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Utilization of information and communication
technologies to monitor grazing behaviour in sheep

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CHAPTER 1

Review of the literature

1. INTRODUCTION

In all production systems, especially in extensive ones, it is economically convenient to maximize the proportion of forage in the diet to minimize feeding costs (Baumont et al., 2000). The study of the feeding behaviour of grazing ruminants is of fundamental importance for the improvement of pastures management and animal productivity, respecting environment and animal welfare (Blomberg, 2011; Swain and Friend, 2013). The information about feeding behaviour of herbivores allows indeed to evaluate the degree of use of the pasture by the animals and to avoid localized overgrazing (Bailey, 2004; Wark et al., 2007) or undergrazed areas, both of which may favour shrub encroachment and woody phytomass, increasing fire hazard (Jáuregui et al., 2009).

Overgrazing indeed, by excessively reducing the biomass, can cause the collapse of grass cover, favouring the development of shrubs as dominant vegetation (D'odorico et al., 2012), as well as the undergrazing. Moreover, overgrazed areas can reduce biodiversity and plant cover and are often associated on one side with risks of soil erosion and on the other with nutritional imbalances of the flock or herd (Braghieri et al., 2005). If grazing does not allow the animals to satisfy their nutritional requirements, it may be necessary to supplement their diet to avoid decreases of growth and of productive and reproductive performances (Gordon, 1995).

The productivity of grazing animals depends indeed on feed intake, which is a difficult parameter to measure, especially for long periods of time (Milone et al., 2012).

This review aims to investigate the principal parameters that allow a better understanding of grazing behaviour of sheep and the methods used for their detection, highlighting their strengths and weaknesses.

1.1 Grazing behaviour and feed intake

At pasture, the composition of the diet, the intake, and the impact of grazing on vegetation are the result of a complex interaction between social grouping tendencies of the animals and their foraging decisions (Baumont et al., 2000; Dumont and Boissy, 2000). Herbivores, and in particular small ruminants, exploit environmental heterogeneity through selective grazing, which allows them to consume a diet with a higher nutrient quality than that offered, and to distribute their impact on vegetation (Baumont et al., 2000). Predicting intake and animal impact on vegetation requires an understanding of their grazing behaviour; when ruminants start eating, they select a feeding site and then a patch within it, the choice of which is influenced by various factors such as vegetation characteristics, distance from the water, need for shelter and social factors (Baumont et al., 2000).

Dumont and Boissy (2000) for example found that sheep, being typically gregarious animals, chose feeding sites located close to their social group and not those located further away, unless they are followed by other peers.

Bailey and Provenza (2008) found that initially animals graze on areas rich of nutrients, then, when these areas become depleted, they move to other areas characterized by lower quality and quantity of forage. The forage depletion in the area and the expectation of feeding opportunities in other sites, are the motivations that induce the animal to move to another patch (Baumont et al., 2000).

The rate of feed intake and the nutritional content of the diet are influenced by a series of short-term animal decisions that regulate grazing process (Gordon, 1995).

The way animals use the forage resources is affected by many factors, such as pasture composition, plant morphology, forage quality and quantity (Bailey et al., 1996; Harris et al., 2002).

Ruminants, and ewes in particular, generally prefer feeds that are more digestible, richer in energy and which quickly provide a high level of satiety (Provenza, 1995; Baumont et al., 2000; Pulina et al., 2005). The selective grazing of sheep allows them to minimize metabolic discomfort, to reach their nutritional requirements and to avoid toxic matter (Pulina et al., 2005). They use their senses, in particular smell and sight, to select or avoid aliments, according to the post-ingestive effects that they experiment (Bailey and Provenza, 2008). Thanks to the previous learning indeed, herbivores are able to recognize the feeds and to anticipate the nutritional and physiological consequences of their intake, that is essential to determine the motivation to eat and the feed preferences (Baumont et al., 2000). Post-ingestive stimuli are also involved in the control of the satiation process: during a main meal, intake rate is higher at the beginning and then decreases as animal moves towards satiety. The chemoreceptors present in the rumen wall stimulate the rumination behaviour in order to accelerate the digesta outflow and to avoid excesses and nutritional disorders (Baumont et al., 2000).

According to Hodgson (1985), feed intake is regulated by three different factors: bite mass, bite rate and total grazing time:

$$I = BM \times BR \times GT$$

where bite mass can be calculated by dividing daily herbage intake by total daily bites (intake/bites), bite rate can be expressed as the number of bites per unit of time (bites/unit time) and grazing time is the effective time spent grazing.

Bite mass is the variable with the greatest influence on intake and is affected by sward structure, while bite rate and grazing time are compensatory variables (Forbes, 1988).

For example, when food availability is low, such as on pastures with short grass heights, the animal can compensate for the reduced bite mass by increasing daily grazing time, increasing the rate of biting or trying to select higher quality food, in order to compensate for the reduced quantity of forage and to meet its nutritional requirements (Iason et al., 1999).

1.2 Behavioural parameters and measuring techniques

Measuring herbage intake is important to understand the relationship between sward structure and animal nutrition, to assess forage attractiveness and to improve management practices and performances of animals in grazing systems (Gordon, 1995; Undi et al., 2008; Tani et al., 2013).

However, the accurate measurement of feed intake in free-ranging animals is still a challenge, mostly in extensive systems (Undi et al., 2008).

In the past, one of the most used methods for the study of feeding behaviour was the direct and continuous observation (Bourbouze, 1980), that consists in following and recording the activity of a focal animal, chosen randomly, for a time period of at least 8-10 minutes. This method is very labour intensive, can alter animal behaviour and can be difficult to apply in mountain environments, during night grazing or in the presence of adverse weather conditions (Langbein et al., 1996; Sheibe et al., 1998).

To overcome the aforementioned problems, in recent years it has been possible to see a considerable development of automatic systems based on small, light and minimally invasive sensors, able to measure physical or behavioural parameters of individual animals and to convert them into signals for the observer (Scheibe and Gromann, 2006; Rutten et al., 2013; Abbasi et al., 2014).

Automatic recorders like radio tracking devices, pedometers, sound recorders and three-axial accelerometers have been widely used in several works and by farmers for different purposes: to track animals spatial distribution in relation to feeding resources (Bailey and Provenza, 2008), to monitor health status (Ito et al., 2010), to improve fertility (Nebel et al., 2000), to measure metabolic parameters (Edwards and Tozer, 2004) and to study grazing behaviour (Navon et al., 2013; Oudshoorn et al., 2013; Alvarenga et al., 2016).

Over the years various devices have been tested to monitor ruminants behaviour: IGER Behaviour Recorder (Rutter et al., 1997), Tinytag® data loggers (O’Driscoll et al., 2008), IceTag® activity monitors (Trénel et al., 2009; Mattachini et al., 2013) and HOBO® Pendant G Data Logger (Moreau et al., 2009; Nielsen, 2013) showed a high correlation between direct behavioural observations and devices data. However, their use has been limited because many of these systems are expensive, difficult to apply to animals and require experience for interpreting the obtained data (Watanabe et al., 2008; Yoshitoshi et al., 2013).

Nevertheless, there is an increasing trend towards the automation of various farming processes and a greater need to acquire data on animals, in order to improve work conditions, reduce health problems, increase production and improve farm management

by reducing costs (De Koning and Rodenburg, 2004; Rutten et al., 2013). Moreover the development of increasingly powerful electronic tools, with greater sensitivity and data storage capacity, can open new perspectives for the study of animal behavioural activities (Moreau et al., 2009).

The most promising devices in the near future are based on accelerometric sensors, that also in combination with other methods, allow to measure a lot of important parameters such as grazing behaviour (Watanabe et al., 2008; Robert et al., 2009), energy expenditures (Halsey et al., 2011; Miwa et al., 2015) and the rate of dislocation of the animals (Bidder et al., 2014). Indeed the acceleration devices are very small, relatively easy to attach to the animals and offer the opportunity to record a large amount of data. Their use is possible in almost all fields of life and it would be worth implementing it, both on a scientific and business level (Scheibe and Gromann, 2006; Swain and Friend, 2013; Abbasi et al., 2014).

Radio tracking devices and sensors like pedometers, sound recorders and three-axial accelerometers, indeed, have been widely used to determine animals spatial distribution in relation to feeding resources and to study their grazing behaviour (Swain and Friend, 2013).

1.2.1 Global Positioning System (GPS)

Monitoring foraging behaviour of herbivores is important to limit the impact of animals on pastures (Hulbert et al., 1998; Giroux et al., 2012).

Remote control systems can provide researchers to obtain information about grazing behaviour and about interactions with the environment, by evaluating the density of use of the different grazing areas (Turner et al., 2000; Handcock et al., 2009).

Over the years, various technological solutions have been adopted to monitor animal behaviour in different environments. From the early 50s, radio telemetry began to be used for animal monitoring research (Barbari et al., 2007).

Very High Frequency (VHF) technology is based on a radio signal emitted by a transmitter attached to the animal and picked up by a directional antenna connected to a radio receiver. This technique allowed the researchers to follow the animals and to record their activities without altering their natural behaviour, but it has shown several drawbacks due to loss of signal and to errors in estimating the distance travelled by animals (Turner et al., 2000; Barbari et al., 2007).

In addition, the VHF radio technology allows to record only small amounts of data and can receive the signal at a distance of a few kilometres, so it is only applicable to animals that are within limited areas (Barbari et al., 2007).

From the 90s, GPS technology based on satellite telemetry systems have been developed and allowed to overcome many of the limits of the VHF radio telemetry, recording a high number of data on geographical location of animals for the whole day (Rodgers, 2001). Initially the GPS collars, for studies on animal localization, were used to obtain information on wildlife; recently, they were used for works on the determination of best management practices for domesticated grazing animals (Hulbert et al., 1998; Ungar et al., 2005; Schlecht et al., 2009).

Recent advances in GPS technology allowed to update programs in order to obtain collar receivers (Figure 1) suitable for monitoring animal position even at short time intervals (Turner et al., 2000).



Figure 1. Cow wearing GPS collar. Adapted from Turner et al. (2000)

Data can also be imported into a Geographical Information Software (GIS) package, which allows to summarize and analyze many positioning data and to evaluate animal behaviour and pasture utilization with greater precision (Bailey, 2000; Barbari et al., 2007). The main disadvantages of GPS-based monitoring systems are the high energy consumption, resulting in poor battery life and frequent connection losses, mostly in the presence of obstacles or in poor weather conditions (Nadimi et al., 2012; Mason and Sneddon, 2013). For example, Agouridis et al. (2004) found that the GPS collars produced errors on the order of 2.5 times greater under tree cover than in an open field, and on the order of 1.5 times greater near fences than in an open field.

Nevertheless, GPS technology is useful in determining where an animal has grazed, but also monitoring other activities, such as walking, grazing and resting, could provide important information on animal behaviour and could represent a relevant factor in the ecosystem management and conservation (Gervasi et al., 2006).

Since GPS collars provide information on the speed of location changes over time and on distance travelled from animals, Schlecht et al. (2009) assumed that data from GPS recordings could be used as discrimination criteria for the daily activities of grazing animals. However, the same authors pointed out that while walking could be correctly classified for about the 80% of the time, for grazing and standing the previsions are more problematic.

Other researchers conversely, believe that position data alone are not able to correctly discriminate grazing and resting activities and conclude that the integration of GPS systems with motion sensors could provide more accurate data for the determinations of animal activities and the use of environment resources (Ungar et al. 2005; Ganskopp and Johnson, 2007; Ungar et al., 2010).

Gonzales et al. (2015), for example, were able to classify behavioural activities of cows grazing in a paddock by collecting electronic data at high frequency from collar-mounted accelerometers and GPS sensors.

Likewise, Brosh et al. (2006) were able to simultaneously measure the specific energy costs per unit of activity in grazing cows, as well as the daily energy cost of grazing activity, thanks to the combination of the GPS method with heart rate monitors.

1.2.2 Pedometers

Another system for studying animal behaviour is featured by pedometers (Figure 2), which represent a precise and inexpensive method, mainly used in cattle farms, to monitor travel, health and oestrus of animals (Anderson and Kothmann, 1977; Brehme et al., 2008). Indeed animal performances and economic efficiency, especially in dairy

farms, are strongly influenced by good herd health, high reproductive results and also by energy spent travelling (Walker et al., 1985; Brehme et al., 2008).

Animal travel can be divided into at least three categories: foraging, walking and running, characterized by different step lengths, therefore the accuracy of the pedometer readings depends on the category to which the pedometer was adjusted or on the correction factor used to control the readings (Anderson and Kothmann, 1977).

Walker et al. (1985) concluded that pedometers can estimate travel distance with a good accuracy when correctly calibrated.



Figure 2. Pedometer fastened on a cow's leg. Adapted from Kajava et al. (2014)

Regarding health and fertility problems, the late detection of animal diseases and oestrus has a negative impact on milk quantity and quality and on the productive life of the herd, causing substantial financial losses (Brehme et al., 2008).

Pedometers — thanks to the combination of two important physiological parameters, activity and resting time — allow the early and exact prediction of oestrus and health problems, like lameness or metabolic diseases, increasing the sanitary treatments efficacy, and preventing the disease from becoming chronic (Mosaferi et al., 2012; Alsaad et al., 2015). It is indeed well known that during oestrus or illness, animals change their behaviour as an adaptive response to cope the stress, for example increasing or decreasing physical activity, reducing feeding and social behaviour, etc. (Nebel et al., 2000; Owen-Ashley et al., 2006).

Topan et al. (2013), assume that pedometers are useful tools to help farmers decision-making skills and profitability of farms, improving animal welfare through the continuous acquisition of data on their physiological state.

Pedometers are generally attached on the leg of the animals and data can be stored in a micro SD memory card, transmitted to a computer by an antenna, or sent to receiving units installed in the barn or milking support (Brehme et al., 2008; Topan et al., 2013; Alsaad et al., 2015).

According to Walker et al. (1985), the main problem of this technique is the mechanical malfunctioning of the pedometers and the loss of instruments during the experiments; the same authors believe therefore that the use of two pedometers per animal might be useful to avoid losing data due to lost or broken pedometers.

1.2.3 Acoustic telemetry

Another method with great potential is the acoustic telemetry, based on the recording, through a small microphone placed on the nape, on the horns or on the forehead of the

animal (Figure 3), of the acoustic signals produced by the movement of the mouth (Laca and Wallis De Vries, 2000; Clapham et al., 2011; Galli et al., 2011; Navon et al., 2013).



Figure 3. Microphone attached to the forehead of a cow and to the horn of a sheep. Adapted from Navon et al. (2013)

During grazing process, animals continuously move the jaw to select, grab, chew and swallow the forage; however, it is possible to identify two different functions: biting, when the grass is caught and cut, and chewing, when the grass is crumbled inside the mouth (Galli et al., 2011). An additional function can be identified, consisting in chewing and biting with the same jaw movement: chew-bite, that has very important implications for the correct explanation of intake rate and ingestive behaviour (Galli et al., 2011; Milone et al., 2012).

The results of several studies indicate that it is possible to accurately estimate intake by acoustic analysis, indeed biting and chewing sounds differ in spectral composition because the first ones are louder and shorter than the latter (Galli et al., 2011).

The acoustic method is deemed much more reliable to count bites than direct observation, both because it is able to distinguish more clearly the sounds of bites and chews (Navon et al., 2013), and because chew-bites are not visually distinguishable

from bites, inasmuch the grass already in the mouth is chewed at the same time as the fresh grass is cut off (Milone et al., 2012).

However, acoustic monitoring requires further developments and solutions to solve some technical and practical problems (Laca and Wallis De Vries, 2000). The acoustic recordings can indeed be contaminated by background noises, especially in free grazing conditions; some of these noises sound like a bite: drinking, licking a salt block and the direct rubbing of the grass against the microphone (Delagarde et al., 1999; Clapham et al., 2011). Furthermore, to make practical the use of this method, recorded sounds must be automatically decoded by signal-processing algorithms, but this represents a challenge because it implies the isolation of background noises unrelated to the ingestive sounds (Navon et al., 2013).

However, without the automatic processing of sound, the use of this method for periods longer than a few minutes would not be practical, as the decoding of the recorded sounds by an operator takes a long time (Milone et al., 2012).

In a recent study Navon et al. (2013) succeeded to interpret the sound recordings through the use of a sound analysis software that, thanks to interpretive algorithms, allowed to correctly estimate from 84% (sheep) to 96% (goats and cattle) of the jaw movements. Milone et al. (2012) managed to reduce wind impact and other environmental noises by protecting the microphone with a foam cover, and automatically segmenting and classifying the grazing livestock sounds using a Hidden Markov models-based recognition system.

Although the complete automation of sound recordings has yet to be adapted to allow daily measurements of ingestion in free pasture conditions, the acoustic system has

shown good basis for the study of animal behaviour and for a wider application, also through the integration with global positioning systems, to provide more information about ruminants feeding behaviour (Galli et al., 2006; Milone et al., 2012).

1.2.4 Accelerometers

The most promising recording systems to monitor animal feeding behaviour are based on acceleration sensors (Watanabe et al., 2008; Robert et al., 2009).

An accelerometer (Figure 4) is an electronic sensor able to transform the physical acceleration from motion or gravity into waveform voltage signal output, and can measure both the static acceleration due to gravity and the dynamic acceleration due to animal movements (Brown et al., 2013).

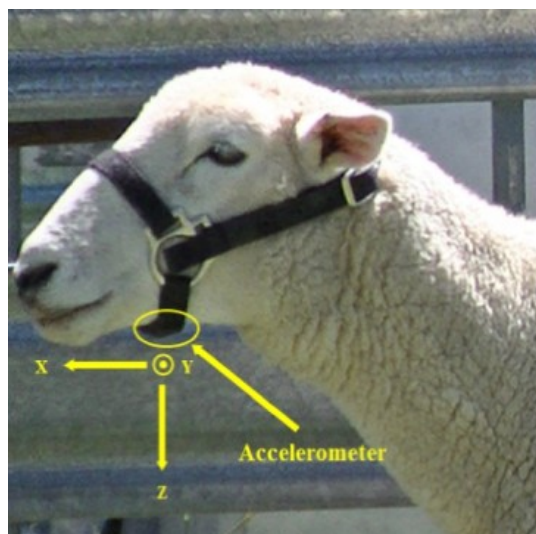


Figure 4. Tri-axial accelerometer attached to an halter; the arrows indicate the direction of the x, y and z axes on the recorder. Adapted from Alvarenga et al. (2016)

These tools usually incorporate wireless communication technologies like ZigBee, Bluetooth, Wibree and WiFi, that allow to download the data acquired from the

accelerometer from a good distance and are commonly used in sensor network based researches to transmit sensor data (Abbasi et al., 2014).

The first devices for the detection of animals grazing activity were based on pressure sensors that measured a jaw movement as a change of electrical resistance (Penning et al., 1984; Rutter et al., 1997; Rutter, 2000).

The IGER Behaviour Recorder (Rutter et al., 1997) for example, have been validated in grazing sheep for the digital recording of three behavioural activities: eating, ruminating and other. A high overall index of concordance (91.0%) was found between the IBR data and the observer manual recordings (Rutter et al., 1997; Ungar and Rutter, 2006), but the software program for data analysis (Rutter, 2000) may sometimes misinterpret the activity (Konoff et al., 2002), maybe due to differences in pressure values generated by variations in the adherence of the halter to the animal (Andriamandroso et al., 2016). Moreover, in the first studies on the feeding behaviour of ruminants by automatic sensors, the activities of the animals were estimated on the basis of the visual analysis of the acceleration waveform (Rutter et al., 1997), so the accuracy of the estimates was largely dependent on the reader. To solve this problem, some researchers developed new statistical classification methods, able to automatically discriminate animal activities by setting threshold values on accelerometric signals (Moreau et al., 2009) or by using quadratic discriminant functions (Watanabe et al., 2008).

The advantages of using automatic sensors depend on their simplicity and robustness, the specificity and accuracy of activity detection, the degree of automation for data processing, financial costs and commercial availability (Delagarde and Lamberton, 2015).

Developments in accelerometer technology opened new perspectives for automatic monitoring of animal behaviour (De Passille et al., 2010), and thanks to following advances in microelectromechanical systems (MEMS) technologies it has been possible to reduce the size, weight and energy consumption of the accelerometric sensors, and to guarantee easier assembly on the animals without influencing their behaviour (Watanabe et al., 2008; Büchel and Sundrum, 2014).

Miniaturized accelerometric sensors have therefore started to represent promising tools for measuring free ranging ruminants behaviour and for classifying grazing, ruminating and resting activities (Scheibe and Gromann, 2006; Watanabe et al., 2008; Tani et al., 2013). A large number of studies on the use of accelerometers to monitor cattle behaviour can be found in the literature.

Scheibe and Gromann (2006) found the existence of some differences in acceleration waveform of the main activities (standing, grazing, walking, ruminating, drinking and hay uptake) of cows.

Wark et al. (2007) classified eating, ruminating and sleeping activities in a grazing cow by combining acceleration data with GPS data.

More recently the RumiWatch device (Zehner et al., 2012; Alsaad et al., 2015), which represents an evolution of the IBR, has been developed; it is based on sensors that allow the automatic measurement of feeding, ruminating, drinking and locomotion activities in dairy cattle. The device consists of a muzzle (Figure 5), equipped with a pressure sensor to detect masticatory movements, and of a three-dimensional accelerometric pedometer that detects the movement or resting status of the animals. Data are stored on

an SD card and can be downloaded directly to a PC or sent wirelessly and processed in real time by the evaluation software (Zehner et al., 2012).



Figure 5. Description of RumiWatch noseband sensor. Adapted from Zehner et al. (2012)

Automatic behavioural classification recorders have been successfully used also in sheep studies, although to a lesser extent.

Alvarenga et al. (2016) used a tri-axial accelerometer to identify the main behaviours (grazing, lying, running, standing and walking) of sheep at pasture.

Kuźnicka and Gburzyński (2017) used an accelerometer attached to the lambs neck for the automatic detection of suckling episodes, monitoring lambs welfare during the rearing period and minimizing the need for farmer attention.

Radesky and Ilieski (2017) and Barwich et al. (2018) have recently used a tri-axial accelerometer to classify different gaits and postures in sheep, in order to discriminate lameness from normal walking and to monitor health status of animals.

While in recent years, the amount of studies related to the use of accelerometers in the classification of behavioural activities of grazing ruminants is increasing, only few

references can be found in literature on the use of these sensors to identify and classify masticatory movements of the mouth (Andriamandroso et al., 2016).

However, some authors managed to get a good estimate of the number of bites: Umemura et al. (2009) modified a pedometer in a pendulum under the lower jaw of cattle to monitor jaw movements, being able to count them with a precision of 90% compared to manual counts.

Tani et al. (2013) used a single-axis accelerometer coupled with a microphone, managing to classify with a good accuracy the chewing activities of stabled cows, when the sensor was attached to the cow horn.

Oudshoorn et al. (2013) used a 3-axis accelerometer to record grazing cow bites; a series of thresholds were tested to classify the chewing activities, in order to determine the peak with the best correlation with the observation, but the results indicated the difficulty of counting the bites using an accelerometer in this way.

Therefore, the use of accelerometers for monitoring jaw movements of grazing animals still requires significant hardware and software developments, in particular regarding the automation process and acquisition of data in real time. Moreover, there may be interference in the recordings due to the sensitivity of accelerometric sensors, which could generate unwanted signals during recording sessions due to sudden head movements, for example to drive insects away (Andriamandroso et al., 2016).

Accelerometers, in combination with other devices, have been used to measure also other important parameters such as energy expenditure (Halsey et al., 2011; Miwa et al., 2015) and the rate of dislocation of the animal (Bidder et al., 2014).

Energy costs represent an important parameter to be evaluated in free ranging animals, because daily activities of grazing animals may require more energy expenditures compared to those of confined animals (Brosh et al., 2006; Aharoni et al., 2009).

Such measurements require the registration of the activity for 24 hours period, because ruminants are active even at night (Brosh et al., 2006).

The energy expenditure of animals has been usually estimated by two main methods: the doubly labeled water method and the heart rate method (Butler et al., 2004).

Although many researchers have tested these methods for estimating the energy costs of farm animals, they present some limitations: the doubly labeled water method has high financial costs and cannot make measurements for short periods of time, so it is not able to calculate the energy costs required for specific activities (Butler et al., 2004; Miwa et al., 2015). Also the heart rate method is quite expensive and the estimates can be easily influenced by stressful states of animals, which can cause an increase in energy expenditure; in addition heart rate recorders are difficult to use on free-ranging animals because the electrodes that compose them must be firmly attached to the animal or surgically implanted, to prevent them from falling or breaking (Butler et al., 2004; Miwa et al., 2015). However, surgical implantation in the field is complicated and can also cause infections, therefore alternative techniques are needed (Signer et al., 2010).

Recently Miwa et al. (2015) have tested the potential of an accelerometric technique for estimating the energy expenditure of grazing ruminants, by evaluating the relationship between an acceleration index, the Overall Dynamic Body Acceleration (ODBA), and the heart rate. They concluded that ODBA is a good indicator of the estimated energy costs of grazing animals.

The combination of data obtained from accelerometer devices with those obtained from GPS may instead allow the analysis of temporal space behaviours, in order to allocate the different activities (locomotion, rest and grazing) on specific areas in which they occur, identifying this way grazed areas by those only treaded but not used by the animals, as deemed by researchers like Guo et al. (2009), Spink et al. (2013), Gonzales et al. (2015).

Various factors such as sensor position, sampling frequency and window sizes, can affect classification performances of accelerometers, but these characteristics are rarely described in literature. An accurate selection of above parameters can lead to significant improvements in signal transmission, storage capacity and energy saving (Walton et al., 2018). The sampling frequency of the accelerometer based devices generally range from 1 to 60 Hz (Müller and Schrader, 2003; Martiskainen et al., 2009; Brown et al., 2013; Nielsen, 2013), and its choice depends on the frequency of the activity to be classified, as well as the choice of window size (Walton et al., 2018).

Thanks to technological development, modern accelerometers are now able to record at high frequencies, that generate huge amounts of data, entailing a high cost in terms of energy consumption and making manual processing of recorded data and interpretation of signals based on changes in body movements impractical and difficult. For these reasons many researchers have tested the use of various machine learning approaches to automatically classify accelerometer data (Bidder et al., 2014).

Discriminant function analysis (DA) for example, is a multivariate statistical technique used to determine which continuous variables are able to discriminate between groups and to predict group membership. Once the DA has established which variables are the

best predictors, classification is possible thanks to the canonical functions, which allow to classify subjects in groups in which they had the highest classification scores (Poulsen and French, 2008).

Watanabe et al. (2008) demonstrated that quadratic discriminant analysis is effective for processing accelerometer data and statistically discriminating eating, ruminating and resting activities of cows, obtaining 90% correct discrimination rates when the discriminant functions used the x-axis variables (front-to-back axis).

Yoshitoshi et al. (2013) obtained a discrimination accuracy ranging between 90.6% to 94.6% using a linear discriminant analysis to classify cattle foraging activities based on acceleration data.

Giovanetti et al. (2017) were able to discriminate activities of sheep with a precision of 95% for grazing, 94% for resting and 89% for ruminating by analyzing accelerometer data with DA.

Another good classification method is the support vector machine (SVM), that constructs a hyperplane that best separates and maximizes the distance of observations from this separating hyperplane (Nathan et al., 2012).

Martiskainen et al. (2009) used a three-dimensional accelerometer to classify several behaviours of dairy cows, setting a sampling frequency of 10 Hz and a window size of 10 s for all behaviour classifications and processing accelerometer data using a multiclass SVM classifier, with accuracies ranging from 29% to 86%.

Classification and regression trees (CART) method can be used either for predicting continuous variables or choosing among categories. The cases are grouped through the subsequent division of data into increasingly homogeneous groups. The decision rules

of CART can be applied very quickly and are quite easy to interpret, but sometimes the method can cause over-fitting, which can be mitigated by reducing the number of decision rules incorporated in the tree (Nathan et al., 2012).

Robert et al. (2009) used an accelerometer set to record with epoch length of 3, 5, or 10 s, and generated a classification tree for each epoch to classify cattle activities into standing, walking, or lying. Lying and standing showed excellent agreement with video (99.2% and 98.0% respectively) while walking was significantly lower, with 67.8% agreement. Regarding epochs, classification accuracy was higher in 3 and 5 s compared to 10 s epoch.

Random forest classifiers (RFs) are constructed using a procedure similar to CART, but instead of using all the variables to determine the best division on each group, only a randomly selected subset of variables is used. However, given the stochastic nature of the algorithm, each of its invocations translates into different decision rules and slightly different results (Nathan et al., 2012).

Walton et al. (2018) found that walking, standing and lying activities in sheep can be accurately classified (89–95%) using a RFs. The highest performance of the classifier was obtained when using a sampling frequency of 32 Hz and a window size of 7 s, that allowed also the respect of energy performance.

Artificial neural networks (ANNs) are inspired by the processes of natural networks of biological neurons in the brain and consist in simple computational units called neurons, which are highly interconnected to each other. By adjusting the neurons interconnections weights according to a learning algorithm, neural networks can identify and learn correlated patterns between input data sets and corresponding target

values. After training, ANNs can be used to predict the outcome of new independent input data (He and Xu, 2010).

Nadimi et al. (2012) developed a wireless sensor network to classify sheep behaviours and used an artificial neural network to process data, being able to successfully discriminate 83.8% of grazing and 83.2% of lying.

K-nearest neighbour (KNN) algorithm classifies new data according to the classifications of the k nearest data points from a training set that can be derived from real data obtained with visual observation. The method aims to minimize the variability within clusters and maximize the variability between clusters and has the advantage to require minimal data preparation (Bidder et al., 2014).

Bidder et al. (2014) used a KNN algorithm for identifying animal behavioural activities based on raw tri-axial acceleration data with a mean accuracy of 78%, establishing results comparable to those gained using more complex automated classification methods.

1.3 Practical implications of literature

In conclusion, the literature showed that monitoring the feeding behaviour of grazing ruminants is of fundamental importance to better manage pasture resources and to better meet animals nutritional requirements.

Feed intake at pasture is difficult to measure, especially through direct observation, for this reason, automated systems for monitoring the activities of free-ranging animals have become increasingly important and common.

Some sensors, such as tri-axial accelerometers, showed a good precision and accuracy in the classification of behavioural activities of herbivores, but they do not yet seem

able to discriminate jaw movements, which are of great importance for evaluating animal grazing strategies in different pastures and for estimating the daily herbage intake. Furthermore, although these devices are already used on farms, they still need significant developments, in particular regarding the easiness of installation and use, the real time data acquisition, the sampling frequency of the acceleration signal and the automation process of data classification.

In comparison to the large number of studies on the automatic classification of behaviour in cattle, very few studies have been done in sheep using sensor-based technologies (Walton et al., 2018).

For this reason, during a project (Beharum - Projects CRP-17287 PO Sardegna, FSE 2007–2013 LR 7/2007) financed by the Government of Sardinia, an automatic system (BEHARUM) for the study of the feeding behaviour of sheep was developed.

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OBJECTIVES

The aim of the present thesis was to study grazing behaviour of sheep and to automatically identify and classify their activities through a tri-axial accelerometer based device.

The main objectives of the study were:

- a) to develop a tri-axial accelerometer based sensor for recording and statistically discriminating sheep feeding behaviour into three different classes (grazing, ruminating and resting);
- b) to estimate the rate of biting (number of bites per min of grazing) on the basis of acceleration variables;
- c) to develop a data management software and a remote transfer system of electronically recorded information to a remote control location using wireless networks;
- d) to test different epoch settings in order to optimize device performances and to evaluate their effect on the statistical model applied to the classification of behavioural activities.

CHAPTER 2

Automatic classification system for grazing, ruminating and resting behaviour of dairy sheep using a tri-axial accelerometer

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ABSTRACT

A device based on a tri-axial accelerometer was used to measure behavioural parameters of dairy sheep at pasture. Short tests were performed in grazing conditions to collect accelerometer data simultaneously with video recordings of sheep behavioural activities (grazing, ruminating and resting). The raw acceleration data was processed to create 12 variables: mean, variance and inverse coefficient of variation (ICV; mean/standard deviation) for the X-, Y- and Z-axis and the resultant at 1-min intervals. A database inclusive of the 12 acceleration variables and the three behavioural activities detected for each minute was then created. Three multivariate statistical techniques were used to discriminate the behavioural activities using the acceleration data: stepwise discriminant analysis (SDA), canonical discriminant analysis (CDA), and discriminant analysis (DA). Based on the acceleration variables selected by SDA, the subsequent CDA significantly discriminated the three behaviours by extracting two canonical functions. The first canonical function (CAN1) discriminated the grazing activity from the resting and ruminating, whereas the second (CAN2) differentiated the grazing from the ruminating behaviour. After bootstrap resampling, the DA correctly assigned 93.0% of minutes to behavioural activities. Stepwise regression analysis was used to estimate the

rate of biting (total number of bites/min) using a subset of acceleration data that contained only minutes in which sheep were grazing. In this case, 15 variables were tested and out of them, only one was selected, the sum of X-axis value per minute (SX), which explained 65% of the total variation of the rate of biting.

1. INTRODUCTION

Grazing, ruminating and resting are the main daily activities of ruminants and play a key role in regulating their forage intake.

Monitoring these three activities is important to take management decisions in grazing systems. According to Hodgson (1982), daily herbage intake of grazing animals is equal to $(\text{bites/unit time}) \times (\text{intake/bite}) \times (\text{grazing time})$. The rate of biting (bites/unit time), i.e. the severing of herbage during the act of grazing, together with the bite mass (intake/bite), represents the intake rate (fresh or dry matter/unit of time). The mean rate of biting over 24 h may be calculated from total bites divided by total grazing time (Penning and Rutter, 2004). The total number of bites as well as the behavioural activities of the animals at pasture can be estimated by either direct observation or using automatic recording systems.

The direct and continuous observation of behavioural activities is labour intensive and time consuming (Ungar and Rutter, 2006). Automatic grazing jaw-movement recorders set up around the muzzle of animals based on electric resistance (Penning, 1983; Rutter et al., 1997) or a pressure (Nydegger et al., 2011; Zehner et al., 2012; Ruuska et al., 2016) have been used in discriminating between grazing and ruminating in cattle and sheep with good accuracy. Sound recording has been also identified as a good method

for analyzing the ingestive jaw movements of grazing ruminants (Laca et al., 1992; Delagarde et al., 1999). The method, using an inward-facing microphone, allows to accurately assess bite rate and, more recently, to distinguish among bites, chews and ruminating jaw movement, as well as combined chew-bites (Laca and Wallis De Vries, 2000; Galli et al., 2006; Ungar and Rutter, 2006). Other systems based on electromyography provide estimates of feeding behaviour by positioning electrodes closely attached to the masticatory muscle of the masseter during jaw movement and measuring electrical potential oscillation in dairy cows (Büchel and Sundrum, 2014). Recently the use of tri-axial accelerometers, sometimes coupled with GPS sensors (Wark et al., 2007), has been implemented to monitor cattle behaviour, discriminating among different activities of cows at pasture such as lying, standing, resting and grazing (Seo et al., 2006 cited by Watanabe et al., 2008), or walking, drinking and hay intake (Scheibe and Gromann, 2006). Moreover, accelerometers attached to animals allow the measurement of animal energy expenditure (Halsey et al., 2008; Miwa et al., 2015), travel speed (Bidder et al., 2012), activity and feeding behaviour (Robert et al., 2009; Moreau et al., 2009; Watanabe et al., 2008). The most common sampling frequencies used in studies with accelerometer based sensors were 10, 16 and 32 Hz, depending on the frequency of the movement being classified. These instruments usually incorporate a microprocessor and a memory to store data until the device is retrieved. However some sensors can incorporate wireless communication technologies, (ZigBee, Bluetooth, Wibree and WiFi), and are commonly used in sensor network based research works (Aqeel-ur-Rehman et al., 2014). Recorded data can be then processed to, for example, select threshold values that distinguish the behavioural activities (Moreau et

al., 2009) or develop a quadratic discriminant analysis of transformed variables that automatically classify different behaviours (Watanabe et al., 2008). Other statistical methods such as the classification tree, k-means classifier, multiple-model adaptive estimation approaches, and multilayer perceptron (MLP)-based artificial neural network (ANN) have been also tested (Schwager et al., 2007; Nadimi and Søgaard, 2009; Nadimi et al., 2012).

Despite the growing literature concerning the use of accelerometers in classifying behavioural activities in grazing ruminants, only few references reported the application of this type of sensors to identify and classify jaw movements i.e. bites (Chambers et al., 1981; Umemura, 2013; Oudshoorn and Jørgensen, 2013). The accurate prediction of the number of grazing bites is of great interest to estimate intake in grazing animals but it remains a challenging goal under field conditions. Automatic bite counts was proposed by Chambers et al. (1981) in cattle and sheep to distinguish between the ripping of grass and other jaw movements. This equipment, which combined a micro-switch, an accelerometer and a mercury-switch to detect jaw movements, head movements and head state (up and down) respectively, showed little difference both in cattle and sheep in the agreement between direct observation and bitemeter estimates of the number of grazing bites. Some progress has recently been achieved by Umemura (2013) that found that pedometer values (counts), obtained by attaching the equipment to the neck collar as a pendulum, are well related to the number of visually observed grazing bites in cattle, even though the determination coefficients may vary from 0.79 to 0.90. Finally, Oudshoorn and Jørgensen (2013) used a 3-axis accelerometer to record cow bites and found that the Z-axis had periodic content consistent with the manual bite markings.

Their results were encouraging since the bite frequency automatically recorded was not statistically different from the manual count.

The objectives of this study were: to statistically discriminate the feeding behaviour of sheep into three different classes (i.e. grazing, ruminating and resting) using an X-Bee tri-axial accelerometer based sensor and to estimate the rate of biting (number of bites per min of grazing) on the basis of acceleration variables.

2. MATERIALS AND METHODS

2.1 Experimental site and animal management

The study was conducted at Bonassai experimental farm of the agricultural research agency of Sardinia (AGRIS Sardegna), located in the NW of Sardinia, Italy (40° 40' 16.215" N, 8° 22' 0.392" E, 32 m a.s.l).

The animal protocol below described was in compliance with the EU regulation on animal welfare and all measurements were taken by personnel previously trained and authorized by the institutional authorities managing ethical issues both at Agris Sardegna and the University of Sassari.

This current study was conducted from the 28th of 2013 to the 6th of February 2014 over 6 weeks. Three non-lactating adult Sarda ewes were used in the study with an age of 2.6 ± 0.6 years (mean \pm standard deviation) and live weight of 46.0 ± 1.0 kg. Two animals were used as “focal animal” to monitor the feeding behaviour, the third was used as companion animal. The ewes were kept in a stall and fed ryegrass hay and commercial concentrate in the first three days of each experimental week. Then, for the subsequent three days, they were accustomed to graze from 0900 to 1600 h one of the experimental plots previously established with monocultures of the following forage

species: alfalfa (*Medicago sativa* L., week 1), Italian ryegrass (*Lolium multiflorum* Lam., week 2, 3), sulla (*Hedysarum coronarium* L., week 4, 5) and chicory (*Cichorium intybus* L., week 6). These forages were chosen because they are widespread in Mediterranean sheep production systems and stimulate a wide range of behavioural responses, which have been already explored in micro-sward studies by our laboratory (Giovanetti et al., 2011). The seventh day of each week (test day), the feeding behaviour of the ewes was monitored when they were allocated from 0900 to 1600 h to a 20×20 m observation arena, fenced within each experimental plot using sheep electric net.

2.2 Accelerometer device and feeding behaviour recording

On each test day, one experimental sheep at a time was fitted with a halter equipped with a tri-axial accelerometer sensor, positioned under the lower jaw of the sheep. This device detects the animal's movements by measuring the accelerations on the X (longitudinal), Y (horizontal) and Z (vertical) axis (Figure 1).

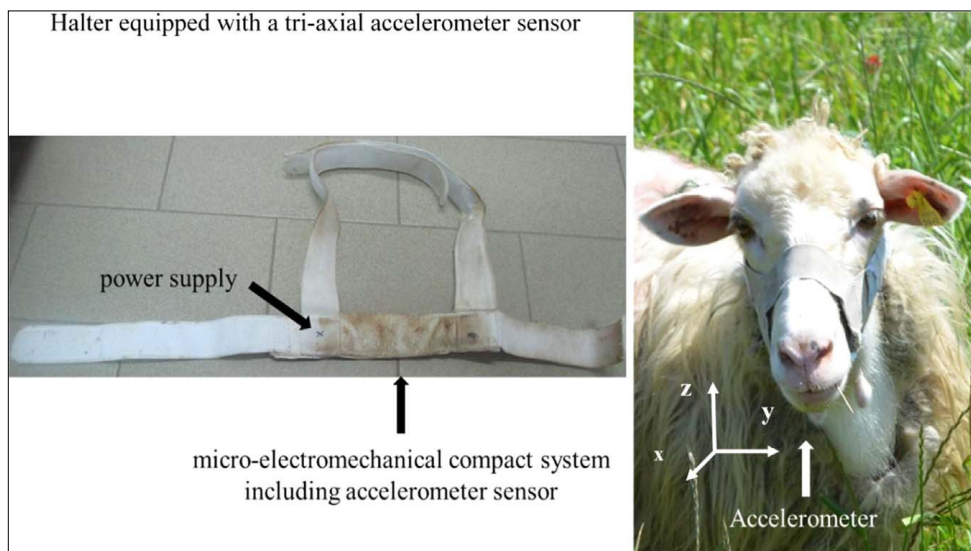


Figure 1. Halter equipped with a tri-axial accelerometer sensor

The sensor is inserted in a micro-electromechanical compact system (MEMS) with on-board peripherals. The central part of the system is an 8-bit high performance microcontroller. It also features integrated modules such as programmers connector, piezoelectric film element, wireless XBee that can transmit up to 1.5 km. The power supply of the system is guaranteed by a Lithium-Polymer (Li-Po) battery, connected via on-board battery connector. The acceleration sensor used was the ADXL335 (Analog Devices, One Technology Way, P.O. Box 9106, Norwood, MA 02062–9106, USA), a complete tri-axial accelerometer with signal conditioned voltage output. It records both dynamic accelerations, related to changes in the movements of the sheep and static accelerations (-9.8 m s^{-2}), caused by earth's gravity. The microcontroller samples raw acceleration data at a frequency of 62.5 Hz and encodes the data, through an analogue-to-digital converter with a resolution of 8 bits, into levels ranging from 0 to 255. Then, the microcontroller selects the first three peaks of accelerations per second and axis. In motionless circumstances, the microcontroller is programmed to return values equal to zero (static accelerations) for the three axes. Acceleration data were sent by a wireless XBee system to a nearby computer equipped with an antenna. A software package (DAS Client, Electronic System), installed on the computer, activates or deactivates the accelerometer device and manages data acquisition.

The feeding behaviour of sheep were video recorded during accelerometer deployment by fixed camera (Sanyo Xacti VPC-TH1, Sanyo Electric Co., Ltd. OSAKA, Japan). Video and accelerometer recordings were split into 30 - 45 min sub-periods during the time on the plots in order to re-align the camera or substitute its memory card. During these recording breaks, the halter with the accelerometer was rotated between the two

experimental sheep. The internal clock of the camera was synchronized with the internal clock of the computer. This ensured both the camera and accelerometer were synchronized in time to allow accurate annotation of the accelerometer data after behavioural recordings were made.

2.3 Preliminary data processing

A file including the three acceleration values for each axis and one of the three behavioural activities (grazing, ruminating, and resting) per second, based on the recorded videos, was created. Behaviour activities were classified according to Gibb (1998). Grazing activity included the act of searching for food while walking with the head down without evidence of biting, or standing still with the head down while biting and chewing either with the head down or the head up.

Ruminating activity included regurgitation, chewing and swallowing of bolus, in lying or standing position. Resting activity included all other activities, basically lying down or standing without rumination, and travelling.

The mean (MX, MY, MZ), variance (VX, VY, VZ) and inverse coefficient of variation (ICVX, ICVY, ICVZ) i.e. mean/standard deviation of acceleration data for each axis and per min as well as the resultant mean (MRES), variance (VRES) and ICV (ICVRES) values of the three axis, per min, were then calculated according to Watanabe et al. (2008).

The video-recorded behaviour of the animals was classified, at 1- min intervals, into one of the three prevailing activities in each minute between grazing, ruminating and resting, therefore the final dataset consisted of the three activities combined with the twelve variables concerning acceleration.

A subset of data that contained only minutes in which sheep were grazing was extracted from the final dataset and the number of bites per minute, counted from video recorded files, was added. Three other variables, the sum of the acceleration values per each axis and per min (SX, SY, SZ), were included to the subset that, at the end, contained 15 accelerometer variables.

2.4 Statistical analysis

An exploratory analysis of the final database was conducted using a one-way ANOVA model to test the effect of behavioural classes (three levels: grazing, ruminating and resting) on each single accelerometer variable. The Bonferroni correction was adopted to control the multiple testing error rate. Three multivariate statistical techniques were used to discriminate the three behavioural activities: stepwise discriminant analysis (SDA), canonical discriminant analysis (CDA), and discriminant analysis (DA).

All statistical analyses were performed by using the SAS software (SAS Inst. Inc., Cary, NC). Twelve variables concerning accelerations (MX, MY, MZ, VX, VY, VZ, ICVX, ICVY, ICVZ, MRES, VRES, ICVRES) and one categorical variable containing the three activities (grazing, ruminating, resting) were used in the analysis. The SDA was exploited to select variables that better discriminated groups. This step was crucial to avoid overfitting problems when new activities are going to be assigned to one of the three behaviours. Moreover, considering that the battery duration depends on the amount of data transmitted or stored, the selection of a reduced set of variables, able to correctly discriminate groups, could partially solve the battery charge problem.

The ability of selected variables in discriminating groups was tested by using CDA (Mardia et al., 2000). In general, if k indicates the number of groups, the CDA derives

$k-1$ linear equations, called canonical functions (CAN) that are used to predict the group to which an object belongs. The structure of a CAN is:

$$\text{CAN} = c_1X_1 + c_2X_2 + \dots + c_nX_n$$

where c_i are the canonical coefficients (CC) and X_i are the scores of the n involved variables. CCs indicate the partial contribution of each original variable in composing the CAN. In consequence, the higher the absolute value of a CC, the higher the weight of the corresponding variable in composing the CAN. The distance between groups evaluated by using the Mahalanobis' distance, whereas the effective groups' separation was tested by using the corresponding Hotelling's T-square test (De Maesschalck et al., 2000). DA was then exploited to classify objects into one of the involved groups. In practice, the canonical functions are applied to each object thus producing a discriminant score. An object is assigned to a particular group if its discriminant score is lower than the cutoff value obtained by calculating the weighted mean distance among group centroids (Mardia et al., 2000).

To validate the derived discriminant functions, the complete dataset was randomly divided into training and validation dataset in the proportion of four to one. This partition of the dataset was iterated 5000 times by using a bootstrap procedure (Efron, 1979). At each run, DA was applied to the training dataset to predict behaviours in the validation dataset and errors in assignment were recorded. To evaluate the performance of DA for discriminating the three behaviour activities, the sensitivity, specificity, precision and accuracy were calculated, based on the error distribution in assignment, using the following equations:

Sensitivity = $TP/(TP + FN)$;

Specificity = $TN/(TN+FP)$;

Precision = $TP/(TP+FP)$;

Accuracy = $(TP+TN)/(TP+TN + FP + FN)$

Where TP, TN, FP and FN are true positive, true negative, false positive and false negative counts respectively.

The k coefficient was calculated (Fleiss, 1981) to evaluate the agreement between observed and model predicted corrected for agreement that would be expected by chance, both for each behaviour and overall. The k values were judged according to criteria of Landis and Koch (1977).

The subset of data containing only grazing behaviour was analysed by regressing the video recorded rate of biting (number of bites per minute) on the acceleration variables (MX, MY, MZ, VX, VY, VZ, ICVX, ICVY, ICVZ, MRES, VRES, ICVRES, SX, SY, SZ) by using a stepwise model to select the best variables subset to predict the number of bites.

3. RESULTS

Since the variation of each variable was great, especially for maximum values, data were edited by using as threshold values the mean of each variable plus two standard deviations. After correction, the final dataset contained 675 min.

The ANOVA model applied to all variables is displayed in Table 1. Apart from VZ and VRES, for which ruminating and resting were similar, the three activities were significantly different. The highest values were obtained for grazing followed by ruminating and resting.

Table 1. The effect of the behavioural activity classes recorded in grazing sheep on the mean (M), variance (V) and inverse coefficient of variation (ICV) of acceleration values per minute along the X, Y and Z-axis and the resultant

	Grazing	Ruminating	Resting	P<
Minutes	247	149	279	0.0001
MX	3.14 ± 0.06 ^a	1.50 ± 0.07 ^b	0.54 ± 0.05 ^c	0.0001
MY	4.40 ± 0.07 ^a	2.74 ± 0.09 ^b	0.78 ± 0.07 ^c	0.0001
MZ	6.95 ± 0.11 ^a	1.41 ± 0.14 ^b	0.88 ± 0.10 ^c	0.0001
MRES	4.83 ± 0.07 ^a	1.90 ± 0.09 ^b	0.73 ± 0.07 ^c	0.0001
VX	29.12 ± 0.65 ^a	9.12 ± 0.84 ^b	5.40 ± 0.61 ^c	0.0001
VY	34.33 ± 0.80 ^a	17.44 ± 1.04 ^b	6.69 ± 0.76 ^c	0.0001
VZ	113.25 ± 2.93 ^a	6.34 ± 3.77 ^b	14.57 ± 2.75 ^b	0.0001
VRES	62.03 ± 1.28 ^a	11.42 ± 1.65 ^b	8.96 ± 1.21 ^b	0.0001
ICVX	0.58 ± 0.01 ^a	0.54 ± 0.01 ^b	0.20 ± 0.01 ^c	0.0001
ICVY	0.76 ± 0.01 ^a	0.67 ± 0.01 ^b	0.26 ± 0.01 ^c	0.0001
ICVZ	0.68 ± 0.01 ^a	0.57 ± 0.01 ^b	0.22 ± 0.01 ^c	0.0001
ICVRES	0.63 ± 0.01 ^a	0.57 ± 0.01 ^b	0.22 ± 0.01 ^c	0.0001

^{a-c}Means followed by different letters differ significantly at P<0.05.

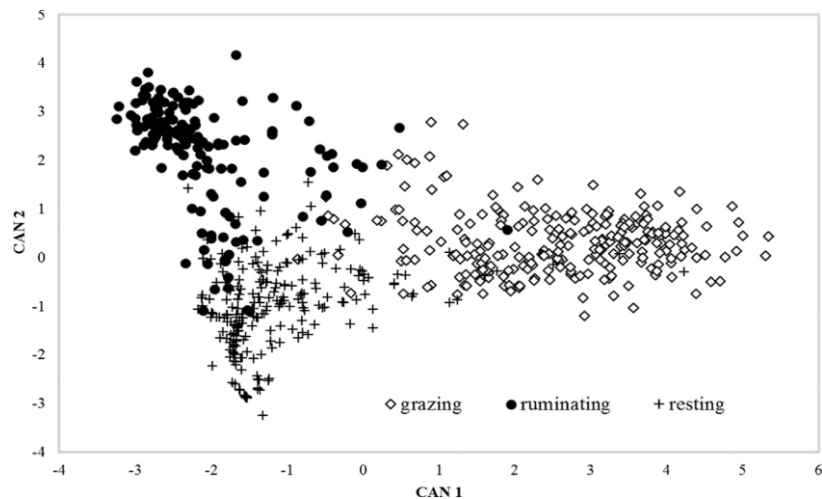
3.1. Discrimination of behaviour activities

SDA applied to the entire dataset selected seven (Table 2) among the twelve original variables. The subsequent CDA significantly discriminated the three behaviours (Hotelling's test P<0.0001) by extracting two canonical functions. The variance explained by CAN1 and CAN2 was 0.68 and 0.32, respectively. Canonical coefficient (CC) values, reported in Table 2, show that both in CAN1 and CAN2, the greatest CCs were for VRES and VZ.

Table 2. Standardized canonical coefficients

Variable	CAN1	CAN2
MZ	4.60	- 3.08
VX	2.74	- 1.72
VY	3.15	- 1.51
VZ	12.80	- 6.50
VRES	- 19.07	10.53
ICVX	- 0.56	1.00
ICVZ	- 0.28	1.69

Figure 2 shows that canonical functions can separate the three behavioural activities. CAN1 discriminates the grazing activity from the resting and ruminating ones, whereas CAN2 differentiates the resting from the ruminating behaviour. In particular, the lowest Mahalanobis' distance was obtained between ruminating and resting (183), whereas greater distances were obtained for grazing and ruminating (324) and grazing and resting (311).

**Figure 2.** Plot of canonical variables (CAN 1, CAN 2) generated from discriminant analysis

After the bootstrap resampling, the DA correctly assigned 93% of minutes to behavioural activities. Errors in assignment, specificity, sensitivity, precision and accuracy of DA for discriminating the sheep behaviour activities and k coefficient of agreement between observed and predicted values are displayed in Table 3.

Table 3. Distribution of the total error and performance in the assignment of behaviour activities, predicted on the basis of accelerometer data

Observed behaviour	Predicted behaviour			
	Grazing	Ruminating	Resting	Total
Grazing	234	8	6	247
Ruminating	1	133	15	149
Resting	9	9	261	279
Total	244	149	282	675
Sensitivity (%)	96	89	93	
Specificity (%)	97	97	95	
Precision (%)	95	89	94	
Accuracy (%)	96	95	94	93 ^a
k	0.92	0.86	0.89	0.89 ^s

^aOverall accuracy of the discriminant analysis.

^sKappa coefficient used as overall coefficient of agreement (P<0.001).

The overall accuracy in DA assignment of minutes to the three behaviours was 93%. The precision was 95% for grazing, 94% for resting and 89% for ruminating. The activity with the highest sensitivity (96%), specificity (97%) and accuracy (96%) of classification was grazing. Ruminating and grazing were predicted with the same specificity (97%). About 10% of rumination was misclassified as resting since these two activities are similar for some of the variables considered in the DA (VRES and VZ).

Resting activity reported lower specificity (95%) and accuracy values (94%) than grazing and ruminating.

3.2. Prediction of rate of biting

Stepwise regression selected only SX and this explained 65% of the total variation for rate of biting. The intercept was not significantly different from zero ($P=0.66$) but the slope (0.06) was highly significantly different from zero ($P<0.001$).

4. DISCUSSION

4.1 Discrimination of behaviour activities

Under the conditions of this experiment, all accelerometer variables had the highest value when the sheep were grazing, the lowest while resting, being the rumination response intermediate (Table 1). Actually when the sheep is grazing, all axes are involved in detecting different dynamic movements with a strong preponderance of vertical axis (Z). Grazing activity is a complex process. The sheep while walking or standing lower and raise its head to search the herbage, gathers and manipulate the herbage with their lips, grips it between its incisors and severe it from the sward (biting) often with a jerk of the head (vertical movement). The herbage severed is then chewed, formed into a bolus and finally swallowed (Penning and Rutter, 2004). Biting and chewing may be carried out simultaneously as “chew-bites” (Laca and Wallis De Vries, 2000). This activity determines horizontal, vertical movements of the head and lateral and vertical movement of the jaw. In ruminating, movements of the head and jaw are more regular related to regurgitation, chewing and swallowing of merycic boluses.

This activity involves lateral and vertical movements of the jaw that are mainly revealed by the Y and Z-axis of the sensor. Resting activity, which includes sheep lying down, standing or travelling in absence of jaw activity, reports the lowest values for almost all variables in all axes with the exception of VZ and VRES values (Table 1) that are similar to ruminating probably because of a lower variability of vertical movements of the head in these two activities.

The SDA selected seven variables (Table 2) out of the twelve original ones. In particular, all variances (VX, VY, VZ and VRES) one mean (MZ) and two ICV variables (ICVX and ICVZ) were included in the final model. Watanabe et al. (2008) in a similar experiment involving grazing cows, found that the best discrimination among the three behaviour classes (95.7% of correct assignment) was obtained by retaining height variables: MX, MY, MZ, MRES, ICVX, ICVY, ICVZ, ICVRES. The above cited authors suggested that mean variables were effective in detecting body posture (static acceleration) whether the ICV variables detected well both differences in body posture and movements (dynamic acceleration) for each activity.

In the present experiment, means are less important than variances and ICVs in detecting activities probably because accelerations related to the head and jaw movements are more variable in sheep than in cows, particularly when grazing. This result is in agreement with Chambers et al. (1981) who reported similar wave forms of the accelerometer outputs during grazing in sheep and cows but with higher variability of acceleration and peak values in sheep than cows.

The authors ascribed these results in part to the greater inertia of the cow's head movement and in part to the typical cow's head movement that involves some circular

movement. In sheep, on the other hand, head movement is essentially backward and forward in the longitudinal axis of the body. Moreover, sheep make greater use of the lips in manipulating herbage that causes a greater ratio of jaw to head movements than cows. Actually cattle are able to open their mouth wider than sheep and to increase the grazed area by extending their tongue thus reaching both an higher herbage mass per bite (bite mass) and an higher herbage intake rate (g DM/min).

Parsons et al. (1994) suggested that the main factor affecting intake rate was the handling time, i.e. the time required to take a bite of herbage of a given mass and to manipulate and chew that herbage before swallowing it. The higher intake rate achieved by cattle than sheep is due to a lower proportion of total jaw movements allocated to masticate herbage during grazing. This is probably related to their higher rumen retention time that allows better fiber fermentation in the rumen and requires a lower number of mastication jaw movements per bite of herbage ingested (Van Soest, 1994). It can be stated the sheep have higher handling costs than cows per unit of herbage ingested that causes a greater variability in jaw/head movements and, as a consequence, in accelerations. Although sheep have probably a different accelerometer pattern than cows while grazing, part of the higher variability of the acceleration detected in this experiment rather than in others focused on cattle (e.g. Watanabe et al., 2008) could be due to the wide range of forage monocultures tested in this experiment. In fact, these forage species are known to give different foraging responses (Giovanetti et al., 2011) and probably affect differently the movements and accelerations associated to their biting and chewing. If we look to the contribution of the X, Y and Z-axes to the discriminant function, we can observe that the Z-axis (vertical body axis) is always

represented with the three categories of variables (mean, variance and ICV). This result can be explained considering that during all monitored behaviours (namely grazing, ruminating and resting) vertical movements are always performed by the animal.

CDA was then exploited to verify if, on the basis of the variables selected by the SDA, minute data came from different behaviour activities. The CAN1 versus CAN2 scatter plot (Figure 2) displayed a clear separation among the three behaviours. In particular, CAN1, which accounted for 68% of total variability, was able to separate grazing from the other two behaviours. This marked difference is confirmed by the ANOVA results that reported greater values in grazing activity than ruminating and resting. To separate the ruminating from resting, the second canonical function (CAN2), which explained the remaining 32% of variance, is needed. In both CAN1 and CAN2, the variables VRES and VZ showed the highest absolute CC values (Table 2).

This result indicates that the separation of the three behaviours is mainly determined by those variables. This was an expected result because VRES combines the variances of the three axis X, Y and Z and VZ is one of the most important variable in the three activities. Furthermore, the Hotelling's test was highly significant for all the Mahalanobis' distances. In our experiment, we found a precision of 95% for grazing, 94% for resting and 89% for ruminating (Table 3). This result is slightly lower than what found by Watanabe et al. (2008) in cattle who reported 98% of correct assignment in grazing behaviour, followed by resting (92.8%) and ruminating (92.3%) activity.

An important outcome of the present study is the high statistical agreement (0.89), determined with k coefficient, which represents a measure of fortuity (or not) agreement between observed and predicted by the model for each behaviour (Table 3).

Among the three behaviours, grazing showed the highest agreement ($k=0.92$). Considering all the performances (sensitivity, specificity, precision and accuracy) in the assignment of behaviour activities (Table 3), the results of this validation exercise overall suggest that the technology developed in the present study is particularly appropriate to precisely and accurately monitor sheep grazing behaviour. Moreover, despite some limitation, the results of this experiment outperform the goodness of fit of the classification of grazing behaviour reported by other authors in sheep, even when no distinction between rumination and resting was tempted. For instance, Marais et al. (2014), placing a tri-axis accelerometer device around the neck of the sheep, were able to identify different sheep behaviours with a high accuracy for standing, walking and running (95.2%, 93.7% and 99.5% of correct assignment, respectively) but grazing was misclassified as lying in 35% of cases.

McLennan et al. (2015), validating an automatic recording system, were only able to distinguish between active (miscellanea including grazing, walking, standing, ruminating and standing) and inactive behaviours (lying ruminating and lying) in sheep without giving any indication of the specific activity performed by the animal.

More recently, Alvarenga et al. (2016), positioning a 3-axis accelerometer under the lower jaw of the sheep, achieved 84.3%, 97.3% and 92.9% for sensitivity, specificity and precision with k of 0.79 in classifying grazing behaviour considering a length epoch of 5 s. Although these authors obtained results similar to ours, they reported an overall lower accuracy (81.9% vs. 93.0%) and were unable to measure rumination activity, which is of high nutritional relevance.

4.2 Prediction of rate of biting

The stepwise regression, applied to the subset of the final dataset, selected only one variable, the sum of acceleration in the X-axis per minute (SX), thus confirming that the head movement during biting is mainly backward and forward in the longitudinal axis of the body. Our results confirm the difficulty to count bites using an accelerometer in field condition, in line with the few studies available in the literature, to the best of our knowledge. The partial agreement with visual observation (65%) obtained in the regression model is probably explainable by different reasons such as: 1) the sensitivity of accelerometers could provide undesirable signals during recording sessions due to head movements not related to grazing activity; 2) the rate of biting is so high in sheep that is sometimes difficult to capture the single bite event, unless video recording is run from very close or under controlled conditions (micro-sward trials); 3) the visual bite count includes also chew-bites that probably produces acceleration signals different from those originated by bites alone.

Finally, we cannot rule out that using raw signal analysis of accelerometer data can provide better prediction of rate of biting or total bite number than using the sum of acceleration values in the X-axis per minute, as found in cows by Andriamandroso et al. (2015) who obtained a mean error of about 5%.

5. CONCLUSIONS

Accelerometers combined with wireless communication technology are useful tools to discriminate grazing, ruminating and resting behaviour activities of grazing sheep. The multivariate statistical approach allowed to reduce the number of variables that are

needed to assign acceleration minutes to the appropriate behaviour classes with a mean accuracy of 93% and k coefficient of 0.89. Some ruminating activities were misclassified as resting probably because the latter included walking and other non classified activities. The sum of accelerations in the X-axis per minute provides a good proxy of the number of bites per minute. Better performances could be obtained in the future by processing data with different time epochs and possibly using raw data instead of minute-based statistical parameters.

Other sensors could be added to this device in order to improve the overall classification accuracy and to effectively drive the management of pastoral resources.

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CHAPTER 3

**The effect of different time epoch settings on the classification of sheep
behaviour using tri-axial accelerometry**

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ABSTRACT

Monitoring behaviour of grazing animals is important for the management of grazing systems. A study was run to discriminate between the main behaviours (grazing, ruminating and other activities) of sheep at pasture wearing a halter equipped with an accelerometer (BEHARUM device), and to identify the epoch setting (5, 10, 30, 60, 120, 180 and 300 s) with the best performance. The BEHARUM device includes a three-axial accelerometer sensor and a force sensor positioned under the lower jaw of the animal. The halter was fitted to eight Sarda dairy sheep that rotationally grazed either a spatial association (mixture) or a time association of berseem clover (*Trifolium alexandrinum* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) for 6 h day⁻¹. The behaviour of the animals was also video-recorded. The raw acceleration and force data were processed for each epoch setting to create 15 variables: the mean, variance and inverse coefficient of variation (ICV; mean/standard deviation) per minute for the X-, Y-, Z-axis and force, and the resultant. Multivariate statistical techniques were used to discriminate between the three behavioural activities: canonical discriminant analysis (CDA), and discriminant analysis (DA). To validate the derived discriminant functions, a bootstrap procedure was run. To evaluate the performance of DA in discriminating

between the three activities, the sensitivity, specificity, precision, accuracy and Coehn's k coefficient were calculated, based on the error distribution in assignment. Results show that a discriminant analysis can accurately classify important behaviours such as grazing, ruminating and other activities in sheep at pasture. The prediction model has demonstrated a better performance in classifying grazing behaviour than ruminating and other activities for all epochs. The 30 s epoch length yielded the most accurate classification in terms of accuracy and Coehn's k coefficient. Nevertheless, 60 and 120 s may increase the potential recording time without causing serious lack of accuracy.

1. INTRODUCTION

Monitoring the behaviour of grazing ruminants is important to understand how animals meet their requirements in pastoral systems and to achieve optimal plant production, animal forage intake and performances (Carvalho, 2013). Since observing animal behaviour is a labour intensive and difficult task, whether it is performed with direct observations or through video recordings, most research has concentrated on recognizing feeding behaviour of ruminants from animal attached sensors. A type of sensor that has recently become widespread in research studies is the tri-axial accelerometer, since it is small, inexpensive, and easy to wear (Brown et al., 2013). Accelerometers have been widely used to automatically detect and classify several behaviours in cattle, e.g. oestrus detection (Ueda et al., 2011), walking (Robert et al., 2009) feeding and standing activities in a free-stall barn (Arcidiacono et al., 2017), sleeping posture (Fukasawa et al., 2018) and time (Hokkanen et al., 2011), and eating,

ruminating and resting activities (Watanabe et al., 2008). Fewer research studies have been conducted to classify sheep behaviours than cattle behaviours.

Umstätter et al. (2008) used integrated pitch and roll tilt sensors, and found that they could distinguish between two categories: active and inactive, with more than 90% accuracy. Other studies on sheep behaviour used the collar attached Actiwatch accelerometer system for classifying activity levels and detecting diurnal rhythms (Piccione et al., 2010; 2011). Other authors (Nadimi et al., 2012; Nadimi and Søgaard, 2009) used the ADXL202 accelerometer to detect grazing, lying down, standing, walking, mating and drinking in sheep with a mean accuracy of 76.2%.

Alvarenga et al. (2016) successfully identified grazing and non-grazing states, with accuracies higher than 83%, in grazing sheep wearing an accelerometer under the lower jaw. More recently Giovanetti et al. (2017a), positioning a device containing an ADXL335 accelerometer sensor in the same place, were able to classify grazing, ruminating and resting behaviour of sheep at pasture with an overall accuracy of 93%. Tri-axial accelerometer based devices can acquire and store information internally, thus consuming very little battery power. However, the amount of data that can be collected is limited by the size of the memory card within the device. On the other hand, data can be directly transmitted to a central receiver for subsequent processing. This practice, however, requires a high power consumption (Vázquez Diosdado et al., 2015).

The sampling frequency of such devices usually ranges from 8 to 100 Hz, thus producing an enormous quantity of data, proportional to the sampling frequency, which can lead to a rapid depletion of the memory device and to high costs in terms of battery consumption caused by sending and receiving large data sets. These restrictions could

be overcome by undertaking some form of preliminary processing of the accelerometer data on the device itself settling and applying to the data stream, for a given sampling frequency, an optimal aggregation window (called epoch).

Optimizing the epoch setting, without compromising classification accuracy, could imply a number of advantages. Short epoch settings could increase the labour involved in processing data, deplete the memory device, decrease the battery duration and may also cause erroneous attribution activities during processing. Actually, if an epoch shorter than the period of time an activity occurs is used, the number of false positive classifications for dynamic activities could increase probably due to transitioning between different activities or body shifts during static activities (Robert et al., 2009). Conversely, optimized longer epoch settings might reduce the memory depletion and increase the battery duration without compromising the performance of the sensor. Nielsen (2013) distinguished grazing from non-grazing behaviour with a 3D activity sensor that correctly classified the behaviours of dairy cows with a relatively high accuracy when the epoch was set at 5 s, 5 or 10 min.

Other authors, as Vázquez Diosdado et al. (2015), while classifying lying, standing and feeding behaviours in dairy cows, reported a small increase in the decision-tree classification algorithm performance at the largest window size of 10 min if compared with 1 and 5 min epoch settings.

In the present research, a customized tri-axial accelerometer based sensor, able to either store data in a micro SD card or send them to a remote computer, was used. In the future perspective of data pre-processing in the device itself, determining the optimum

device settings before field application is crucial, because they could impact on monitoring system accuracy as well as on the effective battery and memory life.

The objectives of this study were: 1) to develop an algorithm based on the multivariate statistical analysis to discriminate the main behaviours (grazing, ruminating and other activities) of sheep at pasture equipped with a customized tri-axial accelerometer based sensor named BEHARUM; 2) to determine the performance of the algorithm in terms of accuracy, sensitivity, specificity, precision and Coehn's k coefficient, at different epoch settings (5, 10, 30, 60, 120, 180 and 300 s); 3) to select the epoch that optimizes the system accuracy of the device.

2. MATERIALS AND METHODS

2.1 Experimental site and animal management

The study was conducted at Bonassai experimental farm of the agricultural research agency of Sardinia (AGRIS Sardegna), located in the NW of Sardinia, Italy (40° 40' 16.215" N, 8° 22' 0.392" E, 32m a.s.l).

The animal protocol below described was in compliance with the EU regulation on animal welfare and all measurements were taken by personnel previously trained and authorized by the institutional authorities managing ethical issues both at Agris Sardegna and the University of Sassari.

The study is part of an experiment conducted in spring 2016, from 1 March to 9 May, with 48 mature lactating Sarda dairy sheep that rotationally grazed berseem clover (*Trifolium alexandrinum* L.) and Italian ryegrass (*Lolium multiflorum* Lam.) for 6 h day⁻¹

¹. Two grazing treatments were used: a mixture of berseem clover and Italian ryegrass, and two monocultures (berseem clover and ryegrass) grazed in succession.

In the latter case, the sheep grazed the first 3 h on the clover and the last 3 h on the ryegrass. The ewes were machine milked twice daily at 0700 h and 1500 h. During milkings, they were individually fed in the milking parlor with commercial concentrate (500 g ewe⁻¹ day) split into two meals. In the remaining daytime, the animals were kept indoors and group-fed 500 g ewe⁻¹ of ryegrass hay and 300 g ewe⁻¹ of alfalfa hay in separate troughs. On four occasions (test days) during the experiment, eight ewes (four per treatment), with an age of 3.1 ± 1.6 years (mean \pm standard deviation), live weight of 41.3 ± 2.8 kg, lactation stage of 73 ± 6 days in milk and milk yield of 2062 ± 362 g ewe⁻¹ day⁻¹, were used. On each test day, after the morning milking, the ewes were carried on a trailer to the experimental plots and equipped with the BEHARUM device before the six hours of access to pasture. At the end of the grazing session, the BEHARUM devices were removed from the animals.

2.2 Description of the BHEARUM device and feeding behaviour recording

The BEHARUM device includes a halter equipped with a three-axial accelerometer sensor and a force sensor positioned under the lower jaw of the animal. Animal head and jaw movements are detected through accelerations measured in the X (longitudinal), Y (horizontal) and Z (vertical) axes (Figure 1) and force exerted by the opening jaw.

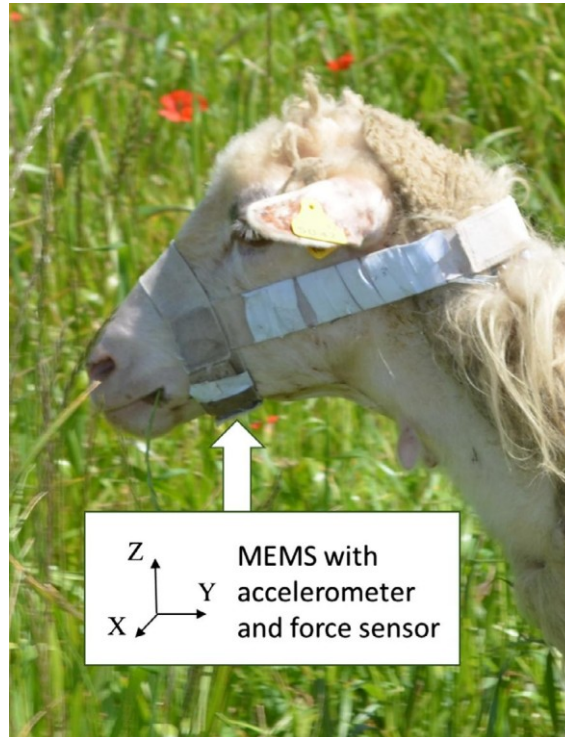


Figure 1. BEHARUM halter with accelerometer and force sensor inserted in a micro-electromechanical compact system (MEMS) positioned under the jaw

The sensors, inserted in a micro-electromechanical compact system (MEMS) with on-board peripherals, sample raw accelerations and force at a frequency of 62.5 Hz, and convert them, through an analogue-to-digital converter with a resolution of 8 bits, in digital levels ranging from 0 to 255. Then the microcontroller selects three converted values per second per axis (Giovanetti et al., 2017a) and force sensor. The converted data could be sent (LoRa wireless system) to a nearby computer receiver equipped with an antenna or to a remote computer through a local server using the GSM services, as well as recorded in a micro secure digital (SD) card inserted in the MEMS.

A software package (DAS Client, Electronic System), installed on the computer, activates or deactivates the BEHARUM device and manages data acquisition. In this

experiment, we adopted the recording of acceleration and force data on micro SD card. On each test day through the 6 h of access to pasture, the feeding behaviour of sheep equipped with the BEHARUM were video recorded, one at time, during accelerometer deployment by fixed camera (Sanyo Xacti VPC-TH1, Sanyo Electric Co., Ltd. OSAKA, Japan). Video recordings of each animal lasted 20–25 min. The internal clock of the camera was synchronized with the internal clock of the computer. This ensured both the camera and accelerometer were synchronized in time to allow accurate annotation of the accelerometer data after behavioural recordings were made.

2.3 Preliminary data processing

On the basis of the recorded videos, a file including the three acceleration values for each axis, the force values and one of the three behavioural activities (grazing, ruminating, and other activities) per second was created, for a total of 69,975 s dataset. Behaviour activities were classified according to Gibb (1998). Grazing activity included the act of searching for food while walking with the head down without evidence of biting, or standing still with the head down while biting and chewing either with the head down or the head up. Ruminating activity included regurgitation, chewing and swallowing of bolus, in lying or standing position. Other activities included all the activities not taken into account in grazing and ruminating, e.g. lying down or standing without rumination, and travelling etc. Mean (MX, MY, MZ, MF), variance (VX, VY, VZ, VF), inverse coefficient of variation (i.e. mean/standard deviation, ICVX, ICVY, ICVZ, ICVF), of acceleration data for each axis and force data, as well as the resultant mean (MRES), variance (VRES) and ICV (ICVRES) values of the three axis and force

(Watanabe et al., 2008), were calculated for the following epoch settings: 5 s, 10 s, 30 s, 60 s, 120 s, 180 s, 300 s.

Video recordings were coded manually assigning to each epoch the prevailing behaviour, that is to say grazing, ruminating or other activities.

We considered as prevailing the behaviour with the highest percentage among the three activities performed by the animal within epoch setting. Overall, the percentage of the prevailing behaviour ranged from 50 to 100%. For that reason, we established three classes of prevalence: 50–75%, 76–99% and 100%. Afterwards we counted, for each epoch setting, how many times the prevailing behaviours were included in one of the three classes and we expressed the values obtained as a percentage of the total.

For each epoch setting, data were arranged in a multivariate manner with seventeen columns including the epoch, the prevailing activity and the fifteen acceleration and force variables (MX, MY, MZ, MF, VX, VY, VZ, VF, ICVX, ICVY, ICVZ, ICVF, MRES, VRES, ICVRES). Eventually, we obtained seven datasets, one for each epoch setting under study.

2.4 Data processing

An exploratory analysis of each dataset was conducted using a one-way ANOVA model to test the effect of behavioural activities (grazing, ruminating and other activities) on all fifteen accelerometer and force variables.

The seven datasets were then submitted to two multivariate statistical techniques to discriminate between the three behavioural activities: canonical discriminant analysis (CDA), and discriminant analysis (DA). All statistical analyses were performed by using the SAS software (SAS Inst. Inc., Cary, NC). CDA was used to test the ability of

the variables involved (the fifteen accelerations and force variables) in discriminating between groups (grazing, ruminating and other activities) (Mardia et al., 2000). In general, if d indicates the number of groups, the CDA derives $d - 1$ linear equations, called canonical functions (CAN) that are used to predict the group to which an object belongs. The structure of a CAN is:

$$\text{CAN} = c_1X_1 + c_2X_2 + \dots + c_nX_n$$

where c_i are the canonical coefficient (CC) and X_i are the values of the n involved variables. CCs indicate the partial contribution of each original variable in composing the CAN. In consequence, the higher the absolute value of a CC, the higher the weight of the corresponding variable in composing the CAN. In the present research, d was equal to 3 (the three behaviours) and, in consequence, two CANs were obtained.

The distance between groups was evaluated by using the Mahalanobis' distance, whereas the effective groups' separation was tested by using the corresponding Hotelling's T-square test (De Maesschalck et al., 2000). DA was then used to classify epochs into one of the three behaviours (Mardia et al., 2000).

To validate the derived discriminant functions, each dataset was randomly divided into training and validation dataset in the proportion of 4:1. This partition of the dataset was iterated 5000 times by using a bootstrap procedure (Efron, 1979). At each run, DA was applied to the training dataset to predict behaviours in the validation dataset and errors in assignment were recorded. To evaluate the performance of DA in discriminating between the three activities, the sensitivity, specificity, precision and accuracy were calculated, based on the error distribution in assignment, using the following equations:

Sensitivity = $TP/(TP + FN)$;

Specificity = $TN/(TN + FP)$;

Precision = $TP/(TP + FP)$;

Accuracy = $(TP + TN)/(TP + TN + FP + FN)$

where TP, TN, FP and FN are true positive, true negative, false positive and false negative counts respectively. The Coehn's k coefficient was calculated (Fleiss, 1981) to evaluate the agreement between observed and model predicted corrected for agreement that would be expected by chance, both for each behaviour and overall. The k values were judged according to the criteria of Landis and Koch (1977).

3. RESULTS

Overall, the distribution of the three behaviours in the datasets is on average 50% grazing, 30% ruminating and 20% other activities. The results of the ANOVA showed that the behaviour activities affected significantly all variables in each epoch apart from ICVX in the 300 s dataset (Table 1). Some variables (MY, VY, MRES and VRES) were always significantly different between the behaviours in all epochs. The same results can be observed for MX, MF and VF with the exception of 300 s, where only grazing was different from the other two behaviours. Differences between the three activities were also found in other variables but only in some epoch (Table 1). In all the other cases, only one behaviour was different from the other two.

Table 1. The effect of the behavioural activities recorded in grazing sheep on the mean (M), variance (V) and inverse coefficient of variation (ICV) of acceleration values per minute along the X, Y, Z-axis, force (F) and the resultant

		MX	VX	ICVX	MY	VY	ICVY	MZ	VZ	ICVZ	MRES	VRES	ICVRES	MF	VF	ICVF
5 s	Grazing	48.3 ^a	3493 ^a	1.2 ^c	71.7 ^a	6821 ^a	1.0 ^b	33.4 ^a	1581 ^c	1.3 ^b	50.6 ^a	3987 ^a	0.8 ^b	48.9 ^a	2098 ^a	1.3 ^c
	Ruminating	17.7 ^b	753 ^b	1.4 ^b	45.7 ^b	4644 ^b	1.2 ^b	29.2 ^b	2965 ^a	1.3 ^b	29.6 ^b	3083 ^b	0.8 ^b	25.9 ^b	1095 ^b	1.7 ^b
	Other activities	7.0 ^c	353 ^c	4.4 ^a	14.0 ^c	1588 ^c	4.5 ^a	19.4 ^c	2345 ^b	3.8 ^a	11.8 ^c	1429 ^c	1.5 ^a	6.8 ^c	271 ^c	7.7 ^a
	P <	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
10 s	Grazing	47.8 ^a	3545 ^a	1.0 ^b	70.9 ^a	6868 ^a	0.9 ^b	33.4 ^a	1640 ^c	1.1 ^b	50.2 ^a	3978 ^a	0.8 ^b	48.8 ^a	2095 ^a	1.2 ^b
	Ruminating	15.8 ^b	624 ^b	1.1 ^b	44.6 ^b	4763 ^b	0.9 ^b	28.7 ^b	3226 ^a	0.9 ^b	28.4 ^b	3109 ^b	0.6 ^c	24.6 ^b	1078 ^b	0.5 ^c
	Other activities	4.5 ^c	198 ^c	3.1 ^a	10.6 ^c	1359 ^c	3.3 ^a	18.2 ^c	2592 ^b	2.7 ^a	9.2 ^c	1311 ^c	1.1 ^a	3.6 ^c	126 ^c	4.4 ^a
	P <	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 1. (continued)

		MX	VX	ICVX	MY	VY	ICVY	MZ	VZ	ICVZ	MRES	VRES	ICVRES	MF	VF	ICVF
30 s	Grazing	47.6 ^a	3625 ^a	0.8 ^b	70.6 ^a	6977 ^a	0.9 ^b	33.4 ^a	1695 ^c	0.9 ^b	50.0 ^a	4004 ^a	0.8 ^a	48.5 ^a	2101 ^a	1.2 ^b
	Ruminating	15.9 ^b	675 ^b	0.9 ^b	44.5 ^b	5066 ^b	0.8 ^b	28.6 ^b	3458 ^a	0.8 ^b	28.4 ^b	3178 ^b	0.6 ^c	24.5 ^b	1146 ^b	0.8 ^b
	Other activities	4.4 ^c	205 ^c	1.8 ^a	10.4 ^c	1431 ^c	1.9 ^a	18.1 ^c	2788 ^b	1.7 ^a	9.1 ^c	1327 ^c	0.7 ^b	3.5 ^c	137 ^c	2.2 ^a
	P <	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
60 s	Grazing	47.3 ^a	3645 ^a	0.8 ^b	70.2 ^a	7004 ^a	0.9 ^b	33.2 ^a	1697 ^b	0.8 ^b	49.7 ^a	3996 ^a	0.8 ^a	48.1 ^a	2090 ^a	1.1 ^a
	Ruminating	15.9 ^b	693 ^b	0.9 ^b	44.6 ^b	5177 ^b	0.8 ^b	28.7 ^b	3594 ^a	0.7 ^c	28.4 ^b	3212 ^b	0.5 ^b	24.6 ^b	1167 ^b	0.8 ^b
	Other activities	5.3 ^c	284 ^c	1.5 ^a	11.7 ^c	1590 ^c	1.3 ^a	18.5 ^c	2939 ^a	1.2 ^a	10.1 ^c	1415 ^c	0.5 ^b	4.8 ^c	221 ^c	1.1 ^a
	P <	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 1. (continued)

		MX	VX	ICVX	MY	VY	ICVY	MZ	VZ	ICVZ	MRES	VRES	ICVRES	MF	VF	ICVF
120 s	Grazing	46.7 ^a	3630 ^a	0.8 ^b	69.9 ^a	7051 ^a	0.8 ^{ab}	33.1 ^a	1745 ^b	0.8 ^a	49.3 ^a	4004 ^a	0.01 ^b	47.5 ^a	2094 ^a	1.1 ^a
	Ruminating	16.4 ^b	762 ^b	0.8 ^b	44.0 ^b	5180 ^b	0.7 ^b	29.1 ^b	3722 ^a	0.6 ^b	28.5 ^b	3217 ^b	0.01 ^b	24.7 ^b	1181 ^b	0.8 ^b
	Other activities	6.2 ^c	356 ^b	1.2 ^a	13.8 ^c	1865 ^c	1.0 ^a	18.2 ^c	2875 ^a	0.8 ^a	11.1 ^c	1499 ^c	0.3 ^a	6.2 ^c	283 ^c	0.9 ^b
	P <	0.01	0.01	0.01	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
180 s	Grazing	46.2 ^a	3624 ^a	0.8 ^b	69.2 ^a	7017 ^a	0.8 ^a	33.0 ^a	1781 ^b	0.8 ^a	48.9 ^a	3993 ^a	0.8 ^a	47.4 ^a	2096 ^a	1.1 ^a
	Ruminating	16.4 ^b	785 ^b	0.8 ^b	43.2 ^b	5103 ^b	0.7 ^b	28.6 ^a	3707 ^a	0.6 ^b	28.2 ^b	3152 ^b	0.5 ^b	24.5 ^b	1172 ^b	0.8 ^b
	Other activities	8.9 ^c	568 ^b	0.9 ^a	18.9 ^c	2484 ^c	0.6 ^b	20.0 ^b	3013 ^a	0.7 ^{ab}	14.0 ^c	1783 ^c	0.4 ^c	8.2 ^c	382 ^c	0.7 ^b
	P <	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Table 1. (continued)

300 s	Grazing	44.3 ^a	3457 ^a	0.8	68.2 ^a	7084 ^a	0.8 ^a	32.5 ^a	1862 ^b	0.8 ^a	47.5 ^a	3940 ^a	0.8 ^a	44.8 ^a	1964 ^a	1.1 ^a
	Ruminating	17 ^b	887 ^b	0.7	43.8 ^b	5216 ^b	0.7 ^b	26.7 ^b	3467 ^a	0.5 ^b	28.1 ^b	3116 ^b	0.5 ^b	24.9 ^b	1229 ^b	0.8 ^b
	Other activities	14.5 ^b	1119 ^b	0.8	22.6 ^c	2673 ^c	0.6 ^b	24.2 ^b	3253 ^a	0.6 ^b	18.5 ^c	2172 ^c	0.4 ^c	16.8 ^b	878 ^b	0.7 ^b
	P <	0.01	0.01	ns	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01

Means followed by different letters differ significantly at P<0.05.

3.1. Discrimination of behaviour activities

Regarding the allocation of prevailing behavioural activity (expressed as percentage of the total), the overall trend was an increment in the low and medium classes of prevalence (50–75% and 76–99%) coupled obviously with a reduction in the highest class (100%), passing from 5 s to 300 s epoch set (Table 2). Actually, the percentage of the prevailing behaviour included in the 100% class decreased from 98.8%, in 5 s, to 60.2% in 300 s.

Table 2. Allocation (expressed as percentage of the total) of the prevailing behavioural activity in the three classes of percentage considered for each epoch setting

Epoch	50–75%	75–99%	100%	n
5 s	0.6	0.7	98.8	13,995
10 s	1.8	1.0	97.4	6992
30 s	3.5	3.3	93.3	2334
60 s	4.5	6.6	88.3	1167
120 s	5.6	13.7	80.2	585
180 s	7.9	19.2	72.6	390
300 s	11.9	26.3	60.2	233

In developing multivariate techniques, the MRES variable was discarded from the analysis because it made the (co)variance matrix singular due to linear dependencies with other variables. The CDA significantly discriminated between the three behaviours (Hotelling's test $P < 0.0001$) by extracting two canonical functions for each epoch set. The variation λ_1 accounted for CAN1, ranged among epochs between 0.78 and 0.93 whether the variation λ_2 explained by CAN2 ranged between 0.08 and 0.22 (Table 3).

Table 3. Variance explained (λ_1, λ_2) and standardized canonical coefficients at different time epoch settings

	5 s		10 s		30 s		60 s		120 s		180 s		300 s	
	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2
λ_1	0.88		0.91		0.93		0.93		0.93		0.88		0.78	
λ_2		0.12		0.08		0.07		0.07		0.07		0.12		0.22
Variables														
MX	0.52	-0.15	0.48	-0.72	0.36	-1.52	0.17	-1.95	-0.07	-2.45	-0.77	-1.78	0.34	-1.96
VX	0.35	0.98	0.57	1.52	0.59	2.29	0.60	2.78	0.81	3.14	0.99	2.98	0.83	2.93
ICVX	-0.05	0.23	-0.03	0.22	-0.09	0.59	-0.10	0.68	-0.05	0.52	0.02	0.72	-0.03	0.41
MY	0.95	0.57	1.29	0.55	1.15	1.03	0.82	1.61	0.86	1.66	0.62	3.28	1.44	2.66
VY	0.36	0.63	0.58	0.99	0.64	1.10	0.60	1.29	0.78	1.27	0.02	0.94	1.35	0.87
ICVY	-0.08	0.30	-0.03	0.18	0.02	-0.12	0.01	-0.05	0.02	-0.19	0.03	-0.29	0.22	0.63
MZ	0.92	0.87	1.49	0.86	1.98	1.09	2.33	1.21	2.35	1.06	2.04	1.49	2.08	1.57
VZ	-0.16	-0.03	-0.34	0.30	-0.87	0.37	-1.49	0.53	-1.38	0.55	-1.71	0.69	-0.58	0.41
ICVZ	-0.06	0.15	-0.03	0.06	-0.02	0.07	-0.03	0.11	0.02	0.02	-0.30	1.06	0.65	1.42

Table 3 (continued)

	5 s		10 s		30 s		60 s		120 s		180 s		300 s	
	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2	Can1	Can2
λ_1	0.88		0.91		0.93		0.93		0.93		0.88		0.78	
λ_2		0.12		0.08		0.07		0.07		0.07		0.12		0.22
Variables														
VRES	-1.64	-1.86	-2.41	-2.23	-2.26	-3.00	-1.82	-3.81	-2.02	-3.69	-0.82	-4.89	-3.46	-4.55
ICVRES	-0.002	-0.09	-0.24	0.49	-0.14	0.26	-0.04	-0.22	-0.08	0.17	0.50	-2.05	-1.30	-3.59
MF	0.81	-0.34	0.90	-0.54	0.98	-0.76	0.92	-0.95	0.65	-1.17	0.66	-0.26	0.88	0.70
VF	-0.02	0.52	0.09	0.65	0.01	0.90	-0.09	1.29	0.12	1.43	-0.16	1.26	0.06	0.88
ICVF	-0.13	0.64	-0.03	0.30	-0.003	0.22	0.001	0.35	0.01	0.29	-0.05	0.20	-0.11	0.09

The lowest value of error in assignment, after the bootstrap resampling, was obtained in the 30 s epoch whereas the highest value was at the 300 s epoch (Figure 2).

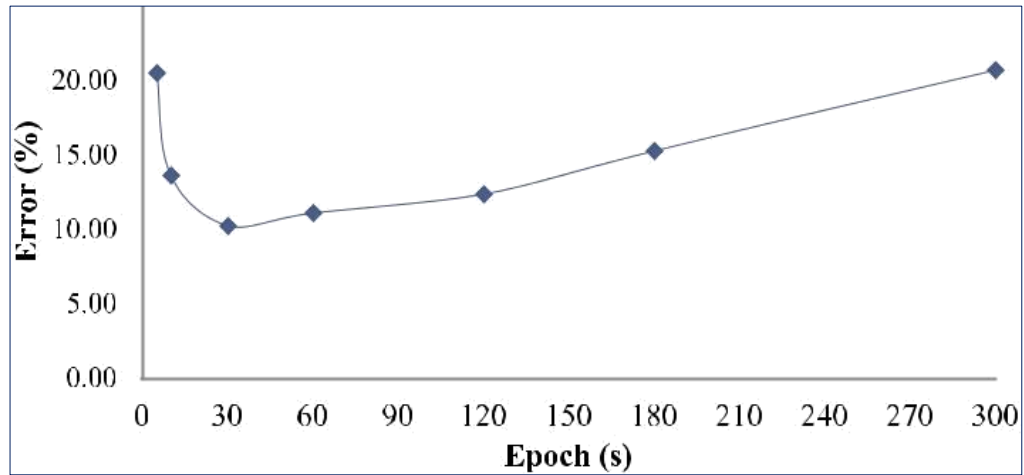


Figure 2. Distribution of errors in assignment of DA after bootstrap procedure among epoch settings

VRES and MZ showed the highest canonical coefficient values in CAN1 in nearly all the epochs with the exception of 5 s and 180 s, which recorded biggest values in VRES and MY and MZ and VZ respectively (Table 3).

The highest canonical coefficient in CAN2 was found in VRES in all the epochs.

In all time epoch settings canonical functions separated the three behavioural activities ($P < 0.001$, Figure 3).

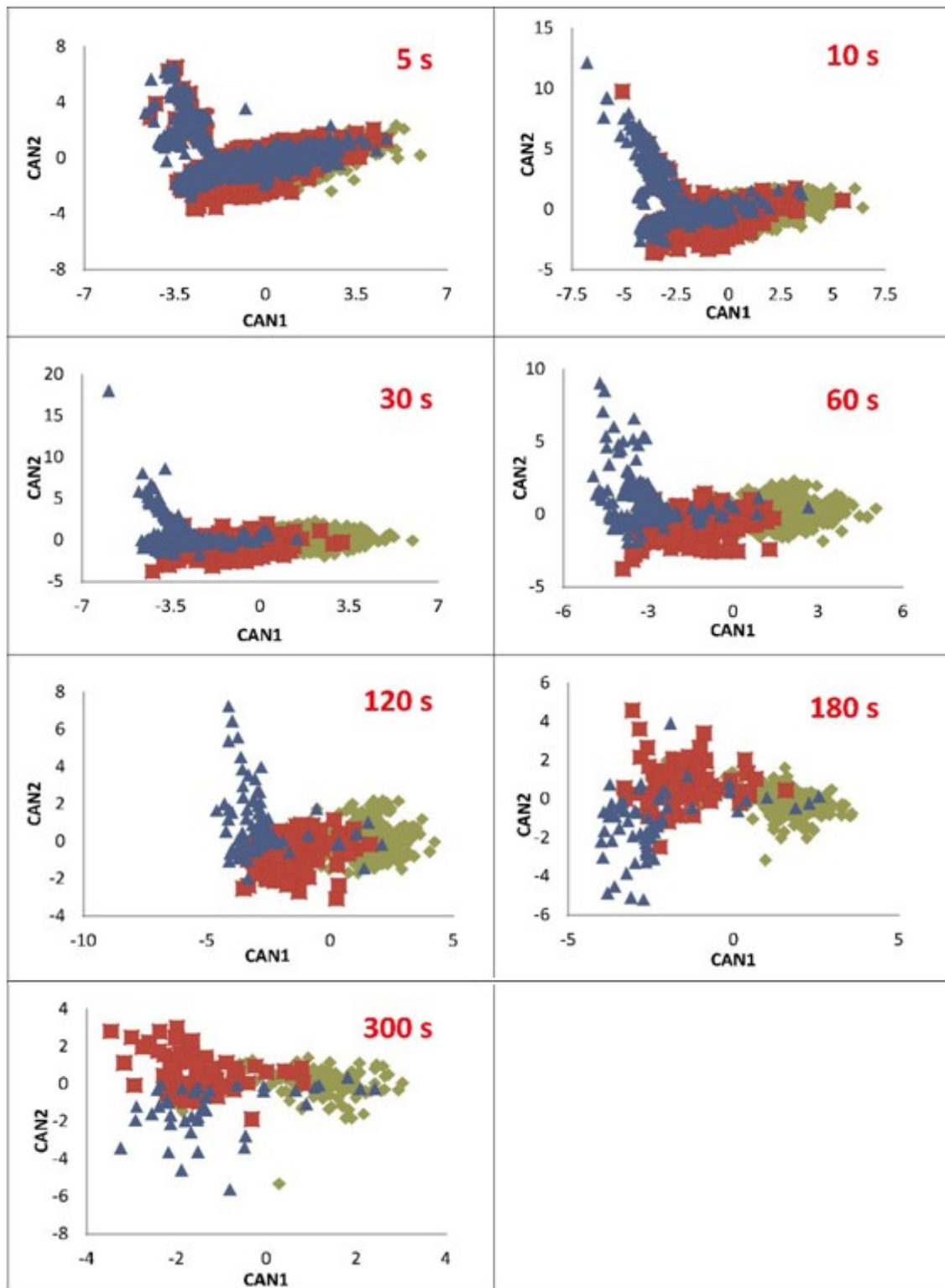


Figure 3. Plot of canonical variables (CAN 1, CAN 2) generated from discriminant analysis for different time epoch settings (5 s, 10 s, 30 s, 60 s, 120 s, 180 s, 300 s)
 ▲ blue=other activities, ■ brown=ruminating, ◆ green=grazing. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

In particular, CAN1 discriminates the grazing activity from the other activities. Ruminating is an intermediate process, and this is confirmed by the Mahalanobis' distances (Table 4). In fact, the highest values were observed between grazing and other activities with the only exception of 300 s where it is slightly higher than the distance between grazing and ruminating.

Table 4. Mahalanobis distance from main behavioural activities at different time epoch settings

		Ruminating	Other activities
Time epoch settings			
5 s	Grazing	4	10
	Ruminating	0	3
	Other activities		0
10 s	Grazing	7	17
	Ruminating	0	4
	Other activities		0
30 s	Grazing	11	23
	Ruminating	0	5
	Other activities		0
60 s	Grazing	12	24
	Ruminating	0	5
	Other activities		0

Table 4. (continued)

		Ruminating	Other activities
Time epoch settings			
120 s	Grazing	11	20
	Ruminating	0	4
	Other activities		0
180 s	Grazing	10	17
	Ruminating	0	5
	Other activities		0
300 s	Grazing	8	7
	Ruminating	0	4
	Other activities		0

3.2. Performance of the discriminant analysis model

The performances of the DA model, displayed in Table 5, show that the sensitivity of the model to predict grazing was the highest in the 60 s epoch set and the specificity in the 120 s epoch set. When the model was used to predict the rumination, the specificity reached the maximum value in the 30 s setting whereas the sensitivity in the 120 s epoch setting. The 30 s epoch length was the best to predict other activities, both in terms of sensitivity and specificity. The highest precision of the models to predict grazing was recorded in the 120 s set while the highest accuracy was obtained in 30- and 60 s, the k value being 0.9 in 30-, 60-, 120- and 180 s.

Ruminating and other activities behaviours were predicted with the highest precision and accuracy in the 30 s epoch with a k value of 0.8 and 0.7, respectively. The overall accuracy values of the prediction models were similar in the range between 10 s and 120 s epochs, peaking in the 30 s epoch. The Coehn's k coefficient reached a plateau value (0.8) in the 10-, 30-, 60- and 120 s epochs.

Table 5. Performance of the model in the assignment of the behaviours at different time epoch settings

		Sensitivity	Specificity	Precision	Accuracy	Cohen's k
		%	%	%	%	
Grazing	5 s	88.1	86.9	86.9	87.5	0.7
	10 s	94.7	90.8	91.9	92.8	0.8
	30 s	94.8	93.0	94.1	94.0	0.9
	60 s	95.0	92.9	94.0	94.0	0.9
	120 s	92.8	94.2	95.3	93.4	0.9
	180 s	91.6	92.4	93.6	91.9	0.9
	300 s	88.0	89.6	91.6	88.7	0.8
Ruminating	5 s	67.9	88.8	78.4	81.0	0.7
	10 s	73.5	93.9	86.8	86.7	0.8
	30 s	80.4	94.7	88.1	90.0	0.8
	60 s	79.8	93.9	86.3	89.4	0.8
	120 s	82.2	91.6	79.9	88.9	0.7
	180 s	78.3	90.2	76.9	86.7	0.7
	300 s	73.5	89.5	74.1	84.9	0.6

Table 5. (continued)

		Sensitivity	Specificity	Precision	Accuracy	Cohen's k
		%	%	%	%	
Other activities	5 s	79.6	92.0	59.6	90.4	0.5
	10 s	87.9	94.0	67.2	93.2	0.6
	30 s	92.3	96.0	77.9	95.5	0.7
	60 s	86.6	95.7	76.5	94.5	0.7
	120 s	79.2	95.5	75.9	93.0	0.7
	180 s	72.7	94.2	69.6	90.9	0.6
	300 s	57.0	89.7	48.9	84.9	0.4
Overall	5 s				79.4	0.7
	10 s				86.4	0.8
	30 s				89.7	0.8
	60 s				88.9	0.8
	120 s				87.6	0.8
	180 s				84.7	0.7
	300 s				79.3	0.6

4. DISCUSSION

4.1 Discrimination between behaviour activities

In this study a multivariate statistical algorithm was developed, by using tri-axial acceleration data obtained from an under lower jaw mounted sensor, to classify grazing, ruminating and other activities of dairy sheep. The CDA successfully distinguished the different behaviours, although the CAN1 vs CAN2 scatter plots (Figure 3) showed

different levels of separation according to the time epoch length. This fact could be due to the variation (λ_1) explained by CAN1 that reached higher values in 30-, 60-, and 120 s than in the other time epochs (Table 3), whereas CAN2 was not able to separate behaviours (Figure 3).

The Mahalanobis' distance was greater between grazing and other activities than that between grazing and ruminating in all epochs with the exception of the 300 s epoch. This is probably related to the low prevalence in this dataset, with only 60.2% of records classified in the highest class (100%, Table 2). As a consequence, the contribution of the “non prevailing activities” (i.e. the complement to the prevailing activity) within records, was higher in this case than in the other epoch sets. This indicates that increasing the aggregation time window for calculation of the means, variances and ICV from 120 s to 300 s probably flattened data distributions and limited the ability to discriminate between different behaviours such as other activities and rumination. In fact, the results of the ANOVA on the effect of behavioural activities (Table 1) show that increasing the aggregation time window (the epoch length) increases the number of variables that show at least two behavioural classes to be not significantly different. In 5 s epoch set, for example, among the fourteen variables under scrutiny, only three variables (ICVRES, ICVZ and ICVY) had, at least, two values undifferentiated between behaviours, while in 10 and 30 s epochs the variables with this response were four (ICV, ICVY, ICVZ and ICVF) and so on for the other epochs. These results highlight the limits of the ANOVA, which analyzes each variable individually, in separating the three behaviours. In consequence, the adoption of a

multivariate approach, that conversely uses a set of variables to separate and assign new observations to groups, is fully justified.

VRES and MZ, showing the highest standardized canonical coefficients for CAN1 (Table 3), are the most important features for behavioural classification in all epoch settings. Therefore, grazing activity, that includes various dynamic movements such as biting, chewing and head shaking while lowering the head, can be differentiated from other behaviours by VRES that measures the total amount of variance of the acceleration signal through three dimensions, and by MZ that, in turn, represents the mean of head/jaw vertical accelerations.

In CAN2, which explained a very low part of the total variation ($0.08 < \lambda_2 < 0.22$), VRES showed the highest standardized canonical coefficient at all epochs, in agreement with what reported by Giovanetti et al., (2017a) who used BEHARUM device to monitor the feeding behaviour of dairy sheep, using a 60 s time epoch. The importance of these variables in discriminating sheep behaviours was also reported by Barwick et al., (2018).

Conversely, Watanabe et al., (2008), who studied behaviour in grazing cattle, found the highest discriminant scores using the means and ICV as explanatory variables. González et al., (2015), with a triaxial accelerometer attached on a collar around the neck of steers, were able to separate cattle foraging behaviour from other activities, at 10 s epoch, using the X-axis mean which corresponds to our MZ variable.

Umstätter et al., (2008) found similar results (epoch settings 30 s), with pitch features (head up and down), our Z-axis, as the most important factor for behavioural classification for sheep wearing a collar equipped with a sensor.

Alvarenga et al., (2016), conversely, reported that the most important feature was the means of the X-axis (forward/backward acceleration) to capture head position and level of activity related to grazing for different epoch settings (3-, 5- and 10 s).

The reason of this inconsistency is unclear but may possibly be related to the different methods of accelerometer deployment, or to the different number of behavioural activities considered as well as to the different classification criteria. Moreover, some sources of signal variation may arise from differences in physical structure of animals affecting sensor orientation.

To the best of our knowledge, we did not find any result on the use of force sensor in animal behaviour studies. This sensor, that measures the force exerted by the opening jaw movements during feeding related activities, showed the highest MF and VF values when the animals performed dynamic movements (i.e. grazing and ruminating). Although the canonical coefficients of force variables were low, their contribution to the discrimination of the three behaviours has been revealed important.

4.2 Performance of the discriminant analysis model

The performance of the model changed according to the predicted behaviour (Table 5). In fact, the grazing activity showed best performance, in terms of sensitivity and precision, than the other two activities for almost all the epoch set, thus confirming the results of other studies (Giovanetti et al., 2017a; Nadimi et al., 2012). This is probably due to the higher correct true positive classification of grazing than ruminating and other activities, indicating that the classifier had problems predicting positive cases correctly in the last two classes that became most easily confused with other behaviours. Specificity in other activities (i.e. the true negative rate) resulted higher than in

ruminating and grazing for all epochs. Accuracy also was higher in other activities for almost all epochs, except for 180 and 300 s where it reached the highest value in grazing activity. The unequal number of observations for the different activities could partially explain the above modelling performance. In fact, other activities did not exceed 20% in the whole our dataset. Therefore, sensitivity and precision are probably more informative than accuracy and specificity in order to summarize performance results. Grazing behaviour was predicted in 5 s epoch dataset with higher sensitivity (88.1% vs 84.3%) but lower specificity (86.9% vs 97.3%) and precision (86.9% vs 92.9%) than in the work by Alvarenga et al., (2016) on Merino ewes.

The precision in the current study resulted anyway higher than that found by Marais et al., (2014) using a 5.12 s epoch, which was c.a. 66%.

The 10 s epoch length, conversely, showed higher sensitivity (94.7% vs 91.7%) and precision (91.9% vs 89.8%) but lower specificity (90.8% vs 96.2%) in comparison with data by Alvarenga et al., (2016).

Barwick et al., (2018) with the same epoch setting of 10 s found different performances in discriminating sheep grazing behaviour according to deployment position (ear, collar, leg). They actually reported the best performance in ear deployment that produced better results than our study, apart from sensitivity (92.0 vs 94.7%).

Rodriguez et al. (2017), in an experiment with sheep, dividing their dataset in epochs of 14 s, reported performance similar to our 10 s epoch for grazing behaviour recognition. They actually found a sensitivity of 91.3%, a precision of 90.9%, a specificity of 91.9% and an accuracy of 91.6%.

Moreau et al., (2009), even if in a study conducted with grazing goats wearing the Hobo® G Pendant Data Logger, reported a true recognition rate of eating behaviour (grazing or browsing) similar to our results for 5 (89% vs 87% respectively) and 10 s (91% vs 92% respectively). We encountered some difficulties in comparing the performance of the BEHARUM device for epochs larger than 20 s with the literature, since few papers regarding the use of accelerometer sensor in small ruminants have been published. Moreover, a comparison of results obtained in sheep with those obtained with large animals could not be appropriate. This assumption is supported by the results observed in a study conducted with the BEHARUM device placed under the jaw of cattle at pasture. Giovanetti et al., (2017b), using the same device (BEHARUM), time epoch setting and statistical data analysis as in the present experiment, found that overall accuracy of 5, 10 and 30 s epochs in sheep showed higher values than those reported in cattle (79.4%, 86.4% and 89.7%, vs 77.5%, 82.2% and 88.8% in sheep and cattle, respectively). This was probably due to the better capability of the model, for those epochs, to predict grazing activity in sheep than cattle. In the present experiment, the performance of the model was explored in relation to the choice of the best epoch setting. If we consider the overall model performance in discriminating the three behaviours, the best overall accuracy and k coefficient was found at 30 s epoch setting (Figure 1, Table 5) and the worst performances at the smallest (5 s) and biggest epoch setting (300 s). In sheep, contrarily to what happens in cattle (Giovanetti et al., 2017b; Vázquez Diosdado et al., 2015), when epoch setting is above 30 s, increasing the time epoch length, decreases the correct classification of behaviour. Our findings of the ANOVA analysis (Table 1) indicate that this could be probably related to the lack of

significant differences of behaviours for many variables within the longer time frames, as in the case of 120 s, 180 s and 300 s epoch settings. Looking towards the 60 s epoch we can observe a slightly lower overall accuracy (88.9% vs 93.0%) and Coehn's k coefficient (0.8 vs 0.9) than those found by Giovanetti et al., (2017a) and this may be due to the bigger and more varied dataset utilized in this paper than in the previous study. As a matter of fact, in the present research sheep grazed mixed grass and legume pastures whereas, in the work by Giovanetti et al., (2017a), animals were fed on pasture monocultures only. Despite this slight decrease of the model performance in this study, the 60 s and 120 s epochs cannot be discarded a priori, because they actually show a good k coefficient (0.8 for both) as well as accuracy, which values are 88.9% and 87.6% respectively. In this current study, the overall k value indicates substantially greater classification agreement than that would be expected to occur by chance, thus indicating that the classification success of our model could be considered reasonable for most of the epochs. Therefore, if the user's aim is to get from BEHARUM device applied to sheep the best performance of classification during short grazing periods (say one day or less), the 30 s epoch should be chosen. Alternatively, if the user's goal is mostly practical, i.e. to record good quality data for a longer periods (days or weeks) then 60 and 120 s epochs should be chosen, with the aim to save battery energy, allowing for a longer recording time.

5. CONCLUSIONS

Our results showed that the discriminant analysis of data from an under lower jaw tri-axial accelerometer can accurately classify important behaviours such as grazing and ruminating in sheep at pasture.

The prediction model performed better in classifying grazing behaviour than ruminating and other activities for all epochs. The 30 s epoch length yields the most accurate classification in terms of accuracy (89.7%) and Coehn's k coefficient (0.8). Nevertheless, 60 and 120 s, may increase the potential recording time without causing serious lack of accuracy, and could be adopted for most practical purposes for monitoring sheep behaviour in extensive conditions.

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CHAPTER 4

Relationship between accelerometer features and behavioural traits in Sarda dairy sheep submitted to short term grazing tests

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ABSTRACT

The accurate estimation of herbage intake is key to adequately feed grazing ruminants. Ten dairy Sarda sheep fitted with a halter equipped with an accelerometer (BEHARUM device) were allowed to graze for 6 min micro-swards of Italian ryegrass (*Lolium multiflorum* L.), alfalfa (*Medicago sativa* L.), oat (*Avena sativa* L.), chicory (*Cichorium intibus* L.) and a mixture (Italian ryegrass and alfalfa). Accelerometer data and video recordings of behaviour were collected simultaneously. The raw acceleration data was processed to calculate 15 variables: mean, variance and inverse coefficient of variation (ICV; mean/standard deviation) for the X, Y and Z axis and the resultant. A database was then created inclusive of the acceleration variables and herbage intake (DMI, g), intake rate (DMIR, g/minute), bite mass (DMBM, g) and the logarithm of number of bites (LB) measured during the tests. Partial least square regression analysis (PLSR) was used to verify if the acceleration variables could be used as predictors of behavioural traits. The precision and accuracy of PLSR were evaluated implementing the Model Evaluation System, in which predicted values were regressed against observed ones, based on r^2 , root-mean-square error of prediction (RMSEP) and Dent & Blackie test.

The PLSR showed an overall good accuracy (Dent & Blackie test $P=1$) and was proven precise for the estimation of LB ($r^2=0.86$, RMSEP=3%), DMI and DMIR ($r^2=0.71$, RMSEP=22%), but not of DMBM ($r^2=0.32$, RMSEP=26%).

To conclude, BEHARUM can accurately estimate with high to moderate precision number of bites and herbage intake of sheep short term grazing Mediterranean forages.

1. INTRODUCTION

Studying the feeding behaviour of ruminants and monitoring the energy intake during grazing is of fundamental importance to improve feeding efficiency, animal productivity and pastures management, respecting environment and animal welfare (Blomberg, 2011; Oudshoorn et al., 2013; Swain and Friend, 2013).

The productivity of grazing animals depends indeed on feed intake, which is a difficult parameter to measure, especially for long periods of time (Milone et al., 2012).

Various techniques have been developed to estimate daily intake rate at pasture, but they are often invasive for the animals and difficult to apply on rangeland (Bonnet et al., 2015). The grazing process involves selecting, severing, chewing and swallowing the herbage, so it can be defined as a sequence of bite and chew jaw movements that could have important implications for the correct estimation of intake (Milone et al., 2012; Navon et al., 2013). An additional function, “chew-bite”, can be identified and consists in chewing herbage already in the mouth while the fresh herbage is severed, with the same jaw movement (Ungar et al., 2006a; Galli et al., 2011; Milone et al., 2012).

Jaw movements are of great importance to assess animal grazing strategies when grazing in different types of pastures and to better estimate their intake (Andriamandroso et al., 2016).

Intake can be defined as the product of bite mass, bite rate and grazing time (Hodgson 1985), but its prediction is very difficult because bite mass is the most variable component of grazing behaviour, being influenced by a lot of parameters such as pasture characteristics, animal motivation, grazing time, diet supplementation level, etc. Several techniques have been developed to monitor animal jaw movements and to detect bites, that are the elementary components of the grazing process (Ungar et al., 2006a; Andriamandroso et al., 2016).

Automatic recorders (Rutter et al. 1997) or acoustic monitoring (Ungar et al., 2006b) allowed to evaluate bite rate and to distinguish among the different jaw movements of ruminants, such as cropping, chewing and even chew-biting, but these advances are still limited to simplified and controlled grazing conditions (Bonnet et al., 2015).

Acoustic analysis allows to differentiate with good accuracy the different jaw movements (Ungar et al., 2006b) using the Hidden Markov model (Milone et al., 2012), able to estimate sequences of bites, chews or chew-bites not observable directly, through their acoustic spectrum characteristics. However, the outdoor application of microphones can be disturbed by environmental noises, so automatic recording and analyses of sounds still requires significant developments (Andriamandroso et al., 2016). Also accelerometer sensors have been tested to automatically count jaw movements (Umemura et al., 2009; Oudshoorn et al., 2013; Rombach et al., 2018) however, as for sound sensors, the sensitivity of accelerometers could provide

interferences and undesirable signals during recording sessions, therefore significant developments are required in order to isolate the signal relative to the jaw movements of grazing animals (Andriamandroso et al., 2016).

At present, the only method able to allow the continuous estimation of bite mass, time per bite, instantaneous intake rate and selection of plant species and parts, especially in complex feeding environments, seems to be the “continuous bite-monitoring” method tested by Bonnet et al. (2011), that achieved a good accuracy combining preliminary estimates of bite mass, performed by the hand-plucking method, and continuous bite monitoring. The advantages of this method are that it is non-invasive, less costly, applicable in almost any type of environment and able to give a lot of information about foraging process. However, the continuous bite monitoring has limitations and is not suitable for all situations, it is indeed time consuming, as an observer can follow only one animal at a time and it is difficult to use with wild herbivores or during the night, because it requires the use of powerful flashlights that can disturb the animals (Bonnet et al., 2015).

In complex grazing environments it could be convenient to combine the continuous bite monitoring method with information provided by different sensors, as GPS, accelerometers and microphones: for example, the combination of acoustic monitoring, able to identify the exact number and time in which bite and chewing events occur, with continuous bite monitoring, able to give information on bite mass and on selected plants, can help to overcome the challenge of grazing intake estimation (Bonnet et al., 2015; Andriamandroso et al., 2016).

The objective of this study was to derive a model to predict sheep behavioural variables as number of bites, bite mass, intake and intake rate, on the basis of variables calculated from acceleration data recorded by a customized tri-axial accelerometer based sensor named BEHARUM.

2. MATERIALS AND METHODS

The animal protocol below described was in compliance with the EU Council Directive 98/58/EC that regulates the use of animals for experimental and other scientific purposes.

2.1. Forage species

The study was conducted at Bonassai experimental farm of the agricultural research agency of Sardinia (AGRIS Sardegna), located in the NW of Sardinia, Italy (40° 40' 16.215" N, 8° 22' 0.392" E, 32 m a.s.l) in April 2016.

Five treatments, four monocultures and one mixture, established by sowing in paired boxes the forage species to create micro-swards (Orr et al. 2005), were compared. The monocultures were: Italian ryegrass (*Lolium multiflorum* L., LL), alfalfa (*Medicago sativa* L., AA), oat (*Avena Sativa* L., OO) and chicory (*Cichorium intibus* L., CC); the mixture was constituted by Italian ryegrass and alfalfa (LA).

An additional specie, barley (*Hordeum vulgare* L.), has been used to adapt the animals to the experimental routine.

These species were chosen because they are widespread in Mediterranean sheep production systems and stimulate a wide range of behavioural responses, which have been already explored in micro-sward studies (Giovanetti et al., 2011).

2.2 Micro-swards establishment and management

The four experimental monocultures and the barley were sown separately, while for the mixture the seeds were intimately mixed before sowing, in polystyrene boxes (28.5 cm x 46.5 cm x 16 cm) weighing 166 g when empty, at the Agriplant garden located at about 10 km far from AGRIS. The boxes had 18 mm drainage holes drilled in the base spaced at 10 cm (10 holes in total). They were filled with a compost (CompaQstrat, N, P, K: 140, 160, 180 mg/l) and then sown in February 2016 (on the 8th of February for LL, AA, CC LA; on the 29th of February for OO and barley) by an automatic planter at a rate of 600 seeds/m² in order to establish a plant density similar to that reported in pastures grazed by dairy sheep (M. Sitzia personal communication).

Alfalfa seeds were inoculated with their specific rhizobium (*Ensifer meliloti*) before seeding. All boxes were subsequently covered with a layer of silice and immediately irrigated. After sowing they were kept in a dark cell at 18°C for three days to promote seed germination.

Overall 275 boxes have been sown (35 oat, 35 ryegrass, 35 chicory, 35 alfalfa, 35 mixture alfalfa + ryegrass, 100 barley). The micro-swards were kept in a politunnel from seed germination until utilization, they were hand-watered when soil surface deemed dry and then transported to AGRIS two days before the experiment started.

2.3 Experimental design

Each treatment has been offered to each animal in a 5x5 Latin-Square with two replications in a 5 days period. Within each replicate, the 5 experimental animals were subjected to the treatments in succession. The order in which the tests on each treatment were conducted within any day were randomized.

2.4 Animals and training

Two replicate groups of 5 animal each of Sarda lactating ewes, homogeneous for age (4 yrs), body weight (43.68 kg), body condition score (2.65), stage of lactation (104 DIM) and milk production (1.270 kg) have been used for the micro-swards test. Five spare lactating ewes were allocated in a box adjacent to the test area in order to substitute some experimental animal if needed.

Before the beginning of the experiment the selected ewes were fed at pasture and then accustomed to consume only hay and concentrate in individual boxes by gradually reducing the time spent at pasture (pre-experimental period, 6 days).

After this period, the training of animals started (adaptation period, 4 days) by offering, after the morning milking at set time, two micro-swards of barley to each subject in a rack for 6 minutes, using the same protocol as during the experimental period. Animals were also trained to wear the BEHARUM device. For the description of the device we refer to the previous published papers (Giovanetti et al., 2017; Decandia et al., 2018).

All the animals were machine milked once a day and fed with a common basal diet consisting of alfalfa hay (0.4 kg/head), ryegrass hay (1.2 kg/head) and a commercial concentrate (0.5 kg/head) split in two meals.

2.5 Measurements

Immediately before each test, the exterior surface of each box has been cleaned to remove any soil, water or any other extraneous material in order to ensure that this material did not become detached during the test and bias results. Then 10 sward surface height measurements have been taken on each micro-sward using a sward stick (Bircham 1981). After the morning milking at set time, animals were worn with the

BEHARUM device and each treatment were offered to each subject (Figure 1) in a rack for 6 minutes (test). After the test the BEHARUM device was removed and *ad libitum* access to hay allowed in order to standardize post-ingestive effects of the swards. The behaviour of each experimental animal has been recorded by a fixed camera (time of ingestion, number of prehension) during the test. The subject could see and be seen by other experimental animals during the trial.



Figure 1. Sheep during the test

The micro-sward boxes were weighed before and after each test with an accuracy of 0.5 g in order to determine the biomass removed. An ungrazed box of the same forage species as that being tested were placed in the same micro-environment as the grazed box and weighed directly before and after each test in order to correct for evapotranspiration losses during the test period.

In two occasions during the experimental period two micro-swards of the sown species were cut at the soil surface, the herbage were then weighed, to measure the herbage on offer, and oven dried at 65°C for 18 hours to determine the dry matter and the chemical composition (NIRS System). A fresh sample of about 50 g was taken and divided in laminae, stems and petiole to determine the sward structure.

Individual milk yield and composition was measured on two occasions, while hay and concentrate intake were measured individually every day.

The following traits were calculated: herbage intake (g) and intake rate, on both fresh (FMI, FMIR respectively) and dry matter basis (DMI, DMIR respectively); bite mass (g) were calculated on both fresh (FMBM) and DM basis (DMBM) from the number of bites, the corrected weight changes of the boxes and the DM determination, bite rate (BR) as the number of bites per minute of feeding.

2.6 Preliminary data processing

Video recordings were coded manually, visually counting the number of bites (including also chew-bites) for each minute, and then summing them to get the total number of bites made during the whole eating time granted by each animal during the test. The number of bites has been transformed in logarithm (LB), in order to obtain a normal distribution of the variable.

Mean (MX, MY, MZ), variance (VX, VY, VZ), sum (SX, SY, SZ), inverse coefficient of variation (i.e. mean/standard deviation, ICVX, ICVY, ICVZ) of acceleration data for each axis, as well as the resultant mean (MRES), variance (VRES) and ICV (ICVRES) values of the three axis (Watanabe et al., 2008), were calculated for the total eating time. A dataset was then created including the behavioural traits (LB, BR, FMI, FMIR, DMI, DMIR, FMBM, DMBM) and the above mentioned acceleration variables calculated for the total eating time of feeding, for a total of 23 (number of variables) per 70 (number of record) dataset.

2.7 Statistical analyses

All behavioural variables (BR, FMI, FMIR, DMI, DMIR, FMBM and DMBM) were analyzed using the proc mixed method for repeated measurement analysis (SAS, 1990) with treatment as fixed effect and sheep, replicate and date as random terms on the basis of the following model:

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

Where:

μ = overall mean

α_i = fixed effect of treatment

β_j = random effect of animal

λ_k = random effect of replicate

ξ_w = random effect of date

ε_{ijkw} = random error

T means were separated by the Tukey test ($P < 0.05$).

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

For this scope the partial least square regression (PLSR) model has been used since it has the ability to handle multivariate regression models with high collinearity among predictors and to make more efficient prediction compared to ordinary multivariate regression or principal component regression (Dimauro et al., 2011).

The general structure of the model is:

$$Y = XB + E$$

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

PLSR extracts a set of orthogonal new variables called latent factors, which are linear combinations of the explanatory variables X , that best model the dependent variable Y .

The maximum number of latent factors depends on the size of X , which has a lower number of columns than Y (Dimauro et al., 2013).

To validate the model a leave-one-out cross-validation has been used. The root-mean-square error of prediction (RMSEP) was used to assess the prediction ability of PLSR. The PLSR was carried out with `pls` function of SAS software (SAS Inst. Inc., Cary, NC). Finally, the precision and accuracy of the model were assessed implementing the Model Evaluation System (MES, release 3.1.16, Tedeschi, 2006) in which the predicted values were regressed against the observed ones. The model evaluation was based on Dent and Blackie test and R^2 .

3. RESULTS

Chemical and structural characteristics of offered forage treatments are presented in Table 1. Differences in chemical composition existing among forage treatments are evident, such as the low dry matter (DM) percentage of chicory, which also reported low neutral detergent fiber content (NDF), and the high values of crude protein (CP) typical of alfalfa. Herbage mass on offer was comparable to that present in a pasture

during the vegetative growth, characterized by a high percentage of leaves and a low percentage of stems in all forage treatments.

Table 1. Chemical composition (on DM basis) of forage species offered as 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	23.24	19.48	20.54	25.46	24.10
EE	4.96	5.03	3.43	3.39	4.11
NDF	46.80	49.74	33.52	36.76	45.53
ADF	26.14	27.35	21.90	24.09	26.65
ADL	0.81	0.37	5.49	4.34	3.03
Ash	12.29	15.20	19.89	12.88	14.69
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	33.7	45.6

OO=oat; LL=Italian ryegrass; CC=chicory; AA=alfalfa; LA=mixture of Italian ryegrass and alfalfa.

SSH=sward surface height; FM=fresh matter; DM=dry matter.

All variables reported in Table 2 were significantly affected by treatments a part from FMI and FMIR. In particular, the number of bites (expressed as logarithm, LB) and the bite rate (BR) were significantly lower in CC than OO, LM and only for BR in LL. Although CC showed the highest fresh matter bite mass (FMBM), it also reported lower dry matter bite mass values (DMBM) than AA, that caused the lowest dry matter intake (DMI) of this essence compared to the other treatments. The dry matter intake rate (DMIR) was also very low in CC although it did not differ from LM.

Table 2. Behavioural parameters of dairy sheep fed with different forage treatments (Lsmeans \pm SE)

	OO	LL	CC	AA	LM	P<
LB	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)	43.27 \pm 4.5 ^a	39.20 \pm 4.4 ^a	25.99 \pm 4.4 ^b	31.75 \pm 4.4 ^{ab}	41.01 \pm 4.4 ^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 0.7 ^a	6.03 \pm 0.7 ^a	3.56 \pm 0.7 ^b	6.12 \pm 0.7 ^a	5.16 \pm 0.7 ^{ab}	0.001

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

Results of the adequacy of predictions of PLSR procedure are reported in Table 3 and plots of the regression equations among predicted and observed values are shown in Figure 2. As demonstrated by the Dent and Blackie Test (P=ns), this procedure was able

to provide accurate estimates, that is how closely model-predicted values are to the true values, of the predicted values for all variables listed. This means that equation parameters, the intercept and slope, were contemporarily not significantly different from 0 and 1, thus indicating that all the equations pass through the origin and the intercept is equal to zero.

Table 3. Results of Model Evaluation System (MES)

Y	Adjusted r^2	Dent and Blackie test P<	RMSEP (%)
LB	0.86	0.88	3.3
FMI (g)	0.66	1.0	20.7
DMI (g)	0.71	1.0	22.2
FMBM (g)	0.41	1.0	22.7
DMBM (g)	0.32	1.0	26.4
FMIR (g min ⁻¹)	0.67	1.0	20.4
DMIR (g min ⁻¹)	0.71	1.0	22.1

LB=logarithm of number of bites; FMI=fresh matter intake; DMI=dry matter intake; FMBM=fresh matter bite mass; DMBM=dry matter bite mass; FMIR=fresh matter intake rate; DMIR=dry matter intake rate.

The degree of precision of the model, that indicate the model's ability to predict similar values consistently, was pretty good although it varied according to the variable considered. The prediction of the number of bites, expressed as logarithm (LB) reported the highest adjusted r^2 (0.86) followed by DMI and DMIR variables ($r^2=0.71$) while the other ones have r^2 values between 0.32 and 0.67 (DMBM, FMBM, FMI and FMIR). The RMSEP was very low when the number of bites were predicted (3%) while it grew up between 20 and 22% for the other variables, reaching 26% for DMBM.

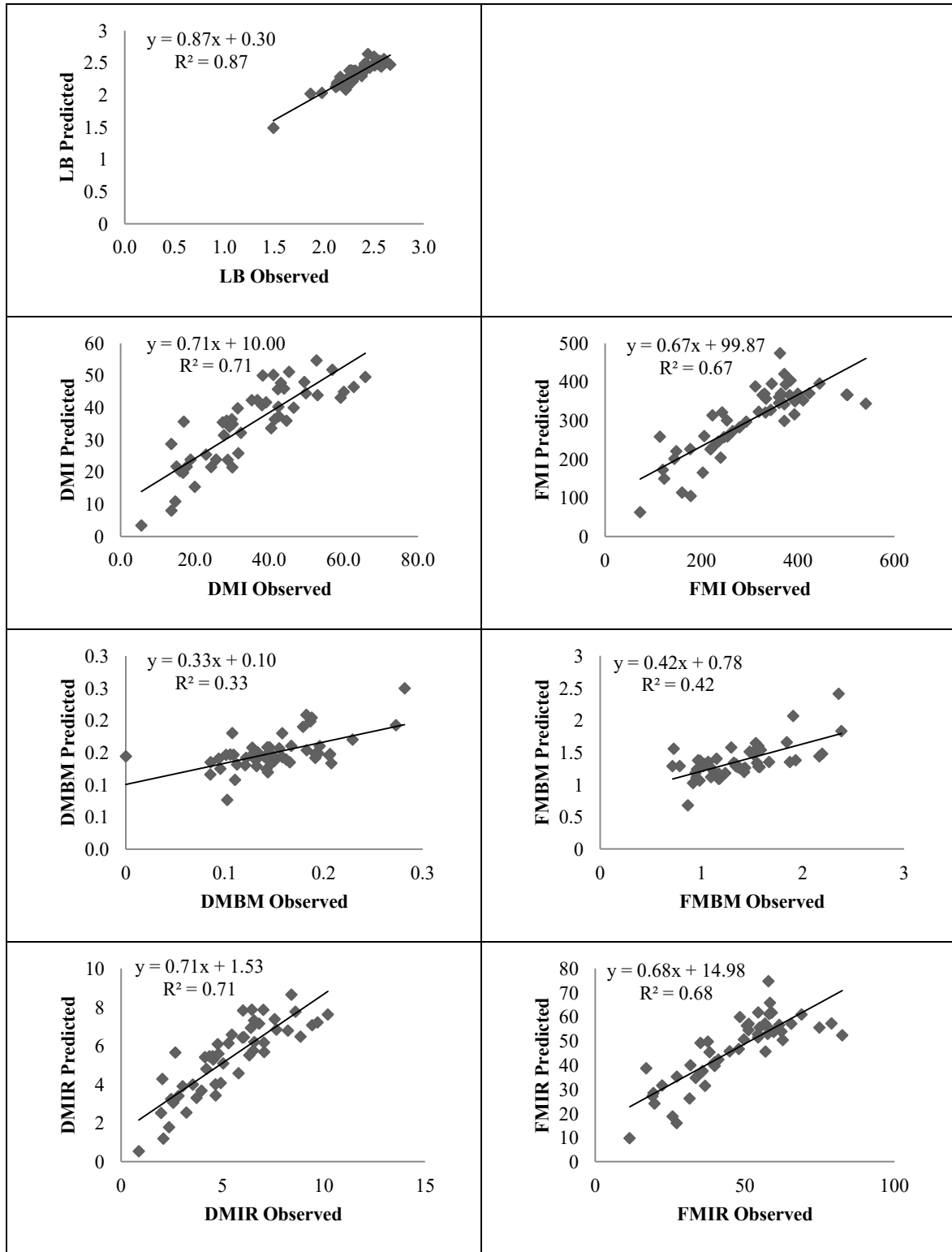


Figure 2. Plots of predicted versus observed values of behavioural variables. LB=logarithm of number of bites; DMI=dry matter intake; FMI=fresh matter intake; DMBM=dry matter bite mass; FMBM=fresh matter bite mass; DMIR=dry matter intake rate; FMIR=fresh matter intake rate

4. DISCUSSION

As expected, the different forage treatments significantly influenced almost all behavioural variables (Table 2), indeed the behavioural responses stimulated by these forages had already been explored in previous micro-sward studies (Giovanetti et al., 2011). CC presented the major differences, showing a significantly lower number of bites (LB) and bite rate (BR) compared to OO, LL and LM. This is probably due to the high fresh matter bite mass (FMBM) of CC, which increased the time required to sheep for chewing each bite. Although CC showed the highest FMBM, it reported lower dry matter bite mass (DMBM) values than AA, that caused the lowest dry matter intake (DMI) of this treatment compared to the others. The dry matter intake rate (DMIR) was also very low in CC although it did not differ from LM. This could be due to the very low dry matter content of CC compared to the other treatments, especially compared to AA that presented instead the highest content of dry matter (Table 1) and consequently the highest DMBM compared to the other treatments.

The aim of this work was to derive a model to predict sheep behavioural variables related with intake on the basis of calculated accelerometer variables.

Several techniques have been developed over the years for estimating bite mass, bite rate and daily intake. Microphone-based methods are the most used devices for this purpose because they showed a good accuracy for jaw movements detection and allow to differentiate three types of jaw movements: chew, bite and chew-bite, that are fundamental components of intake process (Ungar et al., 2006b).

Good results were achieved by combining video and acoustic recordings of ingestive behaviour in short-term studies. Laca and WallisDeVries (2000) for example, were able

to correctly classify chews and bites (accuracy of 94%) and to predict intake with a good accuracy ($R^2=0.90$) by a linear combination of energy flux density in the chewing sounds and average intensity of biting sounds.

Clapham et al. (2011) used an acoustic monitoring system to detect and analyze bites, and were able to differentiate bite and chew using a discriminant function with an accuracy of 94%.

Galli et al. (2011) demonstrated that it is possible to accurately estimate DMI in grazing sheep by acoustic analysis (coefficient of variation=18%, $R^2=0.92$), using chewing energy per bite and total amount of energy in chewing sound as the most important predictors, being able to integrate information about eating time and intake rate.

Only few references can indeed be found on the use of acceleration sensors for the identification and classification of jaw movements. Umemura et al. (2009) succeeded to count cattle jaw movements with an accuracy of 90% compared with manual counts over 10 min segments, modifying a pedometer into a pendulum.

Tani et al. (2013) used a 1-axis accelerometer coupled to a microphone, being able to distinguish cattle chewing activities at 90%, reaching 99% when the sensor was attached to the cow's horn.

Umemura (2013) used three types of pedometers installed on neck collars to determine the accuracy with which the devices measured the number of grazing bites performed by cows. He found that the values recorded by the devices were linearly related to the number of bites recorded by visual observation, but concluded that this technique requires calibration to relate the pedometer values to the number of grazing bites.

Andriamandroso et al. (2015) used a smartphone inertial measurement unit (IMU) which combined accelerometers, gyroscope, magnetometer and location sensors, to count the number of bites through frequency pattern of 1-axis acceleration data, achieving a mean error of 4 to 5% when compared with visual observations.

Oudshoorn et al. (2013) used a 3-axis accelerometer to record cow bites at pasture, testing a series of thresholds values to determine the peak with the best correlation to the observation, but obtained an average correlation coefficient of only 0.65, similar to the partial agreement with visual observation obtained in our previous experiment (Giovanetti et al., 2017) with sheep in grazing conditions. These results confirmed the difficulty to count bites using an accelerometer in free ranging animals, as more recently described by Rombach et al. (2018), that tried to validate the RumiWatch System (RWS; Itin and Hoch GmbH, Liestal, Switzerland) for the measure of ingestive and rumination behaviours of dairy cows during grazing and supplementation in the barn. The algorithms tested in the evaluation software were not able to differentiate between mastication and true prehension bites while eating, indeed the number of prehension bites is overestimated both for grazing and supplemented cows. They achieved a low relative prediction error (≤ 0.10) for the number of rumination boluses, rumination chews, and total eating chews, but a higher error (> 0.10) for the number of prehension bites and time spent in prehension and eating.

As the estimate of free-grazing animals intake is arduous, because of the difficulty of accurately establishing the weight of each bite, in the present study we have chosen to use sown micro-sward boards (Black and Kenney 1984) and to determine the average BM by weighing the micro-swards before and after the animal fed. In complex grazing

environments the best method available to estimate bite mass remains hand-plucking, that simulates a bite by mimicking grass prehension by hand and the estimations accuracy can be as high as 95% for cows and goats with trained operators (Bonnet et al., 2011).

In the present study the PLSR was able to provide accurate estimates of the number of bites, expressed as logarithm (LB) with an adjusted r^2 of 0.86 and a RMSEP of 3%.

This is a good result considering that bite is the elementary unit of the grazing process, therefore, by counting bites it is possible to determine their frequency, which, combined with the bite mass and the grazing time, allows calculation of herbage intake (Hodgson, 1985). Therefore, the accurate detection of grazing behaviour and individual bites is essential for predicting intake.

DMI and DMIR variables showed an adjusted r^2 of 0.71, but a RMSEP of 22%, while the other variables have r^2 values ranging between 0.32 and 0.67 (DMBM, FMBM, FMI and FMIR). The RMSEP reached a value of 26% for DMBM ($r^2=0.32$), confirming the difficult to predict this variable with automated methods.

Bonnet et al. (2015) recently studied the possibility of estimating bite mass combining the hand-plucking method with acoustic sensors coupled to the continuous bite monitoring, achieving an accuracy ranging between 80% and 94%, in a short-term intake rate.

These results suggest that the combination of information provided by different sensors, such as microphones, accelerometers and GPS, can allow better estimates of intake at pasture. Moreover, the lack of differentiation between bite and chew can lead to overestimations of bites or to different accelerations signals from those originated by

bites alone whereby, in agreement with other researchers (Laca and WallisDeVries, 2000; Giovanetti et al., 2017; Rombach et al., 2018) we believe that the differentiation between mastication chews and prehension bites is very important for estimating intake and should therefore be integrated into validation models.

5. CONCLUSIONS

Accelerometer sensors placed under jaw appear promising estimators of the number of bites as well as of DMI and DMIR variables even if at lower level. This is confirmed also by RMSEP that was very low when the number of bites were predicted, while it grew up for the other variables. Overall caution is advised when using accelerometer sensor to estimate intake in grazing conditions.

A combination of information provided by different sensors and the integration of chews and chew-bites differentiation in validation models can allow probably better estimates. This approach warrants further research.

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GENERAL DISCUSSION AND CONCLUSIONS

This work was developed to study the grazing behaviour of sheep, to automatically identify and classify their activities through a tri-axial accelerometer based device and to estimate the rate of biting on the basis of acceleration variables.

The information available in literature (Chapter 1) evidenced that tri-axial accelerometers showed a good precision and accuracy in the classification of behavioural activities of herbivores and in particular of cows, on which numerous studies were conducted using sensor-based technologies, unlike sheep, on which few studies are available. However, accelerometric sensors still do not seem able to discriminate jaw movements, which are of great importance for evaluating animals grazing strategies in different pastures and for estimating the daily herbage intake.

The accurate prediction of the number of grazing bites is of great interest to estimate intake in grazing animals but it remains a challenging goal under field conditions.

Furthermore, although these devices are already used on farms, they still need significant developments, in particular regarding the easiness of installation and use, the real time data acquisition, the sampling frequency of the acceleration signal and the automation process of data classification.

Thus, the aim of this work was to develop a tri-axial accelerometer based sensor for automatically recording and statistically discriminating the feeding behaviour of sheep and to estimate the rate of biting (number of bites per min of grazing) on the basis of acceleration variables.

The short tests performed in grazing conditions using BEHARUM device (Chapter 2) confirmed that accelerometers combined with wireless communication technology are

useful tools to discriminate grazing, ruminating and resting behaviour activities of grazing sheep. In our experiment, we found a precision of 95% for grazing, 94% for resting and 89% for ruminating.

The multivariate statistical approach used in the analysis of data allowed to reduce the number of variables that are needed to assign acceleration minutes to the appropriate behaviour classes with an overall accuracy of 93% and k coefficient of 0.89.

Considering all the performances (sensitivity, specificity, precision and accuracy) in the assignment of behaviour activities, the results suggest that the technology developed in the present study is particularly appropriate to precisely and accurately monitor sheep grazing behaviour.

Modern accelerometers are able to record at high sampling frequencies, generating a huge amount of data which can lead to a rapid depletion of the memory device and to high costs in terms of battery consumption. Better performances can be obtained by pre-processing accelerometer data on the device itself, settling and applying to the data stream, for a given sampling frequency, an optimal aggregation window called epoch, as demonstrated in the second experiment (Chapter 3). The study aimed to develop an algorithm based on the multivariate statistical analysis to discriminate grazing, ruminating and other activities of grazing sheep equipped with the BEHARUM device and to determine the performance of the algorithm in terms of accuracy, sensitivity, specificity, precision and Coehn's k coefficient at different epoch settings (5, 10, 30, 60, 120, 180 and 300 s). The results showed that the prediction model performed better in classifying grazing behaviour than ruminating and other activities for all epochs. The 30 s epoch length produced the most accurate classification in terms of accuracy (89.7%)

and Coehn's k coefficient (0.8). Nevertheless, 60 and 120 s epochs may increase the potential recording time without causing serious lack of accuracy, and could be used for monitoring sheep behaviour in extensive conditions.

Regarding the prediction of biting rate, the results of the experiment performed in grazing conditions (Chapter 2) confirmed the difficulty to count bites using an accelerometer in field condition, in line with the few studies available in the literature.

The partial agreement with visual observation (65%) obtained in the regression model is probably explainable by different reasons such as the presence of undesirable signals during recording sessions, due to head movements not related to grazing activity; the high rate of biting of sheep, that makes difficult to capture individual bite events in field conditions compared to controlled conditions (micro-sward tests); the inclusion in the visual bite count of chew-bites, that probably produces acceleration signals different from those originating by bites alone.

For these reasons, in the successive experiment (Chapter 4) we have chosen to use sown micro-sward boards, with the objective to derive a model to predict sheep behavioural variables related with intake on the basis of variables calculated from acceleration data recorded by the BEHARUM device. The PLSR was able to provide accurate estimates of the number of bites, expressed as logarithm (LB), with an adjusted r^2 of 0.86 and a RMSEP of 3%. This is a good result considering that bite is the elementary unit of the grazing process, therefore the accurate detection of individual bites is essential for predicting intake. DMI and DMIR variables showed an adjusted r^2 of 0.71, but a RMSEP of 22%, while the other variables have r^2 values ranging between 0.32 and 0.67

(DMBM, FMBM, FMI and FMIR). The RMSEP reached a value of 26% for DMBM ($r^2=0.32$), confirming the difficult to predict this variable with automated methods.

These results suggest that accelerometer sensors placed under jaw appear promising estimators of the number of bites as well as of DMI and DMIR variables, even if at lower level. However it is advisable to pay general attention when using accelerometer sensor to estimate intake in grazing condition.

A combination of the information provided by BEHARUM device with those provided by other sensors could improve the overall classification accuracy of sheep grazing behaviour and the estimation of the variables related to intake, allowing to effectively drive the management of pastoral resources.