, with the animal as a random effect. Average daily gain between 45 and 80 was modelled using a fixed effect model, implemented via the *lm( )* function. Differences were tested with a multivariate test for pairwise comparison using the *emmeans( )* function.

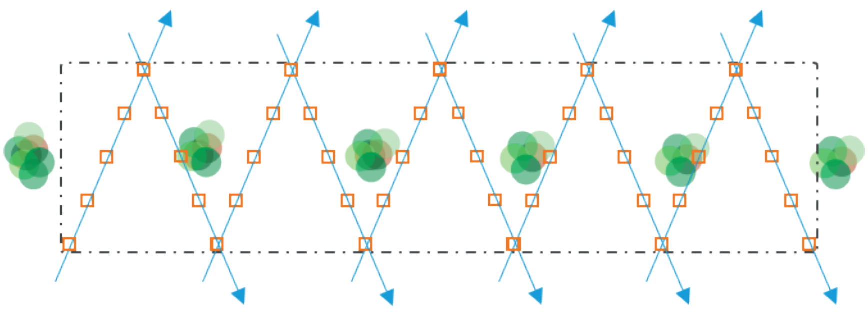


Figure 3: Schematic representation of an M-shape walking pattern to measure the herbage height inside a paddock-spot. Blue arrows represent the walking direction and brown squares the measurement points.

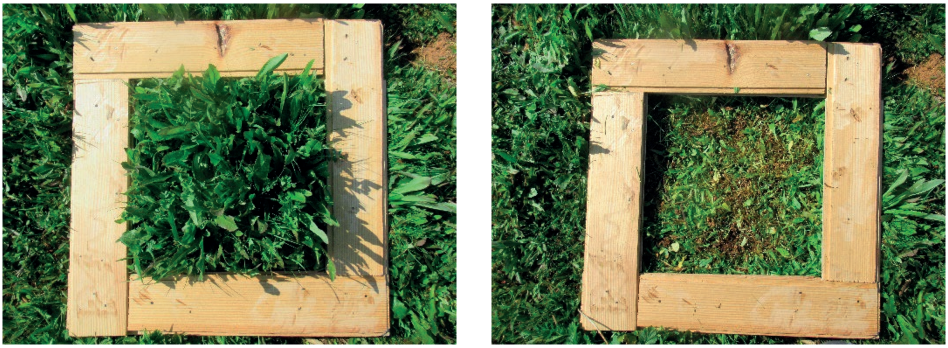


Figure 4: Photo of a sampling location before (left), and after biomass sampling (right). Note that we cut the grass as low as possible, recovering only plants with their roots in the sampling area. We took special care to avoid dirt and tiny rocks. Photos: ®INRAE/Savietto.

We checked the skewness of herbage height measures inside the paddocks using the *skewness( )* function of the R-package *e1071*.

We fitted linear regressions of biomass (kg DM/ha) on herbage height (mm) to estimate the biomass available in each field and paddock-spot using the *lm( )* function of the basic R-package *stats* (https://stat.ethz.ch/R-manual/R-devel/library/stats/html/lm.html).

Influential points were identified using Cook's distance and the difference of fit. We used the R-package *olsrr* to identify these points (see the *measures of influence* vignette at: https://CRAN.R-project.org/package=olsrr).

In total, we set of 36 regression equations. We then used the measurements performed inside each paddock-spot to estimate the biomass available to the rabbits using some of the regression equations we constructed. We also compared the estimated values using a Tukey HSD post-hoc test.

RESULTS AND DISCUSSION

Rabbits' live weight gain and feed intake from 45 to 80 d of age

Rabbits' live weight is shown in Figure 5. At 45 d of age, the live weight (LW) of rabbits placed in the AO was 1319±48.3 g (mean±standard error) and the LW of rabbits in the FL was 1306±43.7 (P=1.00). At 80 d of age, rabbits living in the AO and FL weighed 2217±48.3 and 2308±43.7 g (P=0.18), respectively. Average daily gain of animals in the AO (25.6 g/d) did not differ from animals in the FL (28.6 g/d; P=0.08), despite a higher pellet intake between 45 and 80 d old for rabbits in the FL (3913 kg/rabbit) compared to rabbits in the AO (3294 kg/rabbit). No statistical analysis was performed for pellet intake, as the sample size is equal to one repetition per group; only descriptive. The performance data presented here should be regarded as descriptive, as the sample size (n=12 rabbits per group) is restricted to address further conclusions. For additional information concerning the sample size in nutrition experiments, see Fernández-Carmona *et al.* (2005).

Herbage composition, height measures and herbage sampling

The AO had a majority of grass (52%), some leguminous plants (6%) and other species (42%). Plant species differed in the FL (54% of grass, 17% of leguminous plants and 29% of other species).

Histograms for herbage height measurements taken inside and outside each paddock-spot at AO and FL are shown in Figure 6.

For the AO, height measures for DM estimation taken outside paddock-spots 2, 4 and 5 had a low deviation from height measures performed inside these paddock-spot. For the FL, sampling outside paddock-spots 1, 4 and 5 seems to be accurate.

For the AO, inadequate sampling occurred at paddock-spots 1 and 3, while for FL it occurred at paddock-spots 2 and 3. Average herbage height at paddock-spot 1 at the AO and at paddock-spot 2 in FL was low (high count of herbage height below 20 mm).

The sampling method followed for paddock-spots 1 to 3 on both fields (AO and FL) may explain the observed bias. In these paddock-spots, we began by performing the herbage height measures and grass sampling outside the paddock. In doing so, we arbitrarily applied the five herbage height classes (very low, low, medium, high and very high) with no information on the real distribution of herbage heights inside the paddocks. The absence of numeric information, the high counts of herbage height measurements below 20 mm and a right-skewed normal distribution amplified the sampling error. However, when the herbage height approaches a normal symmetric distribution, the use of an arbitrary herbage height classes with no previous information does not seem to impair the sampling (see paddock-spot 2 for AO and 1 for FL; Figure 6).

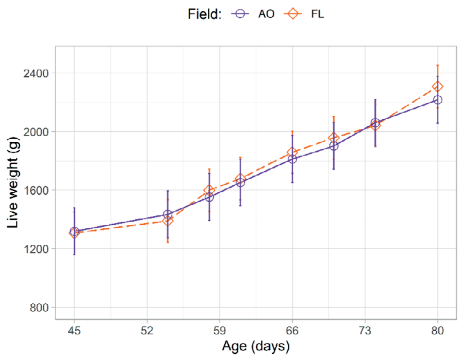


Figure 5: Average live weight (g) of rabbits raised from 45 to 80 d of age in an Apple Orchard (AO, n=12 rabbits) or on Fallow Land (FL, n=12 rabbits)

We identified this issue after analysing the data of the first three paddock-spots in both fields. We then adapted the sampling method. For paddock-spots 4 and 5, we start with measures of the herbage height (at least 115 points) inside the paddock. Based on the information gathered, we searched for a specific sampling location (outside each paddock-spot) with herbage heights within the range of the values obtained inside the paddock-spot. Adaptation of the method resulted in better sampling, as can be seen in the summary statistics presented in Table 1.

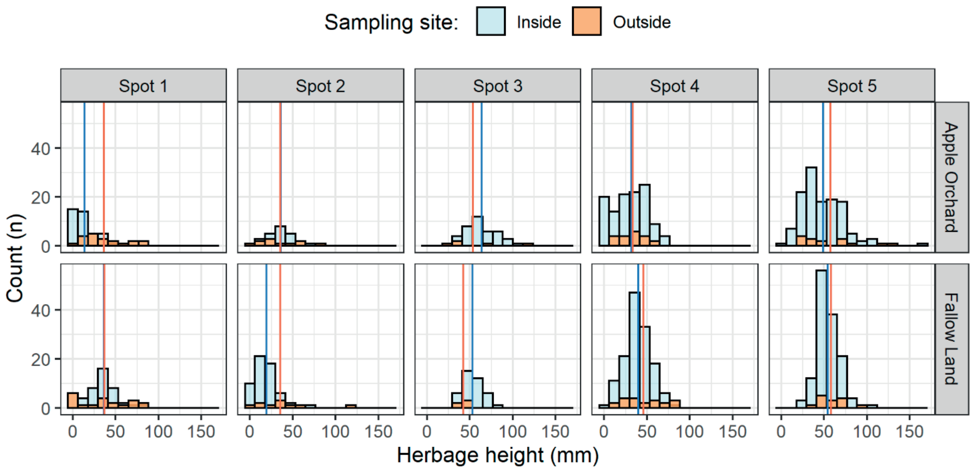


Figure 6: Histograms for herbage height (mm) inside and outside each paddock-spot in the apple orchard and fallow land. The vertical lines represent the mean values of herbage height inside (blue) and outside (orange) each paddock-spot.

Table 1: Sample size (n), mean, and standard deviation of herbage height measurements (mm) inside and outside each paddock-spot for the apple orchard and fallow land.

Field:	Apple Orchard					Fallow Land				
Spot:	1	2	3	4	5	1	2	3	4	5
Inside each paddock-spot										
Sample (n)	40	24	40	115	130	40	59	40	138	135
Mean	13.6	35.7	63.6	31.4	48.6	36.5	19.3	52.9	39.5	54.2
Standard deviation	10.7	15.4	17.4	19.0	26.4	15.2	13.8	11.5	15.4	12.5
Outside each paddock-spot										
Sample (n)	20	11	5	20	15	20	10	5	20	14
Mean	36.4	35.3	53.8	33.4	57.4	36.8	35.4	42.2	45.6	57.6
Standard deviation	25.3	24.2	38.3	15.0	35.1	28.4	31.0	6.72	22.5	14.7

The minimal number of sample size required for an adequate herbage sampling (for DM estimation) appears to be 15 samples, especially when the herbage height is low and the distribution of values is right-skewed (*c.f.* Figure 6). In addition, the rising plate meter we used is sensitive to low height values (below 10 mm).

To avoid unexpected results linked to an inadequate sampling, we advise starting by measuring the herbage heights inside the area available to the rabbits, and based on the information obtained, selecting the points for grass sampling (*i.e.* cut for DM determination).

Regression of biomass on herbage height

Regression equations of biomass (kg DM/ha) on herbage height are in Figure 7. In the left panel, we fitted a single regression line using all sample points (n=140). From this equation, we obtained an intercept of 620.9 kg DM/ha plus 20.7 kg DM/ha per mm of herbage height (Hh). This first equation has an adjusted R^2 of 0.274 and a residual standard error (RSE) of 866 kg DM/ha. Based on this equation, and for an average Hh inside the paddock (all groups combined) of 41.2 mm, the estimated biomass available would be 1475±73.3 kg DM/ha.

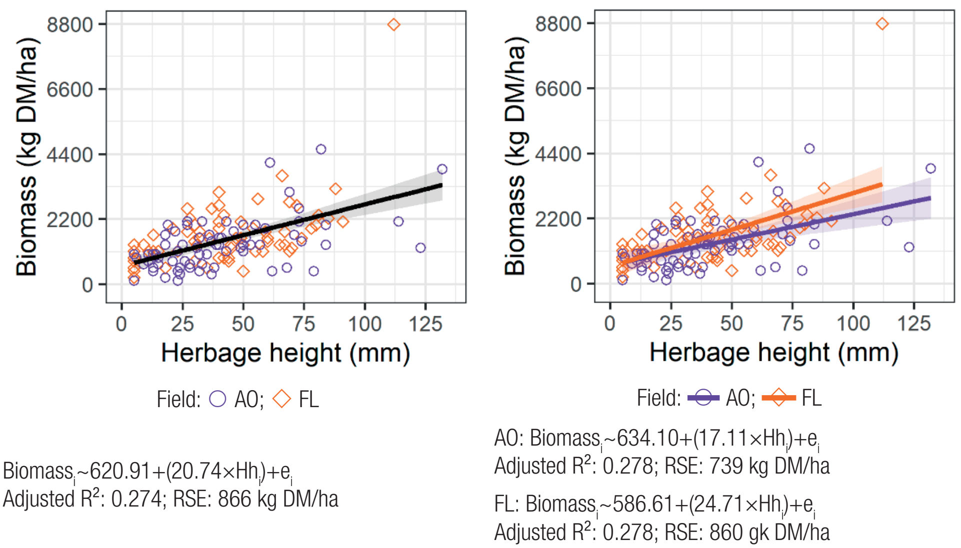


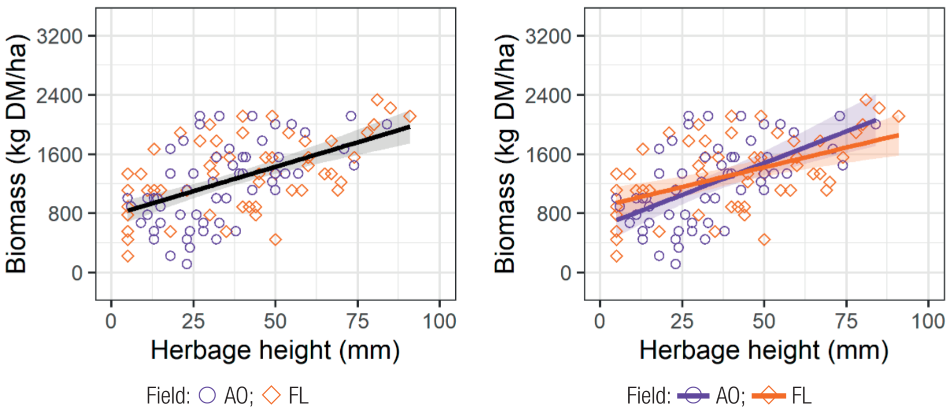
Figure 7: Regression of biomass (kg DM/ha) on herbage height (Hh; mm). Left panel, regression line fitted with all data (n=140). Right panel, one regression line per field: Apple Orchard (AO: n=71) and Fallow Land (FL: n=69). RSE: residual standard error. DM: dry matter.

Regression by fields (Figure 7, right panel) resulted in two distinct equations. For the AO, the intercept was slightly higher (634.1) compared to the intercept for the FL (586.6). However, the slope of the regression of biomass on Hh for FL was steeper (24.7) than the slope obtained for the AO (17.1). It is worth noting the presence of extreme values in both the AO and FL fields. After identifying the outliers and influential points (based on the Cook's Distance and the Difference of Fit), we excluded 26 points (n=14 in the AO and n=12 in the FL) from the original data. This '*clean*' data set had 114 points (AO: 57 and FL: 57).

The new equations obtained after exclusion of outliers and influential points are shown in Figure 8. For the regression on the 114 points, the RSE was 449 kg DM/ha (Figure 8, left). This represented an improvement in the estimation error of 417 kg DM/ha (from the 866 kg DM/ha with the initial data). Considering the same Hh inside the paddock as before (41.2 mm; all groups combined), the available biomass inside the paddock estimated with this new model would be 1313±42.7 kg DM/ha; about 162 kg DM/ha less compared to the initial model (Tukey HSD Post-hoc test; P=0.073).

Regression equations per field after exclusion of outliers and influential points are in the right-hand panel of Figure 8. For the AO, the intercept changed from 634.1 to 621.2 kg DM/ha with no noticeable changes in the slope: from 17.1 to 17.2 kg DM/ha per mm of Hh. The intercept and slope of the FL equation were highly affected by the exclusion of outliers and influential points. The intercept changed from 586.6 to 890.9 and the slope from 24.7 to 10.6, respectively. For both field groups, the exclusion of outliers and influential points reduced the estimation error. For the AO field, the RSE dropped from 739 to 455 kg DM/ha, an improvement in the estimation precision of 284 kg DM/ha, while for the FL the RSE dropped from 860 to 440 kg DM/ha, an improvement of 51.2% in estimation precision.

Comparing the estimation of biomass in the AO field (average Hh of 39.8 mm) before and after data 'cleaning', we obtained similar values: 1339±87.7 and 1328±65.7 kg DM/ha, respectively (Tukey HSD Post-hoc test, P=0.92) For the FL field (average Hh of 43.8 mm), the estimated biomass before and after 'cleaning' the data were 1669±115.6 kg DM/ha and 1356±58.6 kg DM/ha, respectively. A significant difference in the estimation of 313 kg DM/ha (Tukey HSD Post-hoc test, P=0.03).



$Biomass_i$~769.87+(13.18×Hh_i)+e_i
Adjusted R^2: 0.277; RSE: 449 kg DM/ha

AO: $Biomass_i$~621.16+(17.15×Hh_i)+e_i
Adjusted R^2: 0.310; RSE: 455 kg DM/ha

FL: $Biomass_i$~890.86+(10.62×Hh_i)+e_i
Adjusted R^2: 0.236; RSE: 440 kg DM/ha

Figure 8: Regression of biomass (kg DM/ha) on herbage height (Hh; mm) after excluding extreme values and influential points. Left panel, one regression line fitted (n=114). Right panel, one regression line per field: Apple Orchard (AO; n=57) and Fallow Land (FL; n=57). RSE: residual standard error. DM: dry matter.

Comparing the biomass estimations ('*clean*' data; n=114) for an average herbage height of 41.2 mm, we obtained no differences among the three fitted regression (Global: 1312±42.7 *vs.* AO: 1328±66.7 *vs.* FL: 1328±58.2; Tukey HSD Post-hoc test, P>0.90 for all three pairwise comparisons). In this sense, a single 'global' equation could be used in the biomass estimation on both fields.

These results show the need to correctly evaluate the values that influence the regression. This can be performed by calculating the Cook's distance (Cook, 1977) and the difference of fit (Welsch and Kuh, 1977). It is also important to note that different fields, having a slightly different composition in terms of species proportion (grass, leguminous and other plants) may influence the parameter estimations. Although this was not the case here, it is advisable to construct different estimation curves for fields having a different botanic composition, or at least perform a sampling of the biomass in the different fields rabbits will be living in.

Regression of biomass on herbage height per field at different paddock-spots

The final analysis concerns the construction of regression curves for each field at different paddock-spot (Figure 9 and Table 2) and the estimation of biomass available (Table 3). Figure 9 has three panels and 10 regression curves (2 fields×5 paddock-spots).

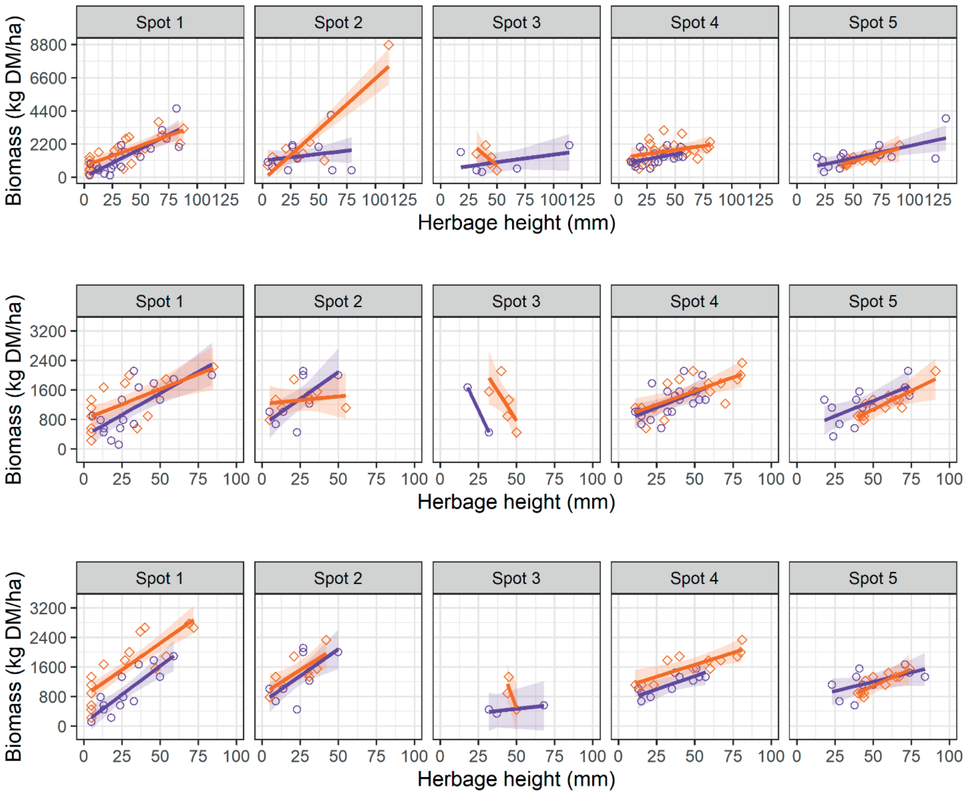


Figure 9: Regression of biomass (kg DM/ha) on herbage height (mm) per field group (○, AO: apple orchard and ◇, FL: fallow land) at different paddock-spots. Top, regression on the original data. Middle, regression on the '*clean*' data from base model (n=114; ○, AO: 57 and ◇, FL: 57). Bottom, exclusion of outliers and influential points per field-paddock-spot (n=95; ○, AO: 45 and ◇, FL: 50). Field: AO; FL.

Table 2: Regression equations of biomass (kg DM/ha) on herbage height measurements (mm) at each field and paddock-spot. Three regression equations were constructed using the original data (n=140) set, the '*clean*' data (n=114) or after excluding outliers and influential values at each field and paddock-spot (n=95).

Field:	Apple Orchard					Fallow Land				
Spot:	1	2	3	4	5	1	2	3	4	5
Regression equations obtained using the original data set (n=140); points of Figure 7										
Sample (n)	20	11	5	20	15	20	10	5	20	14
Intercept	4.11	1086.85	471.60	862.81	457.02	748.67	–244.33	4000.1	1274.6	50.12
Slope	37.53	8.99	10.20	13.26	16.17	26.79	67.54	-64.80	10.78	20.33
Adjust. R^2	0.691	–0.070	-0.022	0.150	0.435	0.570	0.761	0.291	0.082	0.724
RSE	627	1140	819	415	618	648	1155	536	642	182
Regression equations obtained using the 'clean' data set (n=114); points of Figure 8										
Sample (n)	16	8	2	19	12	14	8	5	16	14
Intercept	340.93	632.31	3238.1	703.21	471.17	795.65	1215.5	4000.1	829.00	50.12
Slope	23.31	28.96	–87.3	16.56	16.63	16.45	4.16	–64.80	14.80	20.33
Adjust. R^2	0.483	0.305	-	0.294	0.382	0.344	–0.117	0.291	0.473	0.724
RSE	475	542	-	362	393	522	352	536	365	182
Regression equations obtained after exclusion of outliers per field-paddock-spot (n=95)										
Sample (n)	13	8	3	11	10	15	8	3	12	12
Intercept	79.02	632.31	237.66	637.70	695.37	801.61	856.19	5871.0	1016.7	163.54
Slope	30.87	28.96	4.53	14.30	10.05	28.85	26.35	–107.5	12.92	18.43
Adjust. R^2	0.694	0.305	0.263	0.642	0.244	0.666	0.485	0.210	0.685	0.642
RSE	331	542	95	174	312	489	340	395	217	151

Adjust. R^2: Adjust.- R^2; RSE: residual standard error. DM: dry matter.

In the top panel, regression lines were obtained using the original data. In the middle panel, regressions were fitted using the '*clean*' data (n=114; AO: 57 and FL: 57), while regression on the bottom panel were fitted after exclusion of outliers and influential points performed at each field and paddock-spot.

In the top figures, we noted the high influence of outliers in paddock-spots 2 and 5, and the inadequate sample size in paddock-spot 3. Outliers were presented in every field and paddock-spot, as can be seen in Table 2. In addition, the equation used to identify outlier and influential points altered the final regressions: see Figure 9, middle and bottom panels and equations in Table 2.

Table 3: Biomass estimations (kg DM/ha) from herbage height measurements per field-paddock-spot estimated from equations presented in Table 2 and average herbage height from in Table 1.

Field:	Apple Orchard					Fallow Land				
Spot:	1	2	3	4	5	1	2	3	4	5
Herbage height (mm)	13.6	35.7	63.6	31.4	48.6	36.5	19.3	52.9	39.5	54.2
Estimation from equations obtained using the whole data set (n=140)										
Sample (n)	20	11	5	20	15	20	10	5	20	14
Estimation (kg DM/ha)	903	1408	1123	1280	1243	1726	1077	574	1701	1152
Standard error	110	344	381	94	165	145	117	489	149	50
Estimations from equation obtained using the 'clean' data set (n=114); points of Figure 8										
Sample (n)	16	8	2	19	12	14	8	5	16	14
Estimation (kg DM/ha)	658	1666	-	1224	1279	1396	1296	574	1415	1152
Standard error	159	262	-	85	177	153	133	489	96	50
Estimations from equations obtained after exclusion of outliers per field-paddock-spot (n=95)										
Sample (n)	13	8	3	11	10	15	8	3	12	12
Estimation (kg DM/ha)	499	1666	526	1159	1184	1854	1364	183	1527	1163
Standard error	123	262	83	81	99	134	127	615	68	44

DM: dry matter.

In spite of the different equations obtained, the estimation of biomass at each field and paddock-spot with the '*clean*' data (n=114 points) did not differ from the estimation of biomass after the exclusion of outliers at specific field-paddock-spots (Table 3; Tukey HSD Post-hoc test, P=0.35), except at paddock-spot 1 on the FL (1396±153 *vs.* 1854±134, respectively; Tukey HSD Post-hoc test, P=0.031).

The latter equations (n=95 data) for AO and FL at paddock-spot 1 better recover the real state of the herbage differences among these fields. In the first paddock-spot, the biomass available in the AO was 1355 kg DM/ha lower than that estimated for the FL (Tukey HSD Post-hoc test, P<0.0001). This difference was mainly related to low Hh observed in this first paddock-spot in the AO field.

The comparison of biomass estimations at AO *vs.* FL did not differ when we used the regression equations constructed with the '*clean*' data (n=114) or from the '*final*' data set (n=95), except at paddock-spot 4. The equations fitted with the '*clean*' data (n=114) indicate no biomass difference between AO and FL, while the equations obtained after exclusion of outliers at each field-paddock-spot indicate a significant difference in the biomass present in the AO and FL of 368 kg DM/ha (P=0.002). This result indicates that the data cleaning process should be performed by evaluating the points that may influence the regressions at a specific field and spot.

To demonstrate the need to construct a specific regression model at each field and paddock-spot (which is nested to the date, the progression of the season, and by extension to the herbage growth and/or senescence), we estimated the biomass at each field and paddock-spot from the equation presented in Figure 8 (left panel) and compared the estimations presented in Table 3 obtained from the regression equations fitted with the '*final*' data (n=95). For the AO, we obtained significant differences in the biomass estimations at paddock-spots 1, 2 and 3 of +450, –426 and +1083 kg of DM/ha, respectively (Tukey HSD Post-hoc test; P=0.02). For the FL, estimations differed from these models at spots 1 and 3 (-603 and +1284 kg DM/ha, respectively; P=0.0002). However, the model presented in the left panel of Figure 8, estimates well in spots 4 and 5 on both AO and FL fields, where a better sampling of points was performed (see Figure 6).

Sampling is a key step in biomass estimation using the rising plate meter method. The equation will better reflect the biomass available at a specific field and time (here spot is nested within date) of the year/season when sample size is as high as 15 points (the minimum sample size we recommend). To better reflect the biomass available inside the paddocks, the sampling should consider the real variability in the herbage height measured inside each paddock-spot. Otherwise, the estimations will have an additional unknown error.

Estimation of rabbit herbage intake capacity may also consider the residual biomass inside the grazing area. This should be carried out by fitting regression curves at each field and paddock-spot after grazing. Here, rabbits ingested all the biomass available inside their paddocks (for every field and spot) faster than our ability to move the paddocks to a new location (see Figure 10).

Figure 10: Photos of the Apple Orchard (left) and Fallow Land (right) after 8 d of grazing (paddock-spot 1; n=12 rabbits). Photos: ®INRAE/Savietto.

In this sense, all the estimations of rabbits' herbage intake capacity are underestimations. Roughly, rabbits in the AO ingested 45.2 g DM per day and rabbits in the FL ingested 43.4 g DM per day between 45 and 80 d of age. Their real herbage intake capacity remains unknown. According to Legendre *et al.* (2019), growing rabbits are able to eat as much as 70.3 g DM /d of herbage. In wild rabbits, Cooke (2014) found a herbage DM intake capacity of about 88.8 g/d (adult males only). Both data sustain the observations of herbage restriction in the present study.

Nagy (1987) estimated that a mammalian herbivore is capable of ingesting $0.577\times LW^{0.727}$ g of DM/d (where LW is the live weight in grams). This gives AO rabbits (LW at 80 d of 2217 g) and FL rabbits (LW at 80 d of 2308 g) an ingestion capacity of 156.2 and 160.8 g DM/d, respectively. Considering a DM content in the pellet feed of 89%, the total DM intake observed in rabbits at the AO paddock-spot 5 was 147.8 g/d (105.5 g of pellets plus 42.3 g of herbage). For the FL, paddock-spot 5, the total DM intake was 176.3 g (134.8 g of pellets plus 41.5 g herbage). If we add a daily apple intake of 6.4 g (from the measured intake in the paddock-spot 2 of 32 g/rabbit and a DM content of 20%) to the daily DM intake of rabbits in the AO, paddock-spot 5 would be around 154.2 g. This represents a total DM intake of about –1.3% for AO and +9.6% for FL rabbits, away from the estimations from Nagy's equation. Based on these rough estimations, we argue that the herbage intake obtained here is precise enough to recommend this method for herbage intake measurements for rabbits.

Nonetheless, information on the real herbage intake capacity of pasture raised rabbits is still lacking. Further studies, with adult males, females (at different physiological states) and growing rabbits of different genotypes should be performed. Studies at different locations, with different climatic conditions and/or with different pasture composition are also required. The construction of national and international data sets for cross validation studies on the regression of biomass on herbage height using this technique would also benefit both farmers and scientists trying to develop pasture-based rabbit farming.

CONCLUSIONS

The use of an electronic rising plate meter is a valuable method for biomass estimation for rabbits. The equipment is simple to use and the estimations of biomass availability obtained are acceptable. The rabbit herbage intake calculations achieved with this method are reliable.

Sampling is a key aspect of the method. At least 15 sampling points are required for the construction of regression equations at different locations. Differences in the herbage composition at different fields may also influence the biomass estimations. This should be taken into consideration during sampling. Pasture and herbage composition are affected by herbage growth and senescence, within and between seasons. To correctly account for these sources of variation, a new sampling is recommended whenever rabbits are placed in a new field.

Rabbits are voracious herbivores. In less than 7 d, 12 young rabbits living in 25 m^2 were able to ingest all the biomass to the bare ground. A daily biomass availability below 50 g of DM/d for growing rabbits should be considered as biomass restriction. This threshold may be even higher.

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