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SCUOLA DI DOTTORATO DI RICERCA
Scienze e Biotecnologie
dei Sistemi Agrari e Forestali
e delle Produzioni Alimentari

Indirizzo Scienze e Tecnologie Zootecniche

Ciclo XXVII

INGESTIVE BEHAVIOUR AND PERFORMANCES OF DAIRY EWES PART-TIME GRAZING MEDITERRANEAN FORAGES

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A Dissertation

Presented to the Department of Agriculture of the University of Sassari, Italy

in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

by

Giovanni Molle

November 2014

To Antonio, my father, my friend

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INGESTIVE BEHAVIOUR AND PERFORMANCES OF DAIRY EWES PART-TIME GRAZING MEDITERRANEAN FORAGES

Abstract

Part-time grazing, i.e. restricted time access to pasture, is widespread but its effects on ingestive behaviour and performance of dairy ewes are still overlooked. The objectives of this thesis were: i) reviewing the literature focused on the effects of part-time grazing on ingestive behavior and performance of ruminants and horses; ii) assessing the effects of different time restrictions on the above variables in milked ewes grazing *Lolium multiflorum* Lam (experiment 1) and *Trifolium alexandrinum* L (experiment 2); and iii) modelling the results of the above experiments for predicting herbage intake. The review showed that part-time grazing of pastures by ruminants results in compensatory behaviors such as higher intake rate and grazing intensity and lower energy expenditures, which often bring about mild to nil performance losses as compared with time–unrestricted grazers. Horses are less able in this compensation than ruminants. The experiments on dairy ewes compared 2, 4, and 6 h/d time access to pasture. Their results confirmed the general trend of ingestive behavior, showing that giving access to a moderate quality Italian ryegrass for less than 6 h/d decreased intake and milk yield of ewes in mid lactation, whereas for berseem clover access could be as short as 4 h/d without any loss of intake and performance. The modelling of the data based on both stepwise and partial least square regressions provided good performance in validation and satisfactory sensitivity.

Introduction

Sardinia hosts in its 24,000 km^2 the biggest dairy sheep stock in Mediterranean European Countries, amounting to ~3,200,000 heads, with a milk production above 300,000 t of milk basically devoted to cheese making. Over 11,000 Sardinian farmers with their families make a living from this rural enterprise.

Dairy sheep feeding is based on natural and cultivated pastures and the use of supplements is widespread, with level of supplementation not rarely above of 100 kg/ewe year, representing almost 30 % of total annual energy requirements.

Feeding costs are the highest expenditure of farm budget. It is therefore a must to increase the efficiency of feeding management of sheep in order to curb costs, reducing in the meanwhile the avoidable release of nutrients to the environment.

Intake is regarded as the main driver of animal production but it is difficult to measure it under grazing in experimental farms and still even impossible at farm level.

Although models exist to predict intake of grazing sheep, none of them is able to estimate the intake of sheep with time restricted allocation to pasture below 5-6 h/d. Unfortunately, part-time grazing technique is quite common in Sardinia like in other Mediterranean regions, where dairy sheep are bred. In contrast, part-time grazing has been recently investigated in depth as far dairy cows grazing sytems are concerned, because this technique entails some putative advantage as compared to stall-feeding from one hand, and whole-day grazing to the other.

This thesis is aimed at:

- Reviewing the literature focused on the effects of part-time grazing on intake, feeding behavior and performance of ruminants, with particular reference to sheep (Chapter 1);
- Assessing the effects of different time restrictions to pasture on intake, feeding behavior and performance of milked sheep grazing forage crops, namely a widespread annual grass (*Lolium multiflorum* Lam, Experiment 1, Chapter 2) or a common annual legume (*Trifolium alexandrinum* L, Experiment 2, Chapter 3);
- Developing and evaluating models for predicting the herbage intake of part-time grazing dairy ewes on the basis of the data gathered in the above experiments (Chapter 4).

Chapter 1

Part-time grazing: a review on the effects of duration, timing and frequency of pasture allocation on feeding behaviour, intake and performance by cattle, sheep and horses

Part-time grazing: a review on the effects of duration, timing and frequency of pasture allocation on feeding behaviour, intake and performance by cattle, sheep and horses

1. Introduction

Grazing of ruminants (e.g. Bellarby et al., 2013) and horses (Bott et al., 2013) have been recently re-evaluated in the light of putative benefits entailed by this feeding technique as compared to confinement feeding: lower feeding costs in many grassland-based production systems (e.g. Shalloo et al., 2004), lower footprint (with some exceptions, see Waghorn and Hegarthy, 2011), and overall benefits for animal health and wellbeing (Provenza et al., 2015), provided herbivores are sheltered from extreme weather conditions and adequately supplemented (e.g. Molle et al., 2008; Knowles and Grace, 2013).

The ultimate aim of ruminants' rearing is the product. In some studies, grazing has been proven to be inadequate to sustain individual milk production in high genetic merit cows (Kolver and Muller, 1998). However, in many areas of the world with well-adapted breed strains (Washburn and Mullen, 2014), permanent grassland and annual pastures, either native or cultivated, provide nutrients for attaining high yields of milk and meat. Furthermore, herbage-sourced dairy and meat products are often featured by high contents of micro-components - fatty acids (FA), in particular - known to be healthy for the consumer (Dewhurst et al., 2003, Cabiddu et al., 2005, Buccioni et al., 2012).

Despite the renewed consensus on the putative benefits of pasture-based livestock production systems, grazing management still has to be improved to become as reliable as stall-feeding, being the former complex and dynamic in nature, because it refers to a living ecosystem. Errors in grazing management can, in fact, determine short-term - immediately detectable - but also long-term feedbacks that can impair the efficacy or efficiency of the grazing system (Chilibroste, 1998). For instance, continuous stocking and even intermittent grazing of cattle for 24 h daily on wet soils can bring about a later decrease in primary production due to soil compaction, resulting from herbivore poaching (Shalloo et al., 2004). Moreover, whatever is the adopted grazing method, the allocation of pasture for 24 hours can be inefficient because there are periods within the day when grazing is limited or nil (night hours) and periods when grazing is not efficient (e.g. at dawn when the sward is wet because of dew).

Restricting the time access to pasture, i.e. adopting a part-time grazing (PTG) technique (also named rationed grazing), can often alter and sometimes curb the drawbacks of the unrestricted grazing, in terms of pasture and animal responses.

The putative advantages of PTG versus whole-day grazing can be listed as follows:

i) sparing herbage when herbage growth is low, provided that supplements are fed to meet nutrient deficit; ii) balancing ruminants diet when herbage nutritional composition is featured by excess or deficit of nutrients; iii) enhancing the efficiency of herbage utilisation by reducing damages due to animal trampling, treading and fouling; iv) reducing environmental footprints (e.g. Clark et al., 2010); v) increasing the chances to manage grazing pressure and intensity in time and space and through it the eveness of herbage utilization (Gregorini, 2012a); vi) presumably reducing feeding costs at area unit scale (ha), thanks to a higher stocking rate and a putative higher herbage utilization efficiency.

However, despite many encouraging results, the overall literature does not endorse part-time grazing for all the above adavantages. Why this? To answer this question we must step backward, recalling that this method can be regarded as a case of feed deprivation. In fact, feed deprivation (absolute or relative) consists of one of the following:

- Reduction of the amount of feed on offer;
- Reduction of the quality of feed on offer;
- Reduction of the time access to feed;
- Physical or psychological impairment of the encounter between feed and animal (e.g. distance to feed, distance between feed and shelter, watering points, isolation from herd or flock-mates, scaring effects of predators, other stressors);
- Extreme weather conditions which impair normal feeding behaviour.

All these conditions can operate under PTG, sometimes simultaneously, although some are under partial control of the farmer and others are totally uncontrolled. For instance, the absolute deprivation of any feed (i.e. fasting) for part of the day can be a useful tool to boost the foraging activity in the following meal (Chilibroste et al, 2007).

Time access to feed is a relative feed deprivation, which sometimes gives a similar response to partial fasting, particularly when the excluded time from grazing is spent in pens without access to feed. If supplements are fed as herbage substitutes, then part-time grazing is not a feed deprivation technique per se. Although fasting time and time access to pasture do not overlap and act in a different way on the regulatory mechanisms of intake, many published experiments confound the effects of these factors, impairing data analyses and interpretation (Chilibroste et al., 2007).

This review is aimed at highlighting the main literature results on the impact of PTG on feeding behaviour, intake and performance of grazing cattle, sheep and horses. A side-objective is the assessment of the difference in the response to PTG among animal species with reference to cattle, sheep and horses. In fact, the restriction of time allocation to pasture is expected to affect differently large ruminants, small ruminants and non-ruminant (monogastric) herbivores, whose gastro-intestinal tract, requirements and behavioural pattern are well differentiated (Van Soest, 1994).

Ruminants have a big reservoir (the rumen) to ferment the food rapidly ingested. In particular, rumen capacity is much higher in cows than sheep per unit of energy requirements (e.g. Cannas, 2004). In contrast, ruminants, particularly if hornless, have limited fly and defensive ability towards predators. Horses, the strongest and fastest domesticated herbivores, can extend their foraging to the dark hours, when predation risk is higher. On the other hand, horses have to do so (i.e. foraging for a long time at a slow rate) to compensate for the limited storing capacity of their monogastric gastro-intestinal tract. Therefore, It seems reasonable to evaluate if PTG can differently affect the ingestive behaviour of these animal species.

In the following section, we will sequentially consider the three main components of PTG:

1. The duration of access (hereunder named as time access to pasture (TA, h/d));
2. The timing of pasture allocation (i.e. the actual clock hours of entry to and exit from the paddock);
3. The number of daily allocations to pasture (hereunder named also as frequency of access (FAC, n. allocations/d).

To explore the subject, a database was created encompassing the published papers focused on this topic with reference to ruminants and horses (Appendix 1).

2. Effect of the restriction of time access to pasture

Herbivores' feeding behaviour basically consists of the prehension and mastication of the severed herbage and its subsequent rumination and digestion. In wild and free-ranging domesticated herbivores, these activities follow a well-described pattern (Arnold and Dudzinsky, 1978). This pattern is circadian because it is related to the daily photoperiod, being the main meals and the main rumination periods usually concentrated in the daytime and nightime, respectively (Figure 1a).

However, in domestic herbivores the feeding behaviour pattern is often artificially broken into bouts, due to handling procedures such as milking in dairy animals, supplements offer, corralling to prevent predation or theft and so on (Figure 1b). Thus, in a sense, the restriction of the time available for grazing is very common and millions of herbivores raised in the planet never experience a day-long stay on a pasture, in intensive and extensive farming systems (e.g. Smith et al., 2006).

Time restriction to pasture is not such an obvious concept as someone may think. In fact, the threshold of TA below which the grazing can be probably constrained is unclear. This threshold should be based on proper experimental data (see Erdman et al. (1989) for stall-fed dairy cows). Unfortunately, data on feeding behaviour of ruminants are scanty at local (grazing system) scale and hence authors often refer to more general information. For instance, Arnold and Dudzinsky (1978), comparing populations of dairy cows, beef cattle and meat/wool sheep, reported a median value of grazing time of 8-9 h/day in dairy cows and sheep and of 9-10 h/d in beef cattle. As anticipated, horses need to spread their foraging activity on an even wider time span, with grazing times often greater than 15 h/d (Crowell-Davis et al., 1985). Unfortunately, many factors impinge on grazing time: internal stimuli which motivate feeding, related to animal requirements and sensory appraisal of the feed on offer and external stimuli, such as pasture characteristics season and weather. For this complexity, grazing time is rather unpredictable (Chilibroste, 1999). This is also the case for the length of pasture allocation below which an impairment of feeding behaviour is probable (i.e. when TA < potential grazing time).

For simplicity, we will consider as restricted time at pasture any TA below 22 h/d, which is basically the net time available for expressing feeding or social behaviour at pasture in dairy enterprises, where basic handling procedures usually last c.a. 2 h/d. However, considerations will be made on the adequacy of this conventional threshold, in the light of the examined literature.

In the following sections the effects of time restriction at pasture, in terms of severity (constraint of TA in PTG herbivores measured as h/day) and duration (length of PTG method application measured as days) will be examined, considering several facets of ruminant’s ingestive behaviour and production response.

2.1 Effects on feeding behaviour

In ruminants, feeding behaviour activities are basically classified as grazing, rumination and idling (Gibb, 1998). Grazing time (GT) includes the time devoted to the severing (biting) of herbage and its preliminary mastication and salivation until the bolus is swallowed. Ruminating time (RT) is the time spent for regurgitating the mericic boluses and for their mastication until swallowing. It includes the inter-bolus interval which is the time elapsed from the swallowing of a bolus to the regurgitation of the next one. Idling time is the time devoted to rest (lying or standing), but sometimes it encompasses other activities as well, such as some activities related to grazing, such as walking (searching or not) and social interactions (grooming, playing, mating and so on).

In general, grazing time is split into grazing bouts by idling intervals, whose duration is usually conventionally set to a minimum of 5 min (Rook et al., 1994). Shorter idling periods interspersed among prevalent grazing activities are sometimes computed as grazing as well and named as intra-bout intervals. In this case, grazing time does not overlap with eating time, which is the net grazing time, i.e. the time strictly devoted to the jaw movements associated with foraging (Gibb, 1998). Grazing bouts can be associated with inter-bout times in grazing meals, if grazing is the dominant activity.

According to the mechanistic approach put forward by Allden and Whittaker (1970) and Penning (1986), herbage DM intake (HDMI, g DM/d) can be thought as the product of its main components as follows:

HDMI = GT x HDMIR (Equation 1)

Where:

GT = grazing time (min);

HDMIR = herbage DM intake rate (g DM/ min of grazing).

HDMIR can in turn be considered as the product of bite mass (BM, g DM) and bite rate (BR, n. bites/min graz.), whereas GT is regarded as the product of the daily number of meals (n. meals) and the meal duration (min/meal).

In the case of part-time grazing, GT in equation 1 could also be partitioned in its components as follows:

HDMI = TA × GTP × HDMIR (Equation 2)

Where:

TA = time access to pasture (h/d);

GTP = grazing intensity (as proportion of access time, Smith et al., 2006).

The equation 2 makes clear that, under PTG, herbivore intake is directly affected by TA restriction *in primis* through a putative reduction of GT. In theory, the constraint of TA restriction can be nil, when GT under unrestrained conditions is by far lower than TA. This can occur, for instance, with non-lactating ruminants having access for 7-8 h/d to an abundant pasture resource. However, in many cases, the stopping of grazing in the PTG occurs well before the natural break due to surfeit or satiety. If this occurs, the grazing herbivore can remember this feed deprivation experience, and on the subsequent pasture allocation can adjust its behaviour to the expected duration of TA to pasture. In doing this, it has to face a dilemma: how to re-allocate daily time to the different activities entailed by the feeding behaviour? The physiological 'answer' will depend on the complex balance between hunger and satiety *stimuli* sensed by the body, which in turn result from metabolic and fill drivers modulated by the context of herbivore-pasture inter-play (e.g. herbage accessibility, weather conditions).

Recent research findings suggest that ruminants sense the daily time by perceiving the light-dark cycle that directly impact on the cycle of hormones (namely, melatonin and serotonin) involved in the control of feeding behaviour (Gregorini, 2012a). In particular, neuroscience research results have shown that ingestive behaviour of undisturbed animals, spanning from bees to large mammals, is controlled by the so-called light-entrainable oscillator (LEO), which is anatomically centred in the hypothalamic suprachiasmatic nucleus (SCN) (Meijer and Reitvald, 1989). Light stimulus is sensed by specialized retinal photoreceptors and conveyed to the central nervous system (CSN). This is the master pacemaker for the feeding activity, thanks to its cross-talk with the hunger and satiety centres of CNS (Freedman et al., 1999).

However, when the undisturbed foraging is repeatedly and regularly broken by management cues, such as feed offer, milking or others, these events become "zeitgeber" (time-makers) that promote the activity of another oscillator, named food-entrainable oscillator (FEO; Mistleberger, 1994). This is the "metabolic clock" that, under some circumstances, can be partially or totally uncoupled with LEO (e.g. Piccione et al., 2003). According to some studies, FEO could be anatomically based in the liver (Stokkan et al., 2001), lending support to the key role of liver in the regulation of feed intake, even at meal (hours) scale (Allen, 2014). However, more recent data suggest that FEO could be located in different peripheral tissues and organs (liver included, Antle and Silver, 2009) or, in other words, that there could be a web of interacting FEOs rather one single FEO.

Although the mechanisms underlying these oscillators and their neurological and hormonal network are still far from being fully explored, there is a consensus that time restricted access to feed: i) can be memorized by the animals, thanks to FEO, often in phase with LEO; ii) can result in food anticipatory activities (FAA), expressed as raise in pre-meal locomotion activity, body temperature, gut motility and plasma cortisone levels (Mistleberger, 1994, Feillet et al., 2006). Although part-time grazing can probably elicit FAA, to the best of our knowledge, no studies have specifically addressed this point, showing evidence of FAA under grazing conditions.

In contrast, Gregorini and co-workers (Gregorini, 2012a) have clearly shown a systematic pre-meal raise in plasma ghrelin in part-time grazing cattle, suggesting the role of this hormone in setting the level of the motivation to eat. Other hormones are also probably involved in this mechanism, but their role seems more important for the termination rather than the starting of the feeding process (see reviews by Allen, 2014, Sartin et al., 2010 and Morton et al., 2006).

In general, the restriction of time access to feed impacts on the eating motivation and intake control mechanisms in a way that animals re-allocate the daily time budget among the activity classes, usually prioritising eating rather than resting or rumination in the case of ruminants, as recently backed by Gregorini et al. (2012b). However, the choice in favour of eating is not as obvious as it may appear. In fact, under controlled stall-fed conditions, cows confronted by a restriction of TA to feed did prefer lying on a comfortable mattress than eating in a trough filled up with palatable feed (Munksgaard et al., 2005). Other studies have shown that foraging can be ranked by ruminants less than other needs, such as socialization in gregarious sheep (Dumont and Boissy, 2000).

Nevertheless, in most of part-time grazing studies, while GT obviously decreases in parallel with the severity of TA restriction, the proportion of GT on the time access to pasture (GTP) increases in a linear or exponential way, depending on the study, in both dairy cattle (Figure 2a) and lactating sheep (Figure 2b). This response has also been found in beef cattle and other herbivore species (e.g. goats, Berhan et al., 2005).

Thus, the herbivore usually tends to compensate for reduced time at pasture by re-allocating the time budget in favour of foraging. The plasticity of GT is a measure of the adaptation capability of herbivores to tackle the continuous changes of weather, resource availability and predation risks that they experience in grazing systems.

Grazing time and eating time have been already proven to be sensitive to the shortening of feed resources. For instance, Penning et al. (1991) showed a clear quadratic increase in GT when pasture sward height decreased below 6 cm of sward height, in continuously stocked meat sheep, with time availability for grazing equal to 24 h/d. Likewise, an increase in GTP was observed in continuously stocked lactating meat sheep submitted to a moderate time restriction (9.5 vs. 24 h/d, Iason et al., 1999) or to a more severe TA restriction (2, 4 and 6 h/d) in dairy sheep rotationally grazing Italian ryegrass (Molle et al., 2014).

Grazing time is also affected by the level and quality of supplementation. As found in numerous studies on grazing ruminants (e.g. Bargo et al., 2003), the higher the level of supplementation the lower the GTP. This occurs also in cows submitted to PTG as shown in Figure 3 (Perez–Ramirez et al., 2008). This reduction of grazing intensity is caused by a metabolic feedback coming from the supplement or by the rumen fill increase, particularly if supplementation is based on forages. Cereal grains and starch-based concentrates at high supplementation level can also depress GT, due to a possible impairment of rumen function (sub-clinical or clinical acidosis, e.g. Commun et al., 2009, in free-choice fed sheep).

It is important to remember that within a grazing session the effect of restriction should affect more the tail than the head of the grazing bout. In fact, foraging is a synchronous process (Penning et al., 1993) and herbivores grazing on the same plot tend to start grazing all together at certain clock hours, in relation to photoperiod, if free ranging, or shortly after pasture is allocated, if part-time grazed. However, they usually stop grazing at different times, according to their different production and requirements levels (Penning et al., 1995). In fact, lactating cows and sheep have longer grazing times than non-lactating counterparts (Penning et al., 1995, Gibb et al., 1999). If this theory holds, in severely TA restricted ruminants, the constraint should affect proportionally more high-yielding than low-yielding ruminants. However, to the best of our knowledge, direct and explicit delving into this aspect has been overlooked so far. Under full-time grazing conditions, Sheahan et al. (2011) found that New Zealand strain dairy cows had a slightly longer GT than North-America strain dairy cows (444 vs 420 min/day, P< 0.05), despite the lower milk yield in the former than in the latter strain. However, estimated bite mass and pasture intake were both higher in the North-America strain. These results cast some doubts on the previous research hypothesis (the higher the milk yield the longer the grazing). However, they, confirm that high-producing cows are often more efficient in foraging than lower-producing herd mates, although this can vary with forage availability and accessibility and other environmental conditions, as recently reviewed by Washburn and Mullen (2014).

Some studies have investigated the increase in GTP more in depth, considering the influence of the restriction of TA to pasture on the number of meals, which usually decreases, and meal duration, which, in contrast, increases with the severity of time access restriction (Perez-Ramirez et al., 2008, 2009; Kennedy et al., 2009; Gregorini et al 2009b).

The number of bites per min of grazing (BR) tends to increase in parallel with GTP, along with the reduction of time allocation to pasture (Table 1). The time devoted to bite the herbage is relatively constant (0.68 s, Laca et al., 1994), whereas the time devoted to orally handle/processing the bolus before swallowing it is more variable (Ungar, 1996). So part-time grazing herbivores tend to squeeze chewing in favour of biting, as shown in cattle by the raise of the bite to chew ratio (Kennedy et al., 2009). This effect depends also on forage characteristics: short but dense swards in general favour the increase of BR as compared with tall and sparse swards (Ungar, 1996). Also patchy pastures can decrease BR.

At an upper scale of foraging process, TA to pasture affects the behaviour at feeding station level. The number of feeding stations per grazing session decreased in cows submitted to TA restriction to pasture whereas the number of bites per feeding station increased (Gregorini et al., 2011), showing the tendency of the herbivore to focus on foraging rather than walking and searching for alternate resources. These variables will be discussed also with reference to the effect of part-time grazing on energy expenditures.

In general, bite mass increases with the restriction of time access to pasture likewise the increase in fasting length (Table 1). Bite mass is probably the most sensitive herbage intake driver, accounting for most of the difference between grazing and stall-feeding, being BM in the latter usually unrestrained. In fact, conserved forages and concentrates have ususlly a high DM content and a high density due to processing (chopping, milling, pelletizing). In grazing conditions, BM in turn depends on pasture and animal factors. Herbage bulk density is the most relevant pasture modulator of BM (Ungar, 1996): it changes with forage species, grazing management and within species and grazing technique, across grazing layers and along with the daytime. In other words, many pasture variables can explain the increase of BM in herbivores facing a short time available for grazing. From the animal point of view, if all pasture drivers remain equal, BM can change through an increase of bite depth or bite area. The latter has been found to increase with reduction of rumen fill (Gregorini et al., 2009a).

Although GT and GTP are directly involved in intake regulation, rumination time has also a fundamental impact on ingestive process, because the comminutation of particles play a fundamental role in favouring their degradation by rumen microbes and transit through the gut. Rumination time is usually shorter when the grazing session is restricted: the ruminant tends to postpone rumination to periods when herbage is not accessible anymore (Figure 4). This holds true also for idling as observed in dairy ewes submitted to a severe restriction of TA (2 h/d) to Italian ryegrass (Molle et al., 2014).

2.2 Effects on herbage intake and its rate

Taking into account the effects of part-time grazing on the above feeding behaviour variables, the general trend of herbage DM intake rate (HDMIR), which shows an increase when herbivores are submitted to severe grazing deprivation in time, is not surprising (Figure 5). Intake rate is, in fact, the product of BM by BR, both of which usually increase as time to pasture is shortened. Herbage intake rate varies with hunger level (e.g. affected by supplementation regimen), with usually higher HDMIR in unsupplemented cows or sheep as shown in Figure 6. Futhermore, HDMIR is also higher when ruminants have high herbage allowance under rotational or strip-grazing conditions (Figure 7) or when sward height is above a minimum threshold (c.a. 30 mm for part-time grazing sheep under continuos stocking management, Iason et al., 1999).

Thus, in summary, as ruminants perceive the reduction of the time available to grazing, they compensate for this by eating faster. This trend is sometimes quadratic, showing a time threshold below which the increase is more relevant.

Fasting is also a significant driver of HDMIR, as reviewed by Chilibroste et al. (2007). Usually, the longer the fasting, the more acute the response to time restriction, although only few experiments have disentangled the main effect of fasting from its interaction with the TA restriction to pasture. Very often, the effect of fasting, although evident at the beginning of the grazing session, gradually fades away (transient effect, e.g. Erhard et al., 2001), whereas time restriction to pasture usually has a long-lasting effect on most of behaviour and intake variables (see carry-over effects in section 2.8 below).

In spite of the higher HDMIR, the daily intake of herbage (HDMI) is often reduced in ruminants exposed to severe TA restriction to pasture, although this is not always the case (Figure 8). This suggests that, in practice, we could envisage two classes of time restrictions to pasture: tentatively mild (TA ~10-5 h/d) and severe (TA <5 h/d). However, the diversity of pasture conditions and supplementation treatments among studies suggests adopting a cautionary attitude towards this classification, which warrants a deeper analysis.

Among the factors underlying the variability of herbage intake response to part-time grazing, we can evoke the variability among experimental conditions and treatments and the confounding effect of the replacement of the grazed herbage with the supplement (e.g. Hernadez Mendo and Leaver, 2004, in cows; de Renobales et al., 2012, in sheep). Based on these studies, it is not possible to draw any conclusions on how intake is affected by TA per se but rather by feeding regimen, inclusive of herbage and supplements.

Pasture availability and herbage quality are typical modulating agents of the relationship between time access to pasture and herbage intake (Hodgson, 1990). Dobos et al. (2009) found that herbage intake of dairy cows strip-grazing a C4 grass, kikuyu (*Pennisetum clandestinum*), was not severely reduced if TA to pasture was at least 4 h/d with pre-grazing compressed sward height of 10 cm, but could be even 2 h/d with a higher sward height (13 cm). Also, the modulating effects of level and type of supplementation cannot be neglected. In general, if TA restriction is severe, HDMI is reduced by supplement feeding (e.g. Hernandez-Mendo and Leaver, 2004). In contrast, if TA is not severe, and herbage availability not limiting, it may happen that supplement intake is lowered (Hernandez-Mendo and Leaver, 2004 in silage-supplemented cows; Molle et al., 2014, in hay-supplemented dairy sheep).

Like ruminants, horses are able to increase HDMIR when exposed to restricted TA to pasture (Dowler and Siciliano, 2009, Glunk and Siciliano, 2011a, 2011b). Their voracity is relatively high because they can ingest the herbage needed to cover more than half of their maintenance requirements in the first 4 h of grazing after an overnight fasting (Dowler and Siciliano, 2009). Horses may graze during the night for a high proportion of time, as found in semi-feral Camargue horses (from 49 to 55% of night hours) by Mayes and Duncan (1986). Therefore, we hypothesize that night fasting might have resulted in an increase in HDMIR the day after, in the experiment conducted by Dowler and Siciliano (2009). Nonetheless, HDMI was markedly reduced when horses were exposed to TA ≤ 6 h/d as compared with TA of 9 or 24 h/d (Glunk and Siciliano, 2011b).

If we compare sheep, dairy cows and horses for their hourly HDMI scaled to 100 kg BW ($HDMIh_{bw}$ herbage intake expressed as kg DM/100 kg BW/h of TA), we observe that $HDMIh_{bw}$ seems less affected by severe time access restriction in sheep and cattle than in horses (Figure 9). Interestingly, the regression coefficients of log-log linear regression of $HDMIh_{bw}$ against TA (Figure 10) are significantly different among species (P<0.01). No interaction was detected between animal species and the logarithm of TA (P> 0.10). However, it is important to highlight that the horses database is relatively small and insufficient to enable us to draw firm conclusions on this point.

2.3 Effects on diet quality and diet digestibility

Few studies have assessed the effect of time restriction to pasture on selected herbage quality and hence diet quality and nutrient intake. Ginane and Petit (2005) found that heifers exposed to a TA restriction of 5 h/d gave priority to the quality rather than the quantity of selected herbage, spending proportionally more time grazing vegetative than reproductive patches than counterparts exposed to them for 24 h/d. Similar findings (higher CP and lower NDF levels, on DM basis, and higher DOM in the selected herbage) were obtained in dairy cattle having access to pasture for 6 h/d as compared to 9 and 22 h/d, although, in this case, access was split into two grazing sessions (Kennedy et al., 2009). Even under controlled stall-feeding conditions, goats with access to diversified feed and submitted to restricted time access shifted their dietary choice in favour of high-quality ingredients (Görgülü et al., 2008). This would suggest that ruminants submitted to restricted time access to food tend to compensate for it by increasing energy and maybe protein intake per time unit (hour). However, there is some inconsistency in the literature because other authors found that herbage quality was not affected by the TA restriction (e.g. Perez-Ramirez et al., 2008). Diet selection is a time-expensive process, so it is hardly explainable how a grazer can conceal it with a faster intake rate, unless sward structure can favour the coupling of quantity and quality. For instance, in some species (e.g. white clover) legume leaves are available on the upper grazing layer, favouring the coupling of quantity and quality in herbivores compelled (or motivated) to eat fast (Baumont et al., 2004).

There is also a shortage of information on the fate of the ingested herbage at rumen and post-rumen levels. In general, the shorter the grazing session, the smaller the size of DM and NDF rumen pools, which usually peak at the end of the grazing period (dusk meal; Taweel et al., 2006). Nonetheless, the rate of increase in rumen pools is higher at beginning than at the end of a grazing session (Chilibroste et al., 1998), although the raise can depend, apart from hunger, on the feeding value of forage. For instance, Williams et al. (2014) showed that cows grazing Persian clover had a relatively slow increase in rumen pool sizes after 3 hours of grazing as compared with those measured by Chilibroste et al. (1998) on a grass-based pasture.

Rumination is postponed and preliminary mastication reduced in part-time grazing ruminants. Thus, in severely TA restricted ruminants, digestion and passage rate can be slowed down. Therefore, constraints in time access successfully compensated by the feeding behaviour (higher HDMIR and grazing intensity) can impair digestion later on, particularly if HDMIR had been accelerated by factors such as previous fasting and possibly afternoon grazing meals (see below). This hypothesis has been recently backed by Perez-Ruchel et al. (2013), who found a lower rumination time and slower passage rate in sheep fed fresh *Lotus corniculatus* with restricted access time of 6 h/d as compared with unrestricted counterparts. Also, NDF apparent digestibility was numerically lower in the TA restricted sheep than in unrestricted sheep.

According to Taweel et al. (2006), the rumen fill affects more the termination of the meal preceding dusk (evening meal) than of those following dawn (morning meal) or midday (afternoon meal). Gregorini et al. (2007) highlighted the role of rumen fill in controlling the feeding behaviour, by decreasing grazing intensity, intake rate, searching time and bites per feeding station but increasing bite depth. Furthermore, Gregorini (2012a) recently re-evaluated the above literature outcome, suggesting that meal anticipation, initiation and termination are probably controlled by different mechanisms and rumen fill plays possibly a major role on the termination of the meal, even under conditions of not fasted animals.

Horse data are scanty but reveal that even for this species the severe shortening of time at pasture can have some drawbacks on digestion, with accelerated fermentation and altered fluid balance in the hindgut due to the fast-big meals resulting from part-time grazing. However, no evidence of these problems was detected by monitoring faecal pH and DM in horses with a moderate restriction of TA to pasture (12 h/d vs. 24 h/d; Siciliano and Schmitt, 2012).

2.4 Effects on animal performance

In ruminants, the reduction of time at pasture can result in lower performance. However, the actual response depends on several factors, such as severity of time access restriction, daily period of access (e.g. day vs. night), level of animal requirements, accessibility and quality of the pasture on offer, and supplementation level. As can be seen in Figure 11, overall milk production of dairy cows and dairy sheep often are not apparently affected by TA, even when TA is lower than 6 h/d. In lactating meat goats, Trovar-Luna et al. (2011) showed a lower milk yield in early but not in late lactating does grazing a grass-legume pasture at 12 h/d of TA (night-locked goats) compared with counterparts at pasture for 24 h/d.

In growing cattle, Ginane and Petit (2005) found better performance in the unrestricted (24 h/d) than in the restricted TA (5 h/d) heifers. Contrarily, in supplemented lambs growing on the Chinese steppe, increasing the TA from 2 to 12 h/d did not result in better average daily gains, despite some period x TA interaction (Zhang et al., 2014). Even in growing kids of meat breed, 24 h/d access to pasture resulted in minor, but detectable, advantage as compared with night-locked counterparts in terms of BW daily gain (Trovar-Luna et al., 2011). Berhan et al. (2005), comparing shrunk body weight accretion of growing Boer goats submitted to 4 h/d, 8h/d or 24h/d of TA to pasture found that 4 h/d treatment group showed a numerically lower shrunk BW accretion (69 g/d), without significant differences with the performances of the other groups (85 g/d (8 h/d); and 83 g/d (24 h/d), P>0.05).

Sparse studies on tropical pastures in Africa, overall, lend support to the limited effects of TA restriction on performance (Jung et al., 2002: 9 h/d vs 4.5 h/d, lower BW gain and lower milk yield in the most TA restricted cows) or to its lack of effects. For instance, Smith et al. (2006) did not find any advantage in extending the grazing session from 7 to 11 h/d even when providing roughage as a supplement to night-locked cows. Ayantunde et al. (2008) detected only a small, not-significant increase in BW in cows exposed to 9 h/d rather than 6 h/d TA to pasture. In these studies, however, pasture quality and availability were generally moderate to low, as it can be expected in these farming areas, and cattle had to walk to the pasture and back to the corral even twice a day to drink (Smith et al., 2006), thus exacerbating the increase of energy expenditures associated with the extension of daily grazing sessions.

2.5 Effects on animal product quality

Relatively few studies have fully addressed the effect of TA restriction to pasture on ruminant produce quality. In dairy cows, milk composition has been sometimes influenced by the time access, but overall, there is no apparent trend in milk fat and protein contents along with changes in time restriction (Figure 11). Data on dairy sheep are too few for allowing a proper comparison (Garcia-Rodriguez et al., 2004, Alvarez-Rodriguez et al., 2007, Molle et al., 2014). Although lower values of milk fat and protein contents are detectable in non-restricted as compared with restricted ewes, this is probably basically a dilution or "study effect" (Figure 12).

The effect of restricted or unrestricted TA to pasture on milk fatty acid profile has recently become a popular research subject with reference to dairy cows (e.g. Rego et al., 2008) but findings on dairy sheep and goats lag behind. Addis et al. (2007) and more recently De Renobales et al. (2012) have focussed on the effects of TA to pasture and supplementation on sheep cheese (the former) and milk sheep (the latter), finding an increase of beneficial FA (c-9, t-11 CLA and Ω 3 fatty acids, in sheep grazing at least for 4 h/d without fat-enriched supplements (De Renobales et al., 2012) or 3 h/d with fat-based supplementation (Addis et al., 2007). In these papers, however comparison between part-time grazing and control ewes were absolute (24 vs 3 h/d in Addis et al., 2007 and 0 - stall-fed control vs 4 h/d in De Renobales et al., 2012).

2.6 Effects on energy expenditures (EE)

Data are scanty and refer basically to meat goats. In a long run experiment with goats in different physiological stages, Trovar-Luna et al. (2011), using the heart rate/ O_2 pulse method (Brosh et al, 2004), found a higher ($P< 0.05$) total EE in all-day than night-locked grazing goats (754 vs. 687 kJ/kg BW $^{0.75}$). Furthermore, the EE associated with locomotion was higher (ns) in these goats (64.1 vs. 53.2%, of maintenance requirements, inclusive of thermoregulation, respectively). Also Behran et al. (2005) in an experiment on growing kids found a raising trend of EE with the length of access to pasture, amounting to 4.96 (4 h/d) 5.13 (8 h/d) and 6.19 MJ/d (24 h/d). This result agreed with the longer GT and higher number of steps in the goats exposed to the long TA.

Overall, the response to part-time grazing in terms of EE, or walking activity (e.g. Chen et al., 2013 in lambs) suggests that, in many cases, a moderate time restriction can result in lower EE and possibly in a better efficiency of energy utilization at animal level. Interestingly, in a comparison between full time access to pasture versus zero-grazing (same diet – different feeding management), dairy cows at grazing spent 19% more energy in the first 6-h access period than counterparts fed freshly cut herbage, providing a first approximation of extra EE cost of part-time grazing, independent of diet quality (Dohme-Meier et al., 2014).

2.7 Effects on GHG emissions and N release

De Klein (2001) on the basis of farmlet study and a subsequent modelling exercise showed that part-time grazing is a viable technique to reduce nitrate leaching in New Zealand. The same author (De Klein et al., 2006) in a more focussed study, confirmed that restricting TA to pasture in autumn brings about a reduction of the emissions of N_2O and NO_3 leaching losses. More recently, Clark et al. (2010) found that reducing time allocation of cows to pasture was beneficial to reduce the deposition of urine on the sward and laneways from 89% (control 24 h/d) to about 50 % (2 x 4 h/d or 1 x 8 h/d of TA). In addition, Mufungwe et al. (2013) observed a lower CH_4 emission, as measured using the SF_6 tracer technique, in cows grazing for a restricted time (day or night time with or without access to a total mixed ration in the lock-out period) as compared with stall-fed control cows. This was true at both per animal and per kg of milk levels, although milk yield was not different between stall fed control and the group grazing during daytime and fed the total mixed ration. Data on sheep and goats are scanty. In a recent study the efficiency of nitrogen utilization in dairy sheep was enhanced in the most restricted access time sheep, which was however the group with reduced milk yield and lower recovery of BCS along with the experiment (Molle et al., 2014, and chapter 3 of this thesis).

2.8 Carry-over effects of restricted time access to pasture

The effects of food deprivation and in particular of the restriction of TA to pasture may depend not only on the severity but also on the duration of time restriction. Only few studies have addressed so far this point (e.g. Zhang et al., 2014). The longer the application of the grazing management the more the ruminant can adapt to it, using different behavioural cues and physiological mechanisms to offset the constraints imposed by the limited availability of time for grazing. Conversely, if restriction is severe, it can be argued that the herbivore can experience a marked distress, becoming unable to accommodate the uncomfortable conditions, losing performance and weight. Residual effects of part-time grazing may appear later, and persist even more than a month after its discontinuing. In fact, dairy cows at pasture for 21 h/d that had been submitted for 5 weeks to TA to pasture of 4 or 8 h/d, produced less milk in the following month than control cows kept at pasture for 21 h/d across all the study (Delaby et al., 2008). The loss of milk increased with restriction severity: 2 kg/cow (8 h/d) vs. 3.5 kg/cow (4 h/d). In dairy sheep, a carry-over effect was detected in terms of reproduction performance with a lower fertility (n.s.) and prolificacy ($P<0.05$) at the first mating in the sheep exposed to TA to pasture of 2 h/d as compared to 4 and 6 h/d in the previous two months (Porcu et al., 2014). This is in line with previous results on the carry over effect of grazing management in dairy sheep continuously stocked in pasture kept at 30 mm compressed sward height during mid-lactation (Molle et al., 1995).

3. Effect of the timing of pasture allocation.

This subject has recently gained attention due to some key findings related to the circadian changes in herbage composition (Orr et al., 1997, Delagarde et al., 2000, Gregorini et al, 2008):

- DM content of herbage vary along the day, usually increasing up to a maximum in the afternoon hours;
- Due to photosynthesis, water soluble carbohydrates (WSC) accumulate in stem and leaves during the daytime again peaking in the afternoon. Then they decrease when respiration (so WSC utilization) starts to overcome photosynthesis;
- NDF content tends to passively decrease due to the above quoted trends, with a nadir just overlapping the azimuth of DM and WSC;
- Last but not least, even FA composition of herbage can change, with higher concentration in the first hours of afternoon of those FA which are source of putatively beneficial milk and meat FA.

All these findings pushed research to explore the convenience of concentrating the grazing session in daily periods when many pasture features are in favour of intake, performance and product quality, as recently reviewed by Gregorini (2012a).

Unfortunately, not many studies have addressed this topic in an unconfounded way, since timing and time access are often difficult to disentangle (see Chiliobroste et al., 2007). Therefore we will focus hereunder only on those few studies in which the comparisons between timing of access is independent by the access duration.

Chilibroste et al. (1999) and Soca et al. (1999) compared the timing of access in supplemented dairy cows grazing oats at low herbage allowance (15 kg/cow d) with TA of 6 h/d. The treatments were morning prevailing (6MG: 08:30-12:30; 16:30-18:30) or evening prevailing grazing (6EG 12:30-14:30; 16:30-20:30). The cows of 6EG group had higher grazing intensity but lower ruminating time at pasture (Figure 13) and BR (in interaction with measurement week; Soca et al., 1999) than those of 6MG group. The group 6EG gave better milk performance, although performance were indifferentiated from a control group restricted to 8 h/d at pasture (8MG).

In a subsequent study, dairy cows grazing a legume-based pasture (50% of legumes) and supplemented at a flat level (c.a. 4.5 kg DM/cow d of maize silage and 6.1 kg DM/cow d of concentrate) were fed at pasture either in the morning (MG, 07:00-11:00) or in the afternoon (AG, 11:00-15:00; Mattiauda et al., 2013). The latter cows had higher grazing intensity and lower number of bites than the former. The ratio bites/chews and the bite mass was higher in the afternoon grazing cows. Thus herbage intake rate was higher (P<0.10) in these cows by 25% as compared with the counterparts. Nevertheless, there was no statistical difference in herbage intake and milk yield between morning and afternoon grazing groups. In addition, there was a decrease in rumen pH and a raise of rumen NH_3 and VFA concentrations, more pronounced and faster in afternoon- than morning-fed cows. These results overall suggest that the longer fasting preceding the afternoon grazing session and the higher soluble protein and WSC in the ingested herbage probably elicited this physiological response. These responses at rumen level have been recently confirmed by Gregorini et al. (2008) and Nhikkah (2013).

These results could be modified by weather conditions and other environmental factors (provision of shaded areas in hot periods, flies disturbance) that may occasionally favour or disfavour the afternoon or evening grazing or feeding in general. Ahroni et al. (2005), in fact, found a clear advantage in feeding at night dairy cows exposed to high temperatures and temperature-humidity index during daytime. The night fed cows had a lower intake but their milk yield as good as the morning fed cows, due their lower EE.

Gregorini et al. (2008) in beef cattle compared morning and afternoon feeding (8:00-12:00 vs 15:00-19:00) with or without previous fasting and detected similar grazing intensities (grazing time as proportion of TA) and HDMI but they also found higher BM and BR in the afternoon than morning grazed cattle. Furthermore the unfasted cattle grazing in the afternoon showed a higher OM ruminal digestibility.

Results of the timing access to pasture on small ruminants are still rare. Avondo et al. (2008) found an increase of WSC (20 vs 17 % DM) in the herbage selected by Girgentana goats grazing *Lolium multiflorm* Lam. in the afternoon than in the morning. The former goats showed also higher HDMI (0.825 vs 0.784 kg DM) and a trend to higher milk yield (0.931 vs 0.893, g milk, P<0.09) with higher milk protein and a lower milk urea contents. Interestingly, the milk fat of the afternoon grazing goats was characterized by a higher content of c-9 t-11 CLA and ω 3 fatty acids (Avondo et al., 2008). Similar results were found in a later comparison on *Trifolium alexandrinum* cut in the morning or in the afternoon and freshly fed to Girgentana goats (Pagano et al., 2011).

In a more recent paper, Avondo et al. (2014) compared growing Merinos lambs submitted to 8 h/d TA, 4 h/d TA in either morning or afternoon hours and grazing a perennial ryegrass pasture, finding better performance in the less restricted group, in terms of final body and carcass weights but with undifferentiated dressing percentage among groups. The carcass of the afternoon grazed lambs was featured by an oxidative stability undifferentiated from the unrestricted grazing lambs (Luciano et al., 2012) and a higher content of putatively beneficial FA (Vasta et al., 2012, Luciano et al., 2012). This could be related to the increase of α linolenic and linoleic acids in the herbage during afternoon hours and possibly a less biohydrogenating rumen environment (Gregorini et al., 2008).

Only one study addressed this topic with reference to horses, to the best of our knowledge. Chavez et al. (2011) compared AM (7:00-13:00) vs PM (12:30-20:30) access time of geldings (588 kg BW) to pasture finding a higher intake of fescue pasture in PM than AM grazed horses (6.6 vs 5.6 kg/DM, P<0.05). Despite this result, the authors casted doubts on the benefit of postponing daily turn-out to pasture, due to the higher risk of laminitis associated with higher intake of WSC. In fact, a morning grazing would probably be more safe for this purpose according to Longland and Byrd (2006).

Interestingly, there is no reference in the literature to the evaluation of FAA in grazing studies where part-time grazing is postponed to evening hours. Delaying the access to pasture to evening, theoretically increase the phase angle between LEO and FEO and this could increase the activities that anticipate food provision (FAA). Monitoring these activities and associated physiological variables may help to gain insights into the herbivore motivation to eat, and hence to better explain the variability of animal response to scheduled grazing sessions.

4. Effect of the daily frequency of access to pasture

Overall the increase of the frequency of daily access as far as we know, has less consistent effects on intake and performance of ruminants than the previously quoted factors (TA and timing). Dalley et al. (2001), increasing the frequency of access to fresh strips of pasture from 1 to 6 times per day, did not find any advantage in terms of herbage intake, grazing time or milk production of dairy cows, although BW loss was less with 6 than 1 grazing sessions per day. Even considering experiments where 1 and 2 daily grazing sessions are compared, very rarely a clear advantage has emerged in favour of frequent pasture allocations in terms of behavioural variables, intake or performance (Table 2). This suggests the hypothesis that substituting the autonomous splitting of grazing time into meals with artificially scheduled meals has probably only transient or erratic boosting effects on foraging, if any. However, in some of the studies addressing this topic, the daily frequency of access to pasture was confounded with the distance walked to and from the milking parlour or the hut, in others, environmental condition were unfavourable for some of the grazing sessions (hot temperature or darkness). For instance allocating grazing sessions in the dark hours could be unworthy, particular when herbage allowance is moderate to poor. In fact grazing activity could be reduced or nil at worst, as shown by Williams et al. (2000) in cows grazing Persian clover at low herbage allowance. Another aspect that should be better investigated is represented by the higher energy cost that can result from higher daily frequency of pasture allocation (distance to be walked) and the disturbance that frequent handlings bring about. Disturbance was evoked as a reason for poor performance of cows fed a complete diet 4 times per day instead of once daily (Phillips and Rind, 2001).

5. Modulating effects of the supplementation timing and frequency

As anticipated (Figure 1b), the time unavailable for grazing becomes available for eating supplements, as well as other behaviours (rumination, idling, social interactions). Some studies have focussed on the TA to supplements, as well as the timing and frequency of supplementation. All these may interact with TA restriction to pasture exerting short- or long-run feedback, due to associative effects of supplements on rumen and gut environment.

Part-time grazing experiments in which the timing of concentrate provision is shifted with the aim of better synchronizing energy and N utilization in the rumen are scanty. Trevaskis et al. (2004), superimposed on a comparison between pasture access timing (AM vs. PM) a comparison among timing of concentrate meals. They found no evidence of higher intake or diet digestibility in the synchronous as compared to asynchronous cows fed in the morning. However giving ¾ of barley in the morning milking increased significantly diet digestibility, if cows were grazed in the afternoon. Nonetheless, this was deemed a result of the afternoon grazing more than nutrient synchronisation.

The time not allocated to grazing a forage species could be allocated to grazing another species. This case is not discussed in this review because it is beyond its scope. However, it is important to remind here that part-time complementary grazing can be a sensible way to combine in ruminants diet forage species featured by complementary characteristics such as different CP, NDF or plant secondary metabolites (see review by Chapman et al., 2006, with reference to cattle, and Molle et al., 2008, with reference to dairy sheep).

6. Conclusion

To summarise, the data gathered and analysed so far tend to confirm that the restriction of TA to pasture can be advantageous to ruminants and horses for many facets, provided that restriction is not too severe (depending on animal species), herbage allowance is not limiting or supplementation is adequate to cover the reduction of herbage intake. The effect of animal species should be further explored by the meta-analysis of the collated database.

Timing of the grazing session in the afternoon can be a good strategy to synchronise pasture quality and animal attitude to forage intensively, favouring intake and performance and produce quality, although literature is still poor with reference to sheep (dairy in particular) and horses. In the latter species, the afternoon grazing is putatively risky for the outbreak of laminitis, being this pathology associated to high intakes of WSC and fructan.

The evaluation of the effects daily frequency of access to pasture deserves further research effort, but, particularly in this area, the measurement of ingestive behaviour should be coupled with that of distance walked and EE to gain knowledge on the mechanisms underlying animal response.

Timing, sequence and source of complementary forage and concentrates need also further delving to better understand to what extent the synchronisation of nutrients can be helpful to increase the efficiency of the part-time grazing ruminants and curb their polluting emissions.

The knowledge on the effects of part-time grazing in heterogeneous pastures is still poor. Fast-grazing associated to severe restriction of TA to pasture, apparently fits better to the grazing of monocultures of forage species featured by a moderate-low content of fibre (legumes or good quality grasses) possibly associated as stripes, than monocultures of fibrous grasses or intimate mixtures of forages with different palatability. However this area warrants further research.

Finally a more holistic research is needed to assess these effects at a system level and across the whole grazing season to detect the long term direct and residual effects of part-time grazing.

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. Table 1. Effects of restriction of time access to pasture on bite mass (BM) and bite rate (BR) in part-time grazing ruminants

Reference	Animal	DIM	Initial BW kg	Initial MY Kg/cow d	Exp. Period	Exp. Duration days	Pasture	Grazing method	HM t DM/ha	SH mm	HA Kg/cow c	TA	FA	TIMING		BM	BR
Kristensen et al., 2007	d. cows	96	592	31	g. season	42	LP + TR	CS	1.8	115		4	1	06:30	10:30	0.90	57
									1.6	110		6.5	1	06:30	13:00	0.66	59
									1.5	103		9	1	06:30	15:30	0.66	58
Kennedy et al. 2009	d. cows	202	591	24	March	31		SG	1.3	64	15.4	6	2			0.69b	56
									1.2	62	15.5	9	1			0.48a	58
									1.3	63	15.4	9	2			0.52a	59
									1.2	61	15.5	22	2			0.47a	57
Gregorini et al., 2009	d. cows	35	470	24	Sept-Oct	60	LP 80%	SG	3.2			8	1	08:00	16:00		52b
									3.2			8	2	08:00	16:00		55a
									3.2			22	2	08:00	06:00		47c
Mattiauda et al., 2013	d. cows	60	550	25	May-July		Leg. 50%,	SG	1.7	70	21.4	4	1	07:00	11:00	0.59b	
									1.5	66	20.4	4	1	11:00	15:00	0.71a	
Iason et al., 1999	Lact. ewe		54		May-June	49	LP	CS		3.0		9.5	1	09:30	18:30	0.03	72
										5.0		9.5	1	09:30	18:30	0.04	70
										3.0		24	1			0.02	69
										5.0		24	1			0.03	66
Zhang et al., 2014	Lamb		22		Jul-Sept	99	Steppe	SS				2	1	06:00	08:00		50
												4	1	06:00	10:00		51
												8	1	06:00	14:00		48
												12	1	06:00	18:00		46

Legend: DIM = days in milk; HM = herbage mass; SH = sward height; HA = herbage allowance; TA = Time of access to pasture; FA = daily frequency of pasture allocation

Different letters within study indicate differences among treatment means (P<0.05)

Table 2. Effect of the frequency of daily allocations to pastures on herbage intake (HDMI), milk yield (MY) and milk fat (MF) and milk protein (MP) contents.

Reference	Animal	DIM	Initial BW	Exp. Period	Exp. Duration	Pasture	HM	SH	TA	HDMI	MY	MF	MP
			kg		days		t DM/ha	mm	h/d	kg	kg	g/kg	g/kg
Kennedy et al. 2009	Dairy cows	202	591	March	31		1.22	62	9	12.1	22.4	42	34
							1.31	63	4.5+4.5	12.9	21.5	40	34
Gregorini et al., 2009	Dairy cows	35	470	Sept-Oct	60	LP 80%	3.19		8	12.5			
			470				3.19		4+4	13.9			
	Dairy cows	35	470	Sept-Oct	20	LP 80%	2.68	77	8		20.8	43	34
							2.54	75	4+4		22.1	41	34
	Dairy cows	225	498	April	15	LP80%	3.26	58	8		10.3	55	43
							3.24	58	4+4		10.4	58	41

Legend: DIM = days in milk; HM = herbage mass; SH = sward height; TA = Time of access to pasture; LP =perennial ryegrass; TR = white clover; CS = continuous stocking;

SG = strip-grazing; SS = set stocking

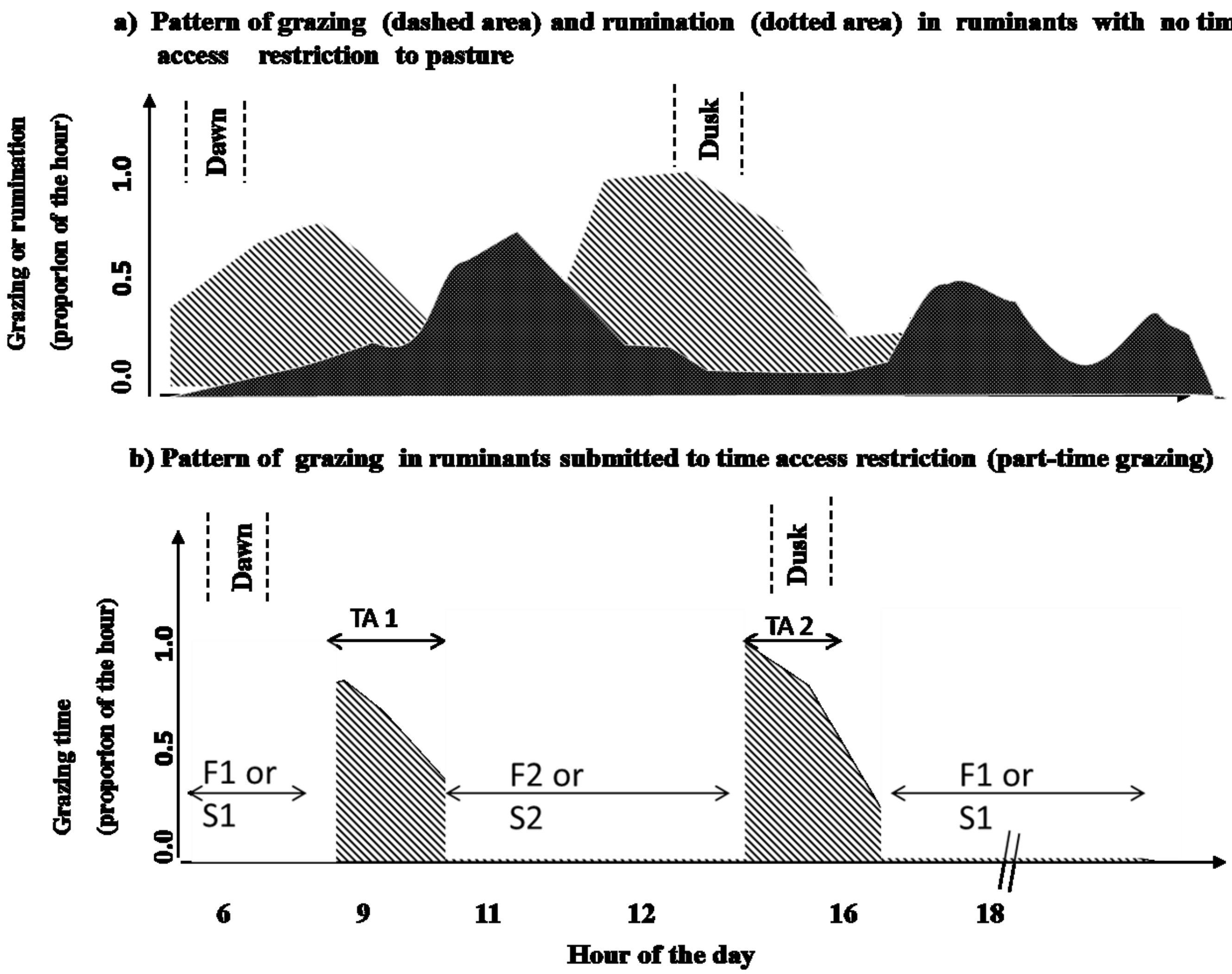
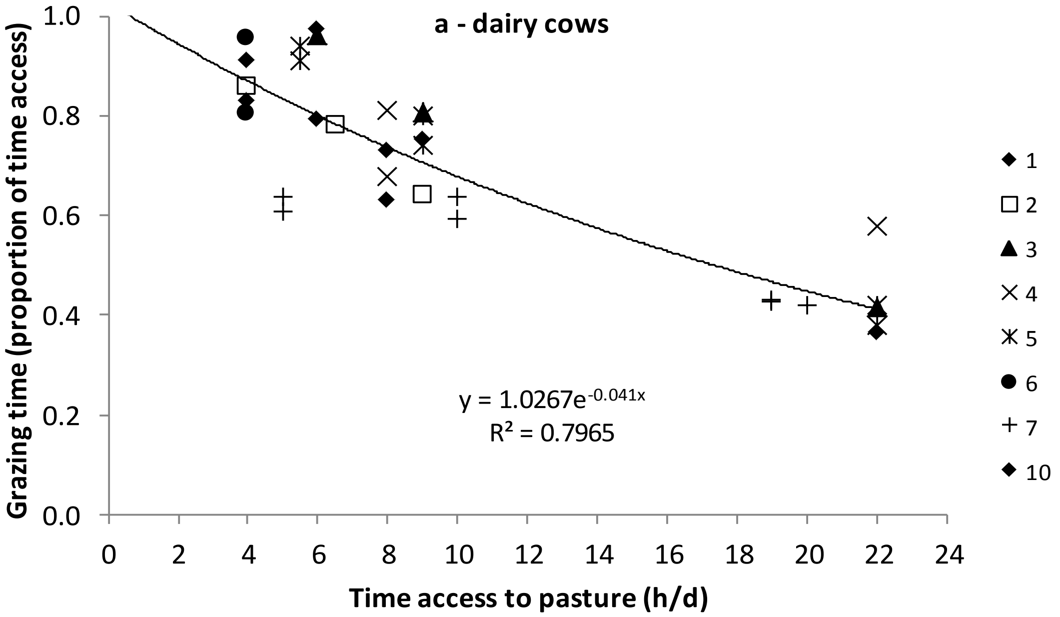


Figure 1. A schematic description of the pattern of feeding behaviour at pasture in ruminants without (a) or with restriction of time of access to pasture (b). In non-restricted time access conditions (a), grazing time is usually concentrated in two or three main grazing meals at dawn, afternoon and evening, before dusk. Rumination activity and idling (not shown) complement grazing. When time at pasture is restricted (b) grazing usually becomes the main or the only activity at pasture. The time complement to 24 hours can be either devoted to fasting (F1 and F2) or available for eating supplements (S1 and S2). In both cases the time complement is also partially available for rumination, idling and social interaction.

Severity of time restriction can be measured as the sum of the time allocation to pasture (TA1 + TA2 in the example) divided by 24 h (4 h/d in the example, the lower the more severe). Timing of time restriction is indicated by the actual clock time of entry to and exit from pasture. Finally, frequency of allocation is the number of grazing sessions in a day (2, in the example). Duration (F1 + F2) and timing of fasting, if any, as well as duration of time access to supplements (S1 + S2), timing and frequency of supplementation, supplementation level and quality all putatively modulate the effects of part-time grazing on ruminant ingestive behaviour and performance.



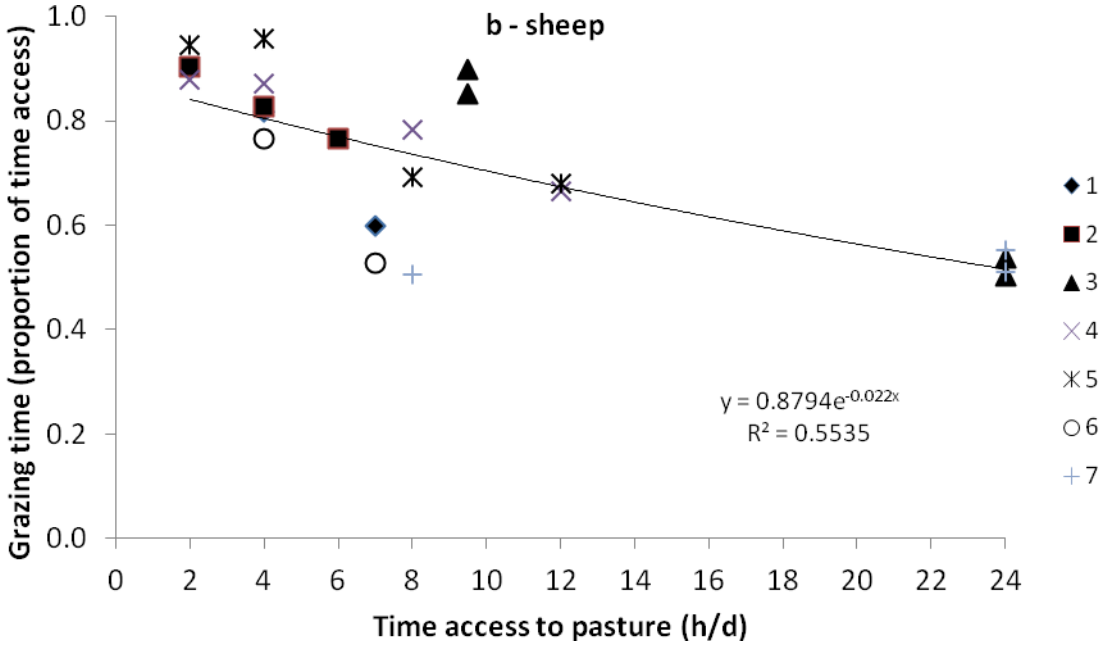


Figure 2. Grazing intensity in part-time grazing cows (a) and sheep (b). For details on the experiments see appendix 1.

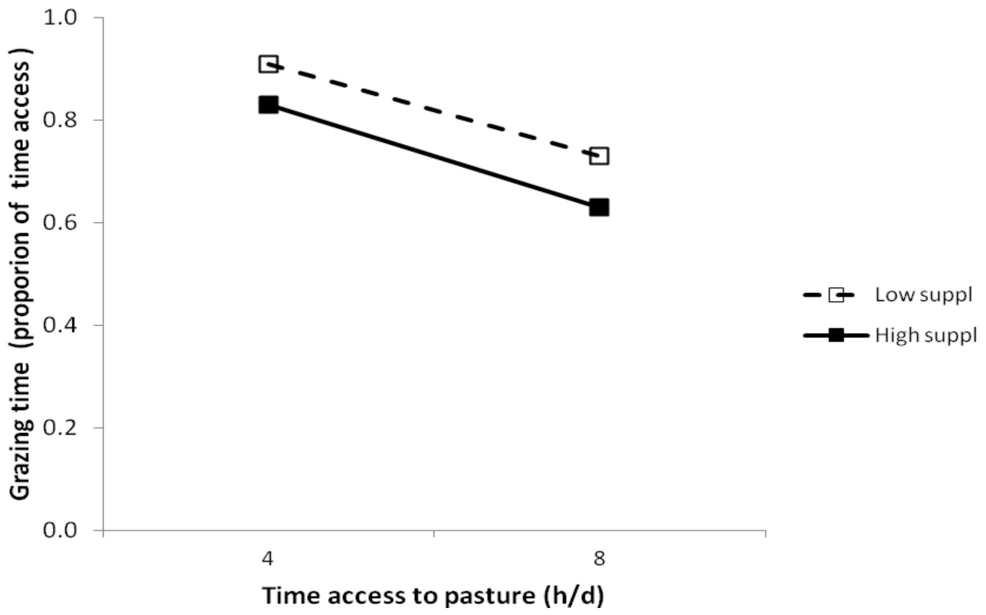


Fig. 3. Effect of the level of supplementation on grazing intensity in part-time grazing dairy cows supplemented either with either 5 (Low suppl.) or 10 (High suppl.) kg DM/day of a maize silage-soyabean meal mixture in the ratio 87/13 on DM basis (Perez-Ramirez et al., 2008). These levels were set to compensate for different herbage allowances (namely 7 and 11 kg DM/cows day above 5 cm, for the high and low supplementation levels, respectively).

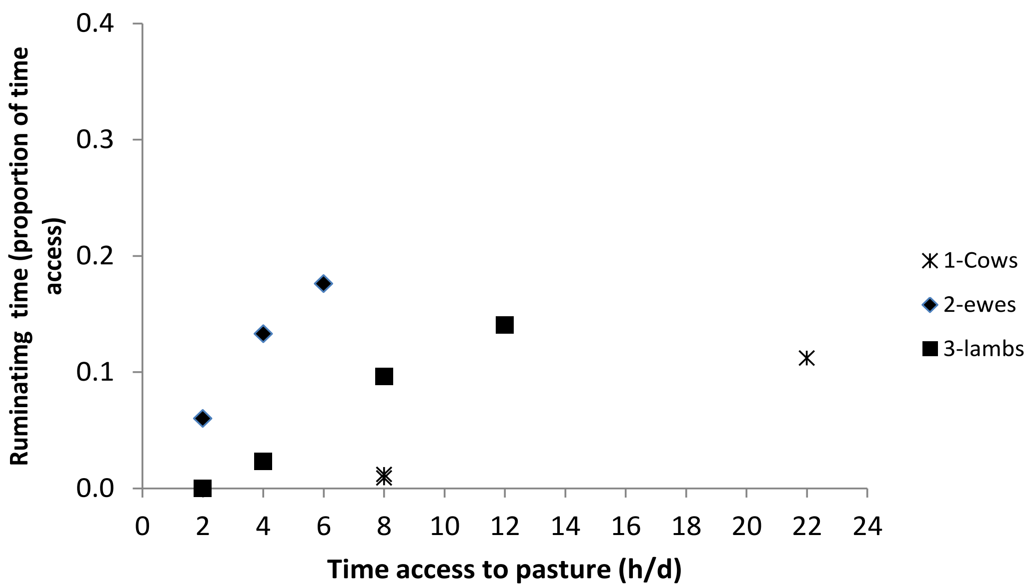


Figure 4. Rumination time at pasture in cows and sheep submitted to part-time grazing (1, Gregorini et al., 2009; 2, Molle et al., 2014; and 3, Chen et al., 2013)

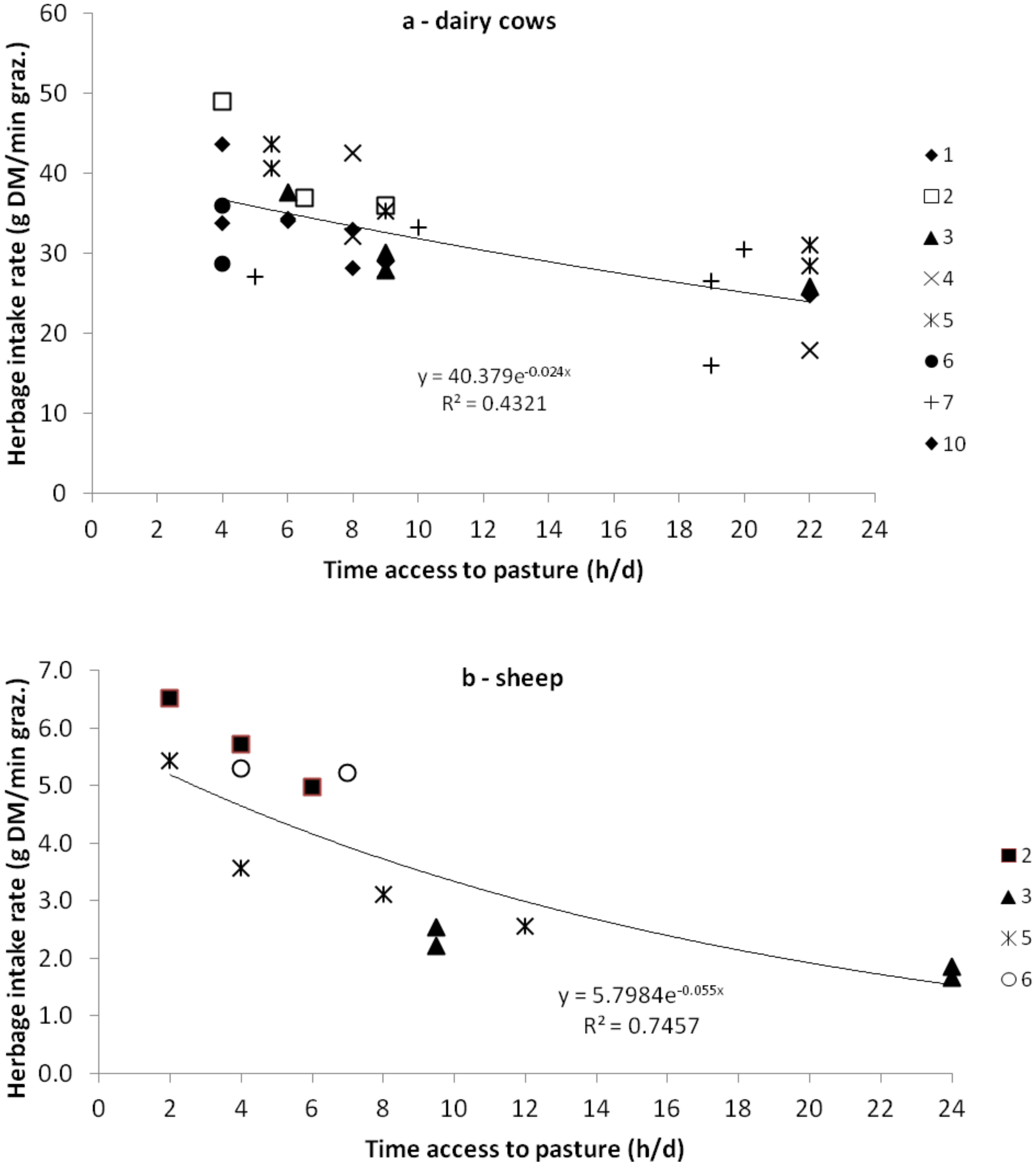


Figure 5. Herbage intake rate in dairy cows (a) and sheep (b) submitted to part-time grazing. For details on the experiments see appendix 1.

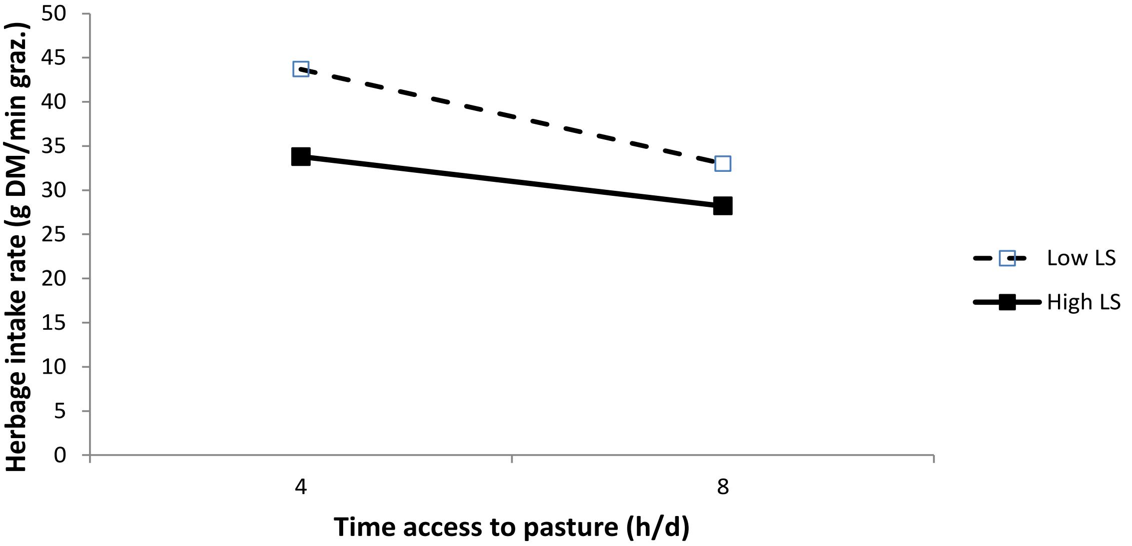


Figure 6. Effect of the level of supplementation on herbage intake rate in part-time grazing cows receiving either 5 (Low LS.) or 10 kg DM (High LS) of a maize silage- soyabean meal mixture(Perez-Ramirez et al., 2008).

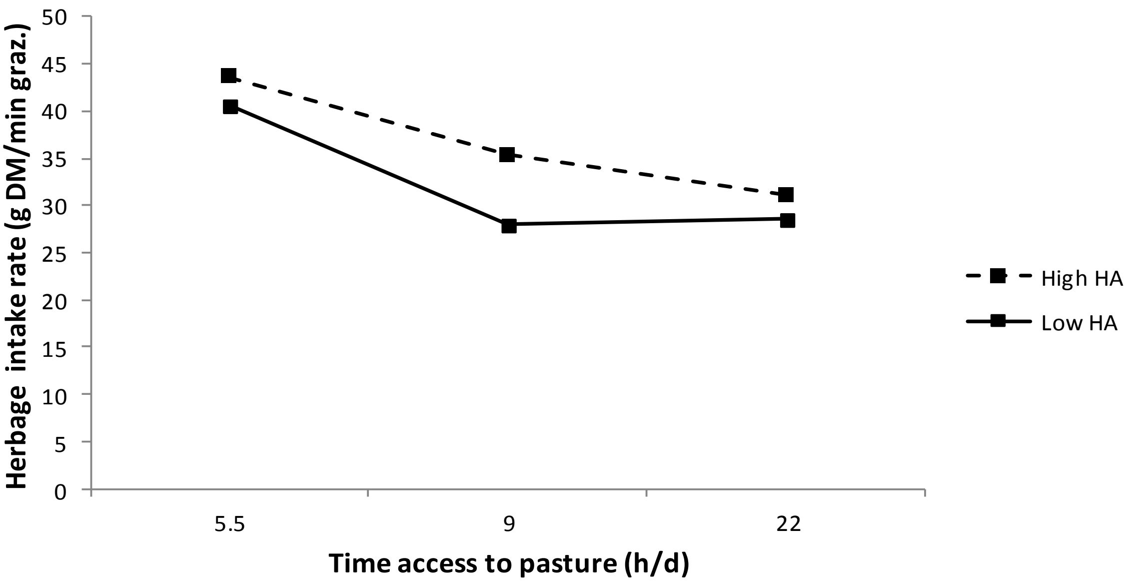


Figure 7. Effect of herbage allowance on herbage intake in part-time grazing dairy cows having either a high (24 kg DM/cow day) or a low (13 kg DM/cow day) herbage allowance at pasture (Perez-Ramirez et al., 2009).

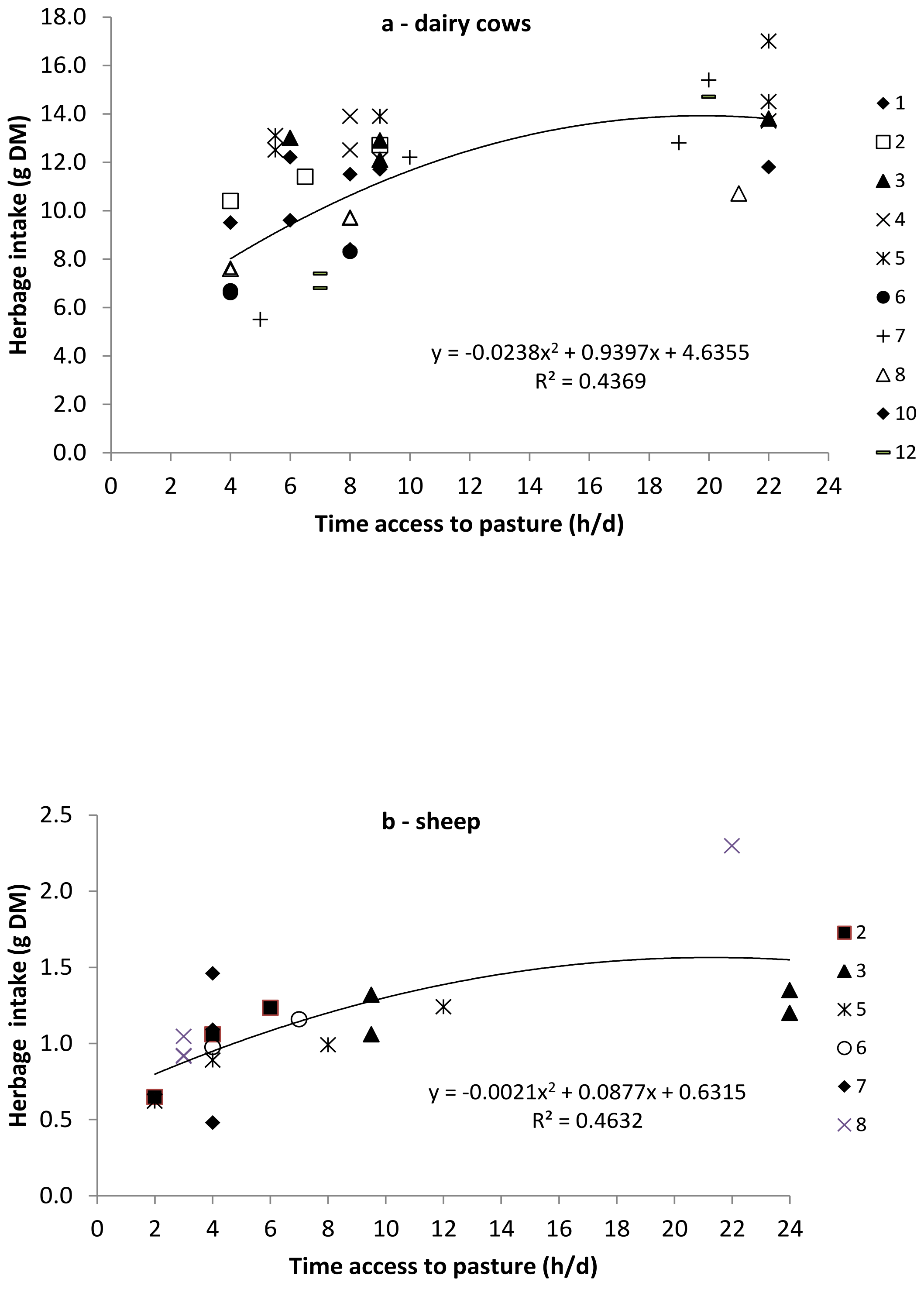


Figure 8. Herbage intake in dairy cows (a) and sheep (b) submitted to part-time grazing. For details on the experiments see appendix 1.

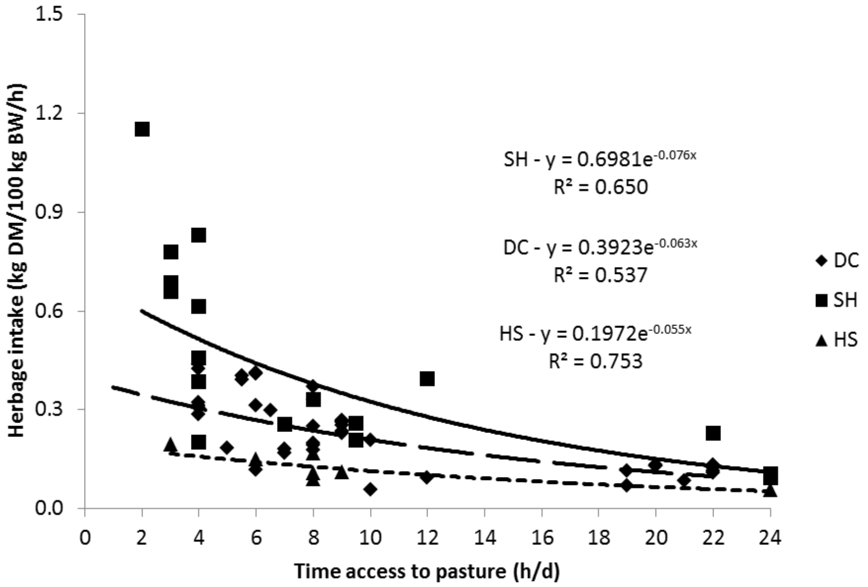


Figure 9. Relationship between the intake per hour of access time scaled to 100 kg BW ($HDMIh_{bw}$) and time access to pasture (h/day) in part-time grazing sheep (SH, continuous line, n = 17), dairy cows (DC, dashed line, n = 40) and horses (HS, dotted line, n=7). Mean data

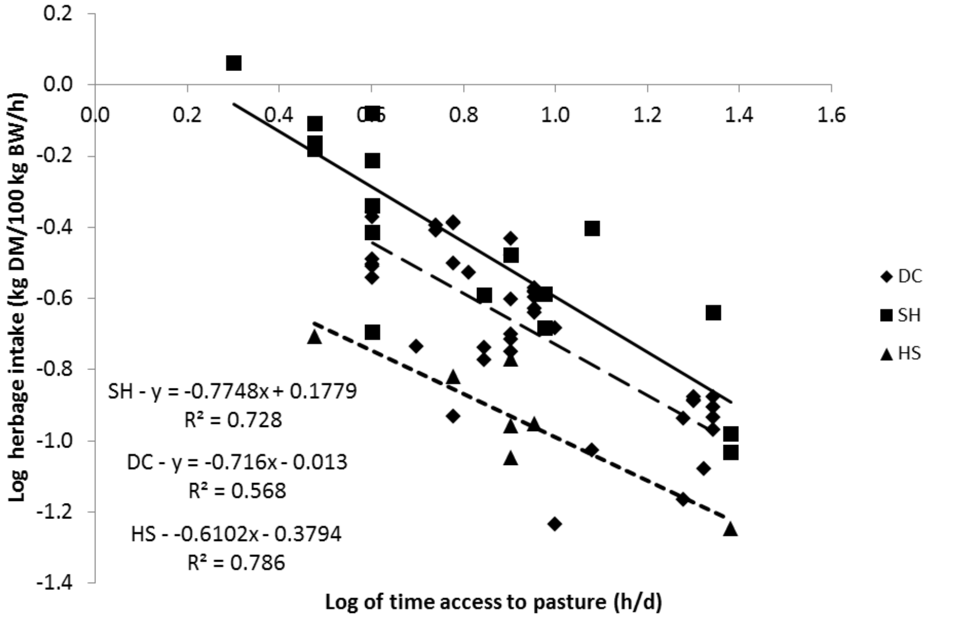
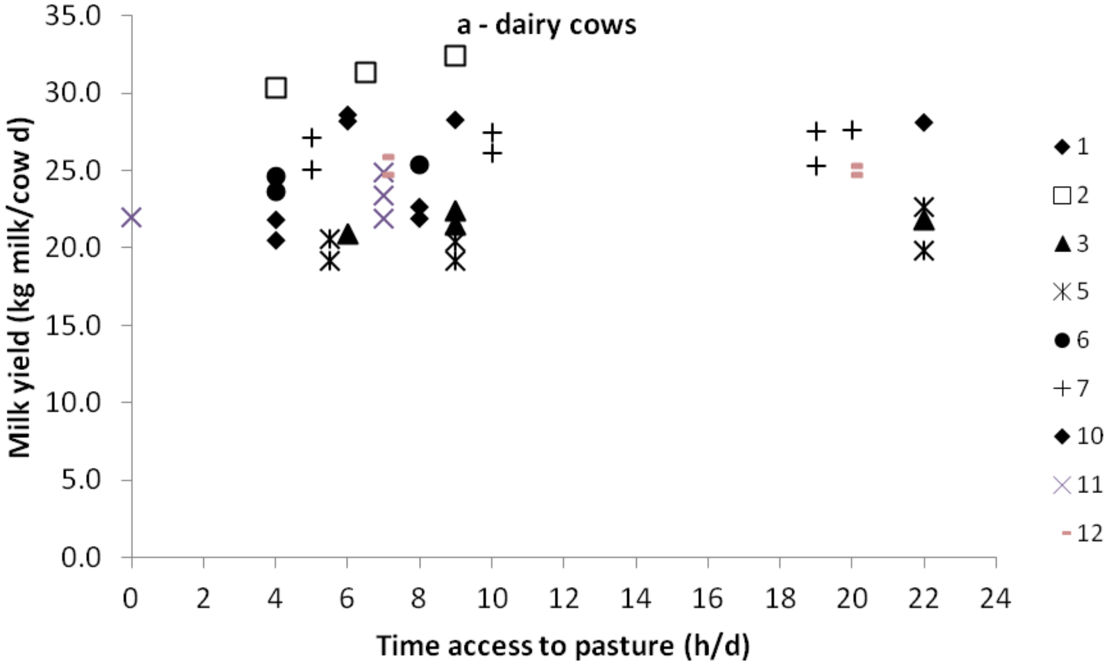


Figure 10. Regession of log transformed intake per hour of access time scaled to 100 kg BW ($HDMIh_{bw}$) upon log transformed time access to pasture in part-time grazing sheep (SH, continuous line, n = 17), dairy cows (DC, ashed line, n = 40) and horses (HS, dotted line, n=7). Regression coefficients are different among species at P<0.01. Mean data.



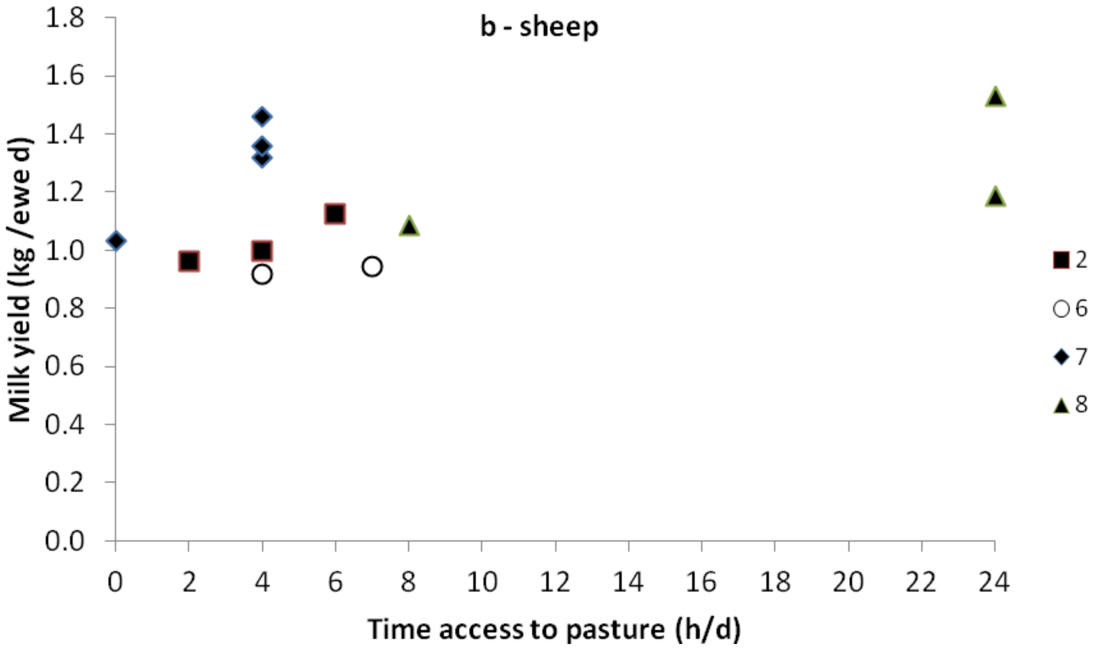
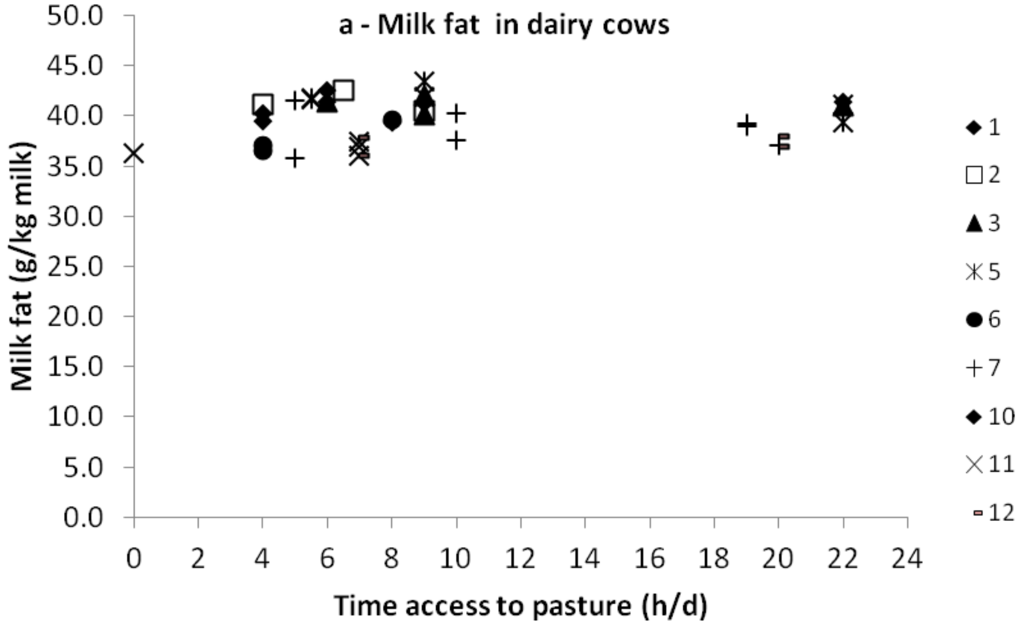


Figure 11. Milk yield in dairy cows (a) and milked ewes (b) part-time grazing. For details on the experiments see appendix 1



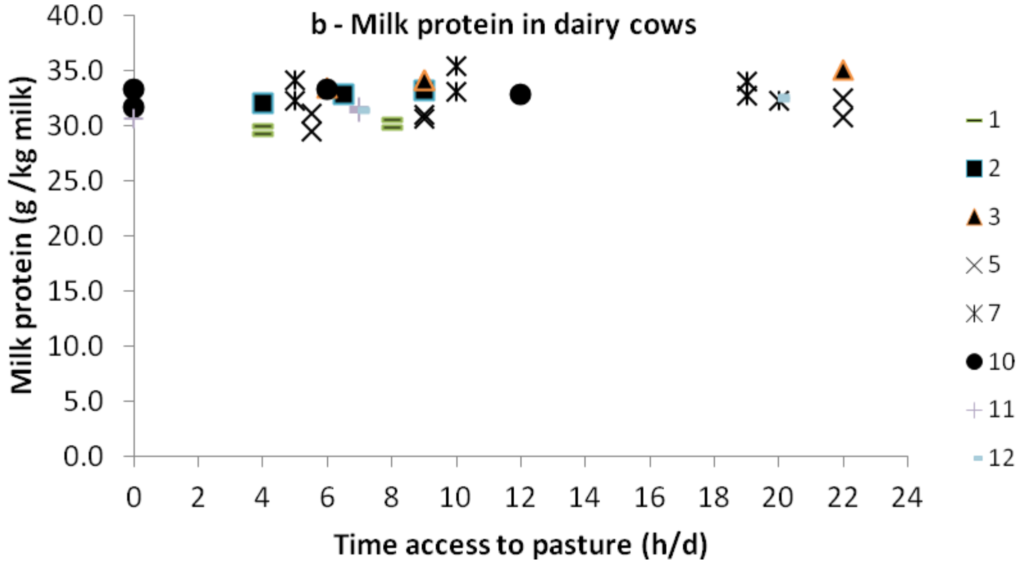
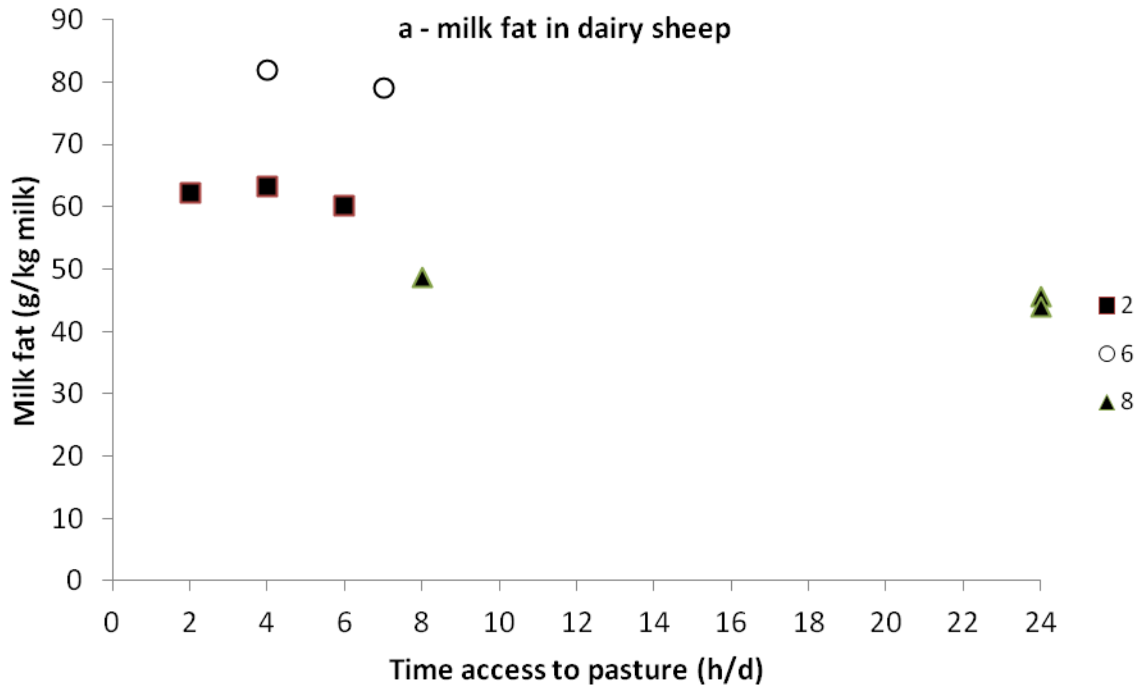


Figure 12. Milk fat (a) and milk protein contents (b) in dairy cows part-time grazing. For details on the experiments see appendix 1



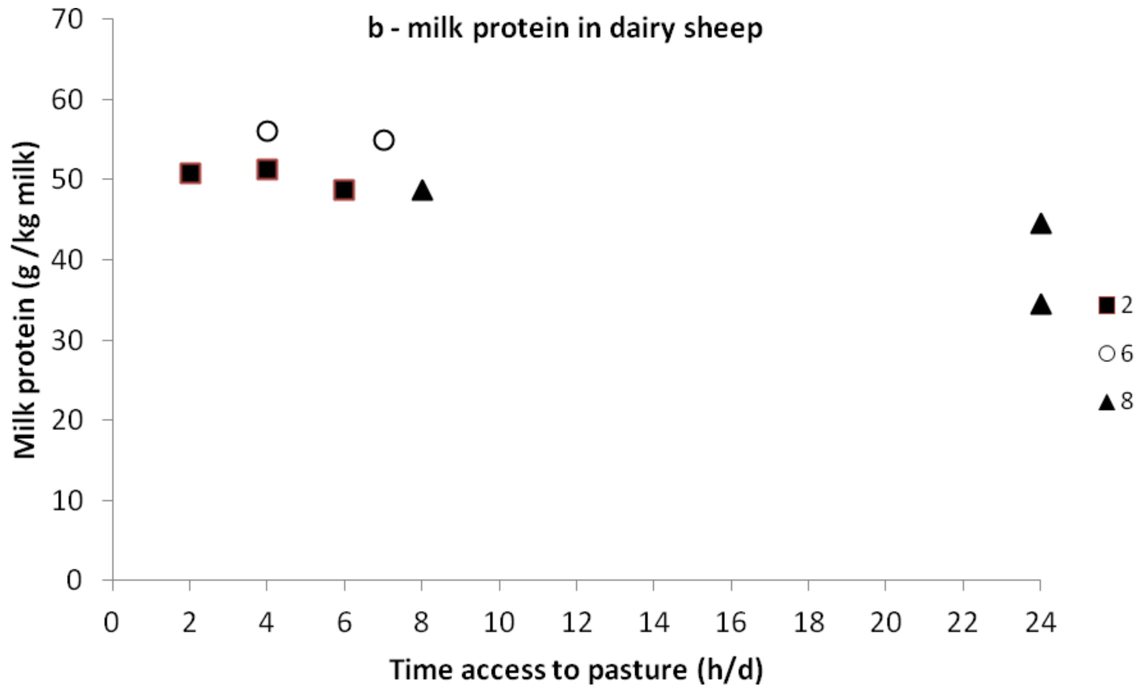


Figure 13. Milk fat (a) and milk protein contents (b) in dairy sheep part-time grazing. For details on the experiments see appendix 1

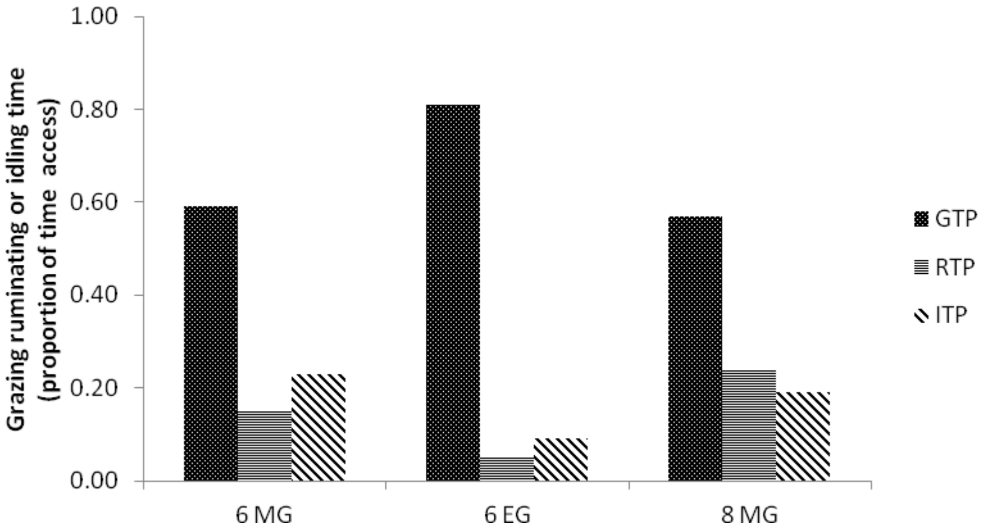


Figure 14. The effect of the timing at pasture in dairy cows grazing 8 h/d from 8 to 16 (8 MG), or for 3 x 2 h/d either during morning (6 MG) or evening hours (6 EG) on the time devoted to grazing (GTP), ruminating (RTP) and idling (ITP), (Mattiauda et al., 2013).

CHAPTER 2

Effects of restricted time allocation to pasture on feeding behaviour, intake and milk production of dairy sheep rotationally grazing Italian ryegrass (*Lolium multiflorum* Lam) in spring

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Effects of restricted time allocation to pasture on feeding behaviour, intake and milk production of dairy sheep rotationally grazing Italian ryegrass (*Lolium multiflorum* Lam) in spring

Abstract

The effects of restricted time allocation (2, 4 or 6 h/day) to pasture and grazing day (Day 1, initial; Day 4, intermediate; Day 7, final) on feeding behaviour, intake and performance were assessed in Sarda dairy ewes, rotationally grazing Italian ryegrass plots for 7 days, with 21 days of regrowth. A randomised block design with two replicates per access time was used with six groups of six ewes each. The ewes were supplemented daily with 400 g/head of a commercial concentrate at milking, 300 g/head of lupin after grazing and 700 g/head of ryegrass hay overnight. Pasture variables, feeding behaviour, herbage and supplement DM intake, and milk yield and composition were measured on 12 days (4 per target grazing day). Plot average data were analysed by a bifactorial model with interaction, which was not significant. Sward height and herbage mass decreased between Day 1 and Day 4 ($P < 0.05$). Leaf area index dropped from Day 1 to Day 7 ($P < 0.05$). Eating time, as proportion of access time, and intake rate were higher in 2 h/day groups than in the others ($P < 0.05$). Nevertheless, herbage and total intake were higher in 6 h/day than in 2 h/day groups, being 4 h/day groups intermediate ($P < 0.05$). Herbage intake decreased with grazing period ($P < 0.05$). Fat normalised milk yield was higher in 6 h/day groups than in the others ($P < 0.05$) and in Day 1 and Day 4 than in Day 7 ($P < 0.05$). To conclude, time restriction below 6 h/day and pasture depletion, in terms of herbage quality, constrained intake and performance of rotationally grazing dairy ewes.

1. Introduction

Restricting time allocation to pasture can tackle herbage shortage during periods of low herbage growth, provided that supplements are offered to fill the nutrient deficit, and can also increase the efficiency of herbage utilization by reducing trampling effect and curbing pollution (Clark *et al.*, 2010). Grazing cows submitted to restricted time access to pasture tend to compensate for the reduction in time allocated to pasture with an increase in intake rate and proportion of time devoted to grazing (e.g., Gregorini *et al.*, 2009), whereas data on sheep are scanty. Orr *et al.* (2001) studied meal duration restriction from 120 min to 15 min in meat sheep grazing either perennial ryegrass or white clover under non limiting sward height conditions. The authors found that the shorter the meal duration the higher the proportion of the time devoted to eating, so that intake rate did not change with meal duration in ryegrass or clover pastures. Iason *et al*. (1999) evaluated access time restriction from 24 h/d to approximately 8 h/d in lactating ewes continuously stocked on a permanent grassland at 6 cm or 3 cm of sward height, and found a significant decrease in herbage intake only in the ewes grazing the shorter sward. Information on the effects of time restriction and its interaction with pasture availability and quality in dairy sheep grazing Mediterranean forages is limited, as reviewed by Molle *et al*. (2004a).

The objective of this study was to assess the effect of time allocation to pasture and pasture availability and quality on feeding behaviour, intake and performance of dairy ewes rotationally grazing Italian ryegrass (*Lolium multiflorum* Lam) in spring.

2. Materials and methods

Experimental design

A randomized block design was used with two replicates per treatment. The treatments were: 2-hour access to pasture (2 h/d), approximately from 0800 to 1000 Central European Time (CET); 4-hour access to pasture (4 h/d), approximately from 0800 to 1200 CET; and 6-hour access to pasture (6h/d), approximately from 0800 to 1400 CET. The grazing day within grazing period (initial day, d1; intermediate day, d4; and final day, d7), an indicator of pasture depletion in terms of herbage quantity and quality, was used as blocking factor. Factors were studied on a balanced set of 12 test-days between 23 March and 30 April 2013.

Experimental site

The study was conducted at the Bonassai research station, in north-western Sardinia (40° N, 8° E, 32 m a.s.l.), from 21 February to 30 April 2013. The climate is Mediterranean with a long-term (1995-2013) average annual rainfall of 568 mm.

Pasture

On 24 October 2012, 1.5 ha of Italian ryegrass (*Lolium multiflorum* Lam. cv. Teanna) were seeded after conventional tilling. Fertilization at sowing consisted of 100 kg/ha of 18 N-46 P-0 K. Seeding rate was 42 kg/ha. No fertilizers were applied after sowing. Pasture was split into 2 blocks of 7500 m^2 each, which were in turn divided into three experimental plots. Each plot was then divided into 4 sub-plots of 625 m^2 each by electric fences and randomly allocated to the treatments. Pasture subplots were rotationally grazed with 7 days of occupation per sub-plot and a recovery period of 21 days. In order to standardize residual sward height, spare sheep were allowed to graze the sub-plots to 3-5 cm sward height after the grazing of the experimental groups.

Animals

Thirty mature and six two-year-old Sarda ewes, previously treated against gastro-intestinal parasites, were selected from the farm flock. On 21 February 2013 the ewes were weighed (mean ± s.d., 42.5 ± 4.0 kg) and their milk yield (1449 ± 206 g/head.d) was measured. From February 22 to March 11 the animals were managed as a flock and adapted to the experimental routine, grazing for 4 h/d and receiving concentrates (700 g/d of a commercial concentrate split into three meals) and ryegrass hay as supplements. On March 12, the sheep were subdivided into six groups, balanced for age and for the pre-experimental measurements and randomly assigned to the experimental plots. During the experimental period, the sheep were machine milked twice daily at 0700 and 1500 h. After the morning milking the groups were carried on a trailer to the plots where they spent the scheduled time. During the remaining daytime the ewes were kept indoors in separate pens. Supplementation consisted of the same concentrate as above (400 g/head.d split into two meals at milkings), 300 g/head.d of lupin seeds after grazing and 700 g/head.d of a ryegrass hay overnight.

Measurements

On each test-day, sward height was measured using a weighted-plate grass-meter (20 measurements/sub-plot) and herbage mass was determined cutting 4 quadrats of 0.5 m^2 per sub-plot. In addition, on four occasions (two on d4 and one on d1 and d7), pasture botanical composition, sown species structure (leaf lamina, stems, spikes and dead matter, as proportion on DM basis) and leaf area index (LAI) were measured on sub-samples of the herbage mass, and leaf lamina mass (g DM/m^2) was then computed. Furthermore, samples of hand-plucked herbage from each grazed sub-plot and of the supplemented feed were taken. Supplement samples were pooled before further processing. All these samples were oven-dried at 65°C and subsequently ground to pass a 1-mm screen to determine the content of DM, CP, NDF, and in vitro DM digestibility (IVDMD) by near infra-red spectrometry (NIRS) for herbage (N = 72) and ryegrass hay (N = 4), or by conventional analyses (AOAC, 1990) for concentrate and lupin seeds (N = 3 for each).

On each test-day, short-term intake rate was measured on 3 ewes per group using the double-weighing technique (Penning and Hooper, 1985). Briefly, herbage intake rate (g /min grazing) was measured weighing the ewes on an electronic scale with a precision of 5 g (Multirange, Mettler Toledo, Novate Milanese, Italy) before and after approximately 1 hour of grazing in the first hour of access to pasture in all groups (IR for groups 2 and 4h/d, and IR_{h1} for group 6h/d) and in the last access hour only in the 6-hour groups (IR_{h6}) to account for possible changes in insensible weight loss (IWL) during the afternoon hours. During each session, all ewes were dressed with disposable diapers to prevent faeces and urine losses. An additional ewe per group, equally dressed, was also harnessed with a muzzle, to simultaneously estimate IWL. In order to account for individual effects on IWL, the four ewes were rotated at each test-day to get a total of 3 measurements of IWL from each animal tested within group.

Feeding behaviour was monitored on the same 3 ewes used for intake rate measurements in each group. Three trained observers recorded the behaviour of 6 ewes, each ewe every 3 min. Feeding behaviour was classified as follows: grazing, if the animal was severing grass or chewing it, ruminating, if the animal was chewing or swallowing a rumination bolus, and idling, if no feeding activity was detected. Feeding behaviour data were accumulated per access hour and test-day before analysis in order to compute the time devoted to each activity (grazing time, GT; rumination time, RT; and idling time, IT) as total (min/24 h) and as proportion of access time. Herbage intake was then calculated as follows: i) HI (g/head d) = IR x GT (min/24 h) for groups 2 and 4 h/d; or; ii) HI = (IR_{h1} x GT_{h1-4}) + (IR_{h6} x GT_{h5-6}) for groups 6h/d, where GT_{h1-4} and GT_{h5-6} are the grazing times (min) in the first four and the last two hours of each test-day, respectively. Weighed mean intake rate of the 6 h/d groups (also indicated as IR) was then calculated as follows: IR (g/min grazing) = HI/(GT_{h1-4} + GT_{h5-6}). Group intake of each supplement was measured at each meal by weighing the offer and the orts. Total intake per group was then computed, summing up herbage and supplement intake.

Milk yield (MY) of the same 3 ewes tested for intake and feeding behaviour was weighed and milk was sampled during the afternoon and morning milkings, following each intake measurement. Milk composition (fat and protein, %) was assayed using the Fourier transformed infra-red method (FTIR, Milkoscan FT+, Foss electric, Hillerød, Denmark). Fat normalized milk yield (FNMY) was calculated according to Pulina *et al.* (1989).

Statistical analyses

All animal data were averaged by date and group before analysis. Data (N = 72 for all variables, with exception of sown species composition and LAI (N = 24)) were analyzed using a GLM model with access time (2, 4 and 6 h/d), grazing day (d1, d4 and d7) and their interaction as fixed effects. Means were separated by Tukey-Kramer t-test only when GLM effects were significant at $P < 0.05$. No effect of interaction was detected for any of the variables studied.

3. Results

The access time did not affect any of the pasture variables (*P* > 0.05). Sward height and herbage mass decreased between d1 and d4 (from 20 to 14 cm and from 1.9 to 1.4 t DM/ha, respectively, *P* < 0.05) but no change was detected thereafter, being d7 (19 cm and 1.8 t DM/ha, respectively) not different from d1 and d4. Herbage on offer per ewe and day, based on herbage mass measurements on d1, did not differ among time access groups, being on average 2.77 (2h/d), 2.86 (4h/d), and 3.00 kg DM/head.d (6h/d, *P* = 0.94). The proportion of Italian ryegrass in the pasture (mean ± s.e., 0.95 ± 0.05) was not affected by the two factors under study (P > 0.10). In contrast, leaf lamina proportion (0.40 (d1) and 0.49 (d4) vs. 0.25 (d7), P < 0.05) and LAI (1.77 (d1) vs. 0.92 (d7), *P* < 0.05; 1.23 (d4), intermediate) clearly dropped along with herbage depletion. Leaf mass per area unit (g DM/m^2) was significantly higher on d1 than on d7 (80 vs. 41, *P* < 0.05), being d4 (58) intermediate.

The nutritional analysis showed a moderate nutritive value of the Italian ryegrass hay (mean ± s.d., CP: 72 ± 8 g/kg DM, NDF: 627 ± 17 g/kg DM, IVDMD: 592 ± 18 g/kg DM) and a standard composition for the concentrate (CP: 170 ± 4 g/kg DM, NDF: 353 ± 7 g/kg DM, IVDMD 774 ± 15 g/kg) and the lupin seeds (CP: 331 ± 5 g/kg DM, NDF: 308 ± 6 g/kg DM, IVDMD 892 ± 9 g/kg). Hand-plucked herbage composition was affected only by grazing day, with an increase in DM between d4 and d7 (179 vs. 202 g/kg, *P* < 0.05) being d1 intermediate (189 g/kg). A reduction of CP after d4 (149 (d1) and 157 (d4) vs. 119 (d7) g/kg DM, *P* < 0.05) and of IVDMD after d1 (852 (d1) vs. 792 (d4) and 781 (d7) g/kg, *P* < 0.05) were also detected. In contrast, NDF concentration increased along with the grazing period (440 (d1) vs. 466 (d4) vs. 493 (d7) g/kg DM, *P* < 0.05)

The access time to pasture affected the time devoted to grazing as proportion of access time (*P* < 0.001), with differences between 2h/d groups and the other groups (*P* < 0.05, Figure 1). Grazing time tended to increase between d1 and d7 (*P* < 0.11, Figure 1).

Also the proportion of rumination time changed with time access (*P* < 0.001) being significantly lower in 2h/d than in the other groups (0.06 (2h/d) vs. 0.13 (4h/d) and 0.18 (6h/d), *P* < 0.01). Idling time tended (*P* < 0.06) to be lower in the 2 and 4h/d treatment groups (0.04 for both) than in the 6h/d groups (0.06). The proportion of idling time was lower (*P* < 0.05) on d7 (0.03) than on d1 (0.06), being the d4 (0.04) not different from either groups. Intake rate displayed an opposite trend to access time, being the highest in 2h/d, the lowest in 6h/d and intermediate in 4h/d (*P* < 0.05, Figure 2). Grazing day also influenced this variable (*P* < 0.001) with intake rates decreasing along with the grazing period (*P* < 0.01 among grazing days, Figure 2).

Herbage intake was also affected by both factors under study, increasing with time allocation on pasture and decreasing with the grazing period, i.e. pasture depletion (Table 1). Interestingly, the ewes experiencing the shortest access time to pasture showed a higher intake of hay, although differences were small in absolute terms ($P < 0.05$, Table 1). No differences were detected in the intake of concentrates, which were eaten almost completely by all groups. In contrast, total intake was affected by both factors under study, with total intake values increasing with the access time to pasture and decreasing with the grazing period ($P < 0.05$, Table 1).

Milk yield and FNMY (1072 (6h/d) vs. 980 (4h/d) and 936 g/d (2h/d), $P < 0.01$) were higher in 6h/d than in the other groups, and in d1 and d 4 than in d7 (Table 1 for MY, ; 1054 (d1) and 1026 (d4) vs. 908 (d7) g/d, $P < 0.001$, for FNMY). Milk fat concentration was lower in the 6 h/d groups than in the 2h/d and 4h/d groups ($P < 0.05$, Table 1) and in d4 than in the other days ($P < 0.05$). Milk protein concentration was lower in the 6h/d groups than in the other groups ($P < 0.01$, Table 1) but was not affected by grazing day ($P > 0.1$).

4. Discussion

Sward height and biomass on offer were basically above the threshold for limiting herbage intake in grazing sheep (Hodgson, 1990). Herbage on offer was also on average above the level of intake expected in sheep, considering that the supplementation level was higher than 1.1 kg DM/head.d. The depletion of pasture in terms of sward height or herbage mass was not evident, possibly for an increase in pasture patchiness along with the grazing period. This lack of clear shortage of herbage on offer probably prevented the interaction between time restriction and grazing day from becoming significant. In contrast, the depletion of pasture clearly worsened both pasture structure and herbage chemical composition. The selected herbage was indeed of medium to poor quality, as evidenced by a markedly lower CP concentration than that usually observed in Italian ryegrass at growing stage (Molle *et al.,* 2008), possibly due to the low fertilization rate applied to the experimental plots.

Restricted time access to pasture and grazing day within the grazing period of the rotational scheme had a clear impact on the feeding behaviour of sheep. The shorter the access time the higher the proportion of time devoted to grazing, in line with previous data in meat (Orr *et al.*, 2001) and dairy ewes (reviewed by Molle *et al.,* 2004a). This proportion tended also to raise along with green biomass depletion, suggesting a trend towards an increase of selective grazing during the last days of the grazing period. Intake rate responded to the restriction of time allocation on pasture similarly to the proportion of grazing time; indeed, the ewes exposed to 2 h/d of access to pasture were more efficient than the counterparts allocated for 4 or 6h/d ($P < 0.05$), confirming the data from Iason et al. (1999). Intake rate tended to drop along with the grazing period, as previously found by Penning et al. (1994) in rotationally grazing lactating ewes. In that case, however, the grazing period was extended above 10 days and sheep tended to reduce their grazing time, expressing a “giving up” behaviour, in the last days. This effect was not found in the current study, possibly due to the lack of sward height or biomass constraints to intake.

Despite this, the marginally higher intake of hay in the groups submitted to the strictest restriction of access time (2h/d) was again a result of ewes’compensatory feeding behaviour. In this study the supplementation level was set to cover the average energy requirements of 4h/d groups and the protein requirements of all groups. Providing *ad libitum* access to hay could have resulted only in slightly different results from the ones observed, given the low quality of the hay on offer.

The rotational scheme brings about an oscillating trend in herbivore intake, which can sometimes result in a drop of nutrient supply below requirements, as highlighted by Molle *et al.* (2004b) in Sarda dry ewes rotationally grazing annual ryegrass (*Lolium rigidum* Gaudin). As observed in our study, this oscillation can negatively influence milk production., quite closely mirroring herbage and total intake data. Actually milk yield and FNMY were constrained by time restriction to pasture below 6 h/d. In Latxa dairy sheep grazing a perennial pasture for 7 or 4 h/d in two subsequent springs, milk yield, but not fat corrected milk yield, was lower in the groups experiencing the most restricted time allocation (Perojo *et al.*, 2005).

To conclude, time restriction to pasture below 6 h/d constrained intake, MY and FNMY of dairy ewes, despite the compensatory increase of the proportion of time devoted to grazing and intake rate which occurred in the groups with the shortest access time. The effect of pasture depletion was evident only with reference to herbage quality, constraining both intake and milk production, particularly in the last day of grazing period.

5. Acknowledgments

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Table 1 – Effects of access time (2, 4 and 6 h/d) and grazing day within a 7 d grazing period (d1, first, d4, intermediate, and d7, last day) on intake and performance of dairy sheep rotationally grazing Italian ryegrass.

Effects	Herbage intake g DM	Hay intake g DM	Concentrate [1] intake g DM	Total intake g DM	Milk yield g	Milk fat %	Milk protein %
Time on the plots (h/d)							
2	648 c	570 a	619	1837 c	961 b	6.23 a	5.08 a
4	1059 b	546 ab	606	2211 b	996 b	6.33 a	5.12 a
6	1233 a	533 b	612	2378 a	1126 a	6.02 b	4.87 b
Grazing day (d)							
1	1106 a	539	608	2253 a	1067 a	6.34 a	5.07
4	920 b	558	612	2090 b	1081 a	5.99 b	5.00
7	914 b	553	617	2083 b	935 b	6.21 a	5.01
Effects (*P* <)							
Access time	0.001	0.01	NS	0.001	0.001	0.01	0.001
Grazing day	0.01	NS	NS	0.05	0.001	0.001	NS
RMSE	217	40	25	221	137	0.30	0.21

[1] Sum of pelleted commercial concentrate and lupin seed intake. Lupin was totally consumed.

[a,b,c] Within column and effect, means followed by different letters differ ($P < 0.05$)

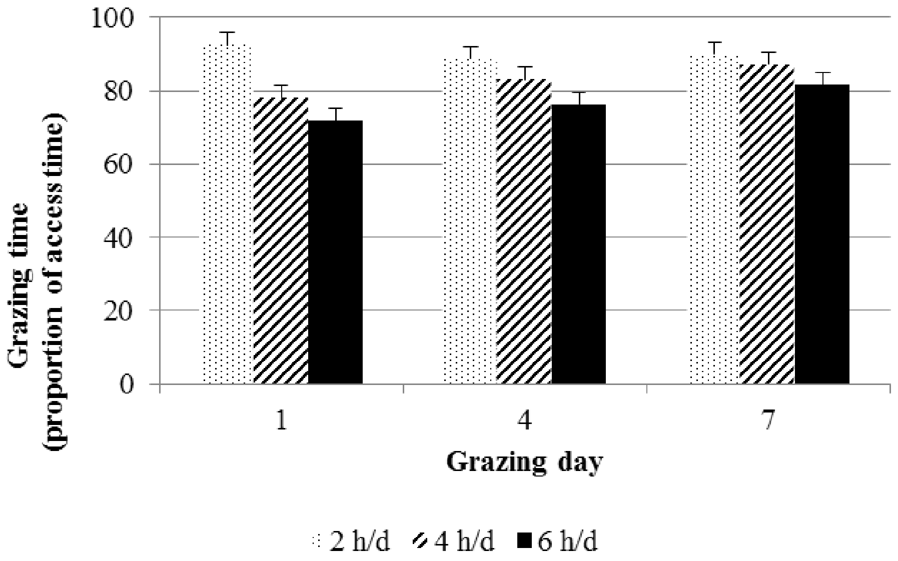

Figure 1. Effects of access time (2, 4 and 6 h/d) and grazing day within a 7 d grazing period (d1, first, d4, intermediate, and d7, last day) on grazing time in dairy sheep rotationally grazing Italian ryegrass. Means ± s.e..

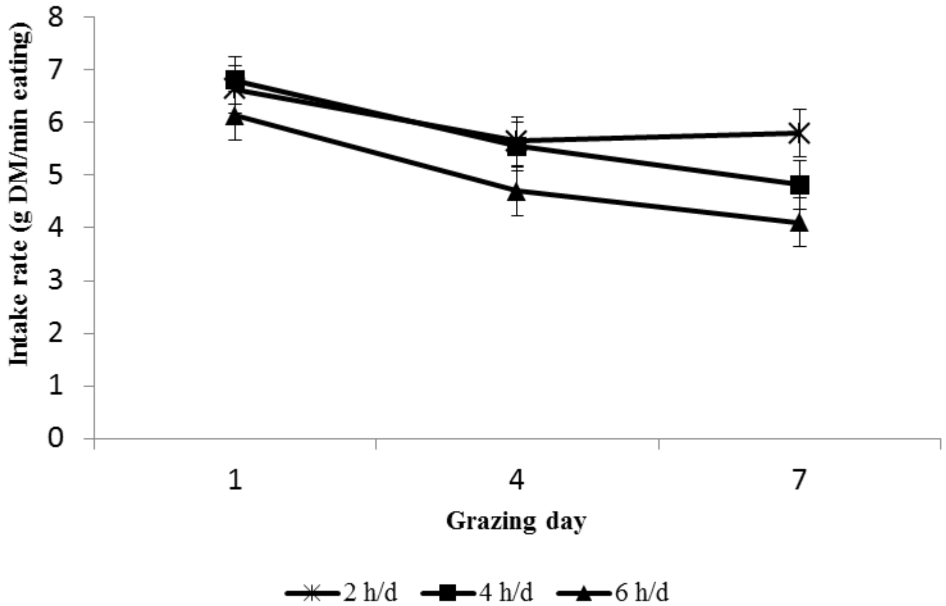

Figure 2. Effects of access time (2, 4 and 6 h/d) and grazing day within a 7 d grazing period (d1, first, d4, intermediate, and d7, last day) on intake rate in dairy sheep rotationally grazing Italian ryegrass. Mean ± s.e. .

CHAPTER 3

Restricted time allocation to *Trifolium alexandrinum* L. pasture in dairy sheep: effects on feeding behaviour, intake and milk production in spring

Restricted time allocation to *Trifolium alexandrinum* L. pasture in dairy sheep: effects on feeding behaviour, intake and milk production in spring

Abstract

The effects of restricted time allocation (2, 4 or 6 h/day) to pasture and grazing day (Day 1, initial; Day 4, intermediate; Day 7, final) on feeding behaviour, intake and performance were assessed in Sarda dairy ewes, rotationally grazing berseem clovers plots for 7 days, with 21 days of regrowth. A randomised block design with two replicates per access time was used with six groups of 4 core ewes each. The ewes were supplemented daily with 400 g/head of a commercial concentrate at milking, 300 g/head of maize after grazing and about 700 g/head of ryegrass-based hay overnight. Pasture variables, feeding behaviour, herbage and supplement DM intake, and milk yield and composition were measured on 12 days (4 per target grazing day). Plot average data were analysed by a bifactorial model with interaction. Sward height and herbage mass decreased between Day 1 and Day 7 ($P < 0.05$ for the former, $P < 0.12$ for the latter). Green leaf proportion and LAI dropped from Day 4 onwards ($P < 0.05$). Grazing time (GTP), as proportion of access time, was affected by the interaction between the factors, with higher GTP in 2 h/day ewes than in the counterparts, particularly on Days 1 and 4 ($P < 0.05$). Intake rate was also higher in the 2 h/day group ($P<0.01$), with only a slight decline along with pasture depletion ($P<0.09$ between Day 1 and Day 7). Nevertheless, herbage and total intake were higher in 4 and 6 h/day groups than in 2 h/day groups ($P < 0.01$). Herbage intake was affected by the day within the grazing period in a non-linear fashion ($P < 0.05$). Milk yield and fat normalised milk yield were higher in 4 and 6 h/day groups than in 2h/day groups ($P < 0.01$). No effect of grazing day on milk yield and composition was apparent, except for a drop in protein and casein content on Day 7 ($P<0.05$). To conclude, time restriction below 4 h/day can constrain intake and performance of dairy ewes rotationally grazing berseem clover. Pasture depletion, mainly in terms of herbage quality, interacted with access time on the feeding behaviour but the effects of this factor on ewe intake and performance were overall mild.

1. Introduction

Feeding is a time-limited process with a circadian rhythm. The timing of feed offer, the access time to the feed and the number and distribution of meals during the daytime and nighttime all impact on feeding behaviour and intake (Nikkhah, 2011a), particularly under grazing conditions in which animals are fully exposed to natural daytime photoperiod (Gregorini, 2012).

When access time to feed is unconstrained (usually more than 8-12 hours/day), animal chronophysiology dictates the feeding behavioural cues. In grazing animals with unconstrained time access to pasture, this usually results in two or three main meals during the daylight period (the main at dawn and at dusk (Gregorini, 2012).

Restricting time access to feed elicits an array of behavioural responses, which might possibly − but not necessarily − compensate for the time restriction. When ruminants, particularly grazing animals, fail to compensate the feeding time gap, intake and performance are often negatively affected (e.g. Kristensen et al., 2007). Despite these putative counter-effects, part-time grazing is a management technique often implemented in dairy cow and sheep farms for some of the following advantages it can brings about as compared with all-time grazing: i) sparing herbage when its growth is low, provided that supplements are fed to meet the nutrient deficit; ii) balancing ruminants diet when herbage nutritional composition is featured by excess or deficit of nutrients; iii) enhancing the efficiency of herbage utilisation by reducing animal pugging effect on wet soils; iv) curbing the emission of pollutants (e.g. Clark et al., 2010); v) reducing feeding costs per farm unit area, if stocking rate is increased.

The response to access time restriction to pasture has focused mainly on dairy cows (e.g. Gregorini et al. 2009a) and beef cattle, whereas data on small ruminants are still scanty. Iason et al. (1999) compared the feeding behaviour of lactating meat ewes with access time to pasture of either 24 h/day or around 9 h/day, finding a significant decrease in herbage DM intake in the ewes continuously stocked at 3 but not 6 cm sward height. In this case, an interaction between time restriction and pasture availability was detected, as also found in Boer goats grazing a ryegrass pasture in China (Zhang et al., 2008).

Data on part-time grazing in dairy sheep grazing Mediterranean forages is almost erratic, as reviewed by Molle et al., (2004a). However, some papers recently contributed to partially fill this gap of knowledge. In particular, de Renobales et al. (2012) compared stall-feeding with a 4 hours/day access to a permanent grassland inclusive of *Lolium perenne* and *Trifolium repens* in Laxta dairy ewes. They found that herbage DM intake was on average 1100 g DM per day and increased along with the decrease in lucerne hay supplementation level (480 g DM with a daily supplementation of 900 g of hay/ewe, 1090 g DM with 600 g of hay/ewe and 1460 g DM with 400 g of hay/ewe). More recently, Molle et al. (2014) found similar average herbage intake (1059 g DM) in Sarda dairy ewes rotationally grazing in spring plots of Italian ryegrass with 4 hours/day of access time to pasture. Furthermore, they found higher (1233 g DM) and lower (648 g DM) intakes in counterparts grazing 6 or 2 hours daily, respectively. The same authors were also able to detect a clear effect of pasture depletion during grazing period: intake was in fact lower from the intermediate day of the grazing period onwards. These response were additional to the ones of the time restriction, with no interaction between the factors under study.

The present work is a follow-up of the study above mentioned, but based on a much higher quality forage. Indeed, the objective of this experiment was to assess the effect of time allocation to pasture and pasture availability and quality during pasture utilization on feeding behaviour, intake and performance of dairy ewes rotationally grazing in spring a widespread Mediterranean legume: the berseem clover (*Trifolium alexandrinum* L).

2. Materials and methods

Experimental design

A randomised block design was used with two replicates per treatment. The treatments were: 2-h access to pasture (2 h/day), from ~ 0800 to ~ 1000 Central European Time (CET); 4-h access to pasture (4 h/day), from ~ 0800 to ~ 1200 CET; and 6-h access to pasture (6 h/day), from ~ 0800 to ~ 1400 CET. The grazing day within grazing period (initial day, Day 1; intermediate day, Day 4; and final day, Day 7), an indicator of pasture depletion in terms of herbage quantity and quality, was used as blocking factor. Factors were studied on a balanced set of 12 test-days between 11 March and 22 April 2014.

Experimental site

The study was conducted at the Bonassai research station, in north-western Sardinia, Italy (40°N, 8°E, 32 m a.s.l.), from 28 February to 30 April 2014. The climate is Mediterranean with a long-term (1995–2013) average annual rainfall of 568 mm.

Pasture

On 29 November 2013, 1.5 ha of berseem clover (*Trifolium alexandrinum* L, cv. Laura) were seeded after minimum tillage. Fertilisation at sowing consisted of 150 kg/ha of 18 N-46 P-0 K. Seeding rate was 40 kg/ha. No fertilisers were applied after sowing. Pasture was split into two blocks of 7500 m^2 each, which were in turn divided into three experimental plots. Each plot was then divided into four subplots of 625 m^2 each by electric fences and randomly allocated to the treatments. Pasture subplots were rotationally grazed with 7 days of occupation per subplot and a recovery period of 21 days. In order to standardise residual sward height, spare sheep were allowed to graze down the subplots to about 8-10 cm sward height after the grazing of the experimental groups.

Animals

On 28 February 2014 thirty-six mature Sarda ewes, previously treated against gastro-intestinal parasites, were selected from the farm flock. On March 4 and 5 the ewes were weighed (mean ± s.d., 41.8 ± 4.1 kg) and their milk yield (MY) (1952 ± 127 g/ewe day) was measured. From February 28 to March 10 the ewes were managed as a flock and adapted to the experimental routine, grazing for 4 h/day on spare berseem clover paddocks and receiving concentrates (400 g/ ewe day of a commercial concentrate split into two meals at milkings and 300 g/ewe day of maize after grazing) and ryegrass hay as supplements (700 g/ewe day). Since preliminary analysis of the ryegrass hay showed a lower CP content (CP = 5 % DM) than expected, on March 4 ryegrass hay was partially replaced by lucerne hay (200 g/ewe day) in order to meet the expected energy and CP requirements of sheep (see below).

On 10 March, the sheep were subdivided into six groups, balanced for age and for the pre-experimental measurements and randomly assigned to the experimental plots. However, since the estimated pasture biomass on offer was below expectations, the group size was prudently reduced to four ewes per group in order to avoid a marked constraint to herbage intake at the end of grazing period. Final 4-ewe groups were also homogeneous for the criteria above mentioned. During the experimental period, stocking density was adjusted according to herbage mass changes, using the so called "put and take" approach (Table 1), being the core ewes (4 ewes per group) submitted to treatments throughout all the experiment with exception of one ewe that exited the experiment for mastitis and was replaced by a counterpart approximately of the same age, body weight and milk yield (MY).

The ewes were machine milked twice daily at 0700 hours and 1500 hours. After the morning milking, the groups were carried on a trailer to the plots where they spent the scheduled time. During the remaining daytime the ewes were kept indoors in separate pens. Supplementation consisted of the same concentrate as above (400 g/ewe day split into two meals at milkings), and 300 g/ewe day of whole maize after grazing. Additionally, they were fed 500 g/ewe day of ryegrass hay and 200 g/ewe day of lucerne hay overnight.

The hay amount was raised to 600 g/ewe day of ryegrass hay and 250 g/ewe day of lucerne hay from the beginning of the second grazing rotation (April 8) up to the end of the experiment, due to the extremely low refusal rate (basically nil) detected in the most time restricted groups (2 h/day).

The experimental supplementation level and composition was set by using Small Ruminant Nutrition System package (Version 1.9) for diet formulation (Cannas et al., 2004; Tedeschi et al., 2010) to balance the expected energy requirements of the intermediate access- time groups (4 h/day) and the expected CP requirements of all treatment groups. The intake of herbage was supposed to be equal to 1.5 times that measured during the previous experiment in the corresponding access-time groups grazing Italian ryegrass (Molle et al., 2014).

Measurements

On each test-day, sward height was measured using a weighted-plate grass-meter (20 measurements/subplot) and herbage mass was determined cutting four quadrats of 0.5 m^2 per subplot. In addition, pasture botanical composition, sown species structure (leaf, stems, inflorescences and dead matter, as proportion on DM basis) and leaf area index (LAI, by an electronic leaf area meter, LI-3100 area meter, LI-COR, 4421 Lincoln, NE US) were measured on subsamples of the herbage mass. Then specific leaf area (cm^2/g DM), leaf lamina and stem mass (t DM/ha) and total and green herbages allowance were computed. Furthermore, on each test-day herbage samples were hand-plucked from each grazed subplot, mimicking the grazing behaviour of the ewes. Supplement feed samples were also taken and pooled before further processing. All these samples were oven-dried at 65°C and subsequently ground to pass a 1-mm screen to determine the content of DM, ash, ether extract (EE) and crude protein (CP, AOAC, 1990), neutral detergent fibre (NDFom for forages or aNDFom for concentrates), acid detergent fiber (ADFom) and acid detergent lignin (ADL, Van Soest, et al., 1991) and *in vitro* DM digestibility (IVDMD, pespsine-cellulase method, Aufrere and Demarquilly, 1989). In addition water soluble carbohydrates (WSC) were measured in herbage samples (Deriaz, 1961). The total number of samples was: N = 72 for the herbage, N = 5 for each hay (ryegrass and lucerne) and N = 4 for each concentrates (commercial concentrate and maize). The chemical composition of the feedstuffs offered as supplements is shown in Table 2.

On each test-day, short-term intake rate was measured on three ewes per group using the double-weighing technique (Penning and Hooper 1985). Briefly, herbage intake rate (g/min grazing) was measured weighing the ewes on an electronic scale with a precision of 5 g (Multirange, Mettler Toledo, Novate Milanese, Italy) before and after about 1 h of grazing in the first hour of access to pasture in all groups (IR for groups 2 and 4 h/day, and IR_{h1} for group 6 h/day) and in the last access hour only in the 6-h groups (IR_{h6}) to account for possible changes in insensible weight loss (IWL) during the afternoon hours. During each session, all ewes were dressed with disposable diapers to prevent faeces and urine losses. An additional ewe per group, equally dressed, was also harnessed with a muzzle, to simultaneously estimate IWL. In order to account for individual effects on IWL, the four ewes were rotated at each test-day to get a total of three measurements of IWL from each animal tested within group.

Feeding behaviour was monitored on the same three ewes used for intake rate measurements in each group. Three trained observers recorded the behaviour of six ewes, each ewe every 3 min, according to Hirata et al. (2002) . Feeding behaviour was classified as follows: grazing, if the animal was severing grass or chewing it, ruminating, if the animal was chewing or swallowing a rumination bolus, and idling, if no feeding activity was detected. Feeding behaviour data (the product of the number of records by the recording frequency (3 min)) were summed up per access hour and test-day before analysis in order to compute the time devoted to each activity (grazing time, GT; rumination time, RT; and idling time, IT) as total (min/day) and as proportion of access time, indicated as GTP, RTP and ITP, respectively. Herbage intake was then calculated as follows: (i) HI (g/head day) = IR × GT (min) for groups 2 and 4 h/day; or; (ii) HI = (IR_{h1} × $GT_{h1–4}$) + (IR_{h6} × $GT_{h5–6}$) for groups 6 h/day, where $GT_{h1–4}$ and $GT_{h5–6}$ are the grazing times (min) in the first four and the last two hours of each test-day, respectively. Weighed mean intake rate of the 6 h/day groups (also indicated as IR) was then calculated as follows: IR (g/min grazing) = HI/($GT_{h1–4}$ + $GT_{h5–6}$). Group intake of each supplement was measured at each meal by weighing the offer and the orts. Total intake per group was then computed, summing up herbage and supplement intake.

In addition to direct observations, on one occasion per target day (April 15 (Day 1), 18 (Day 4) and 22 (Day 7)) the behaviour of the ewes used for intake measurement was video-recorded during the first hour of grazing for about 2-5 min/ewe using a handy-cam (Sanyo VPC-TH1). Groups were monitored according to the order of entry in the subplots, that was randomised among treatments. Video files MP4 were then uploaded to a PC and analysed to count the number of bites and compute the bite rate of ach animal. Bite mass (BM) was then calculated for each ewe, by dividing the intake rate measured in the first grazing hour by the bite rate.

Milk yield (MY) of the same three ewes tested for intake and feeding behaviour was weighed and milk was sampled during the afternoon and morning milkings, following each intake measurement. Milk composition (fat and protein, %) was assayed using the Fourier-transformed infrared method (Milkoscan FT+, Foss Electric, Hillerød, Denmark). Fat normalised milk yield (FNMY) was calculated according to Pulina et al. (1989).

The body weight (BW) was also measured on the initial and final experimental day (March 11 and April 30) in all the ewes submitted to the treatments during all the experiment, except for the first week, when 8 of them (two per each group 4 h/d and 6 h/d) were managed for one week (Table 1) according to the pre-experimental management (4 h/d instead of 2 or 6 h/d). For BW measurement a standard animal scale (Gallagher Europe Bv, Adorp, The Netherlands.), with an approximation of about ±100 g, was used. Furthermore on the same dates, the body condition score (BCS, Russel et al.,

1969) ranging from 1 (extremely thin) to 5 (obese) was estimated by two trained evaluators with an approximation of 0.25 BCS unit. Their scores were averaged prior to data analysis.

The net energy for milk production (NE_L, Mcal/ewe per day) of each feedstuff and thus the intake of NE_L (Mcal) as well as sheep requirements (Mcal/ewe per day, not shown) were calculated by using the equations suggested by Cannas, (2004). In particular, the total digestible nutrients at maintenance level (TDN_m) was calculated for each feed using the following equation:

TDN_m = DMD – ash + (1.25 EE) + 1.9 (Van Soest, 1992)

where DMD (%) is the in vivo DM digestibility. This was in turn calculated using a regression of in vivo DM digestibility coefficients upon corresponding IVDMD (%) values computed on the basis of our laboratory results on dry non-pregnant Sarda sheep:

DMD = 1.08 IVDMD – 9.7674 (n=5, R^2 = 1.00, RMSE = 0.00)

The discount factor (Van Soest et al., 1992) was applied setting the level of feeding equal to 4 times the maintenance requirements to account for the difference between sheep and cattle in anatomy and digestive physiology (Cannas et al., 2007).

Energy requirement were computed on the basis of BW, MY, milk composition recorded on each testing day according to INRA system (INRA, 1989). Allowance for dynamic activity (basically walking) was estimated as a steady proportion of maintenance requirements (i.e. 0.2), irrespective of treatments. Total intake of NE_L was calculated as the sum of the energy intake from each feed, with no allowance for putative associative effects related to diet composition. Energy balance was then calculated as the difference between the intake and the requirements of NE_L. Finally, efficiency of energy and N utilization were calculated by dividing the milk output of energy (1.030 Mcal / kg of 6.5 % fat normalized milk, Cannas, 2004) and N by their respective input (dietary intakes).

Statistical analyses

All animal and pasture data were averaged by date and group or plot before analysis. Data (N = 72) were analysed using a GLM model with access time (2, 4 and 6 h/day), grazing day (Day 1, Day 4 and Day 7) and their interaction as fixed effects. Hourly data of feeding behaviour activities, expressed as proportion of the hour, were analysed by the same model only in the hours 9, 10, 11, and 12 comparing all treatment groups, in the hours 9 and 10 and only 4 h/d and 6 h/d groups in the following hours.

Group average BW and BCS (n = 6) were analysed by a GLM with the treatment as the only fixed factor.

Means were separated by Tukey–Kramer *t*-test only when GLM effects were significant at $P < 0.05$.

3. Results

Pasture characteristics

No effect of access time was detected on any of pasture variables (P > 0.15, Tables 3 and 4) with the exception of the percentage of weeds in herbage mass, which was higher in 2 and 6 h/d than in 4 h/d subplots (Table 3). Weeds consisted of annual ryegrass and unpalatable dicots such as *Veronica* spp. *Papaver* spp., and *Sylibum marianum* L.. There was also a trend to higher specific leaf area in the 4 h/d than 6 h/d subplots (P < 0.05).

In contrast, the grazing day within grazing period affected most of pasture variables. As expected, sward height, herbage mass and herbage and green herbage allowance all numerically decreased along with the grazing period although only sward height decreased linearly and significantly (P<0.01) from Day 1 to Day 7 (Table 3). The percentage of green leaves decreased and those of green stems and dead matter arose from day 4 onwards along with pasture depletion (P<0.05, Table 4). A decline was evident during grazing period for the number of stems per plant and leaf to stem ratio (P<0.001, Table 4). Specific leaf area was unaffected by treatments, whereas LAI trend mirrored that of leaf percentage, with last day featured by a much lower LAI than first and intermediate days of grazing period (P<0.01, Table 4).

Herbage chemical composition and its nutritional value

The restriction of access time to pasture resulted in higher CP and EE levels in the herbage hand-plucked samples of 2h/d groups as compared with counterparts with access time of 4h/d (P<0.05, for CP), or all the less time restricted groups (P<0.05, for EE, Table 5). The grazing day affected most chemical variables with a decrease in CP and EE on the intermediate and final grazing days of the grazing period and, with higher DM and lower fiber and higher WSC contents on the intermediate- than in the first or last grazing days (P<0.01, Table 5). On the whole, these changes resulted only in a very mild decline of IVDMD from the first to the last grazing day (from 85.0 (D1) to 83.6 % (D7), P<0.07, Table 5).

Feeding behaviour at pasture and herbage intake rate

As expected, the measured access time differed among the treatments but a slight discrepancy was noted between planned and actual times (Table 6). In fact the 6 hour treatment tended to have a slightly shorter access time than planned (32 min corresponding to 9% less than planned). This happened as a result of a shorter duration of access in a couple of days, due to high risk of rain in the afternoon hours. Rain in fact can bias significantly the double weighing procedure. Access time on the plots affected all behavioural variables (Table 6). The time devoted to grazing as percentage of total access time (GTP) decreased almost linearly from 2 to 6 h/d groups and increased from Day 1 onwards in response to pasture depletion. The interaction was significant at P <0.001, as shown in Figure 1. It can be noticed that only the groups having access to the plots for more than 2 h/d reacted to the decrease of pasture accessibility and quality increasing their GTP (P<0.01). The percentage of access time devoted to rumination (RTP) was significantly lower in 2 and 4 h/d groups than in the least time-restricted counterparts (P<0.001, Table 6, Figure 1) without any effect of the grazing day. In contrast, the time devoted to idling as percentage of access time (ITP) displayed an opposite trend to that of GTP as shown in Figure 1 (interaction significant at P<0.001). The ewes idled more on treatment 6 h/d and on the first grazing day (Table 6). Then idling time declined but this occurred mainly in the less severely time constrained ewes (Figure 1).

Herbage DM intake rate (HDMIR, Figure 2) was affected by access time (P < 0.001) and tended to be affected by grazing day (P <0.09), with no significant interaction (P>0.15). Interestingly, it dropped linearly from 2 to 6 h/d access time, averaging 10.2 (2 h/d), 8.9 (4 h/d) and 7.3 g DM/min grazing (6 h/d) groups (P<0.01 among groups). The trend of HDMIR along with pasture utilization was as follows: 9.1 at Day 1, 9.0 at Day 4, and 8.3 g DM/min grazing at Day 7, with P = 0.08 for the comparison between the extreme grazing days.

The pattern of feeding behaviour during the grazing session is shown in Figure 3. Up to 10 h in the morning, only the effect of access time impacted on GTP and RTP, with higher and lower proportions in 2 h/d groups, respectively (for GTP, P<0.06 at 9 and P<0.01 at 10, for RTP, P<0.01 at both hours). In the following hours (11 and 12), even the grazing day affected GTP and ITP, with an increase of GTP and a decrease of ITP along with the grazing period (P<0.01 for both variables at both hours, with the exception of GTP at 12: P<0.06). In the above hours 4 h/d ewes grazed more and ruminated less than 6 h/d ewes (P<0.05).

Interestingly, the bite rate measured in the last intake measurement week, showed an increasing trend with pasture depletion, without changes associated to time access to pasture (Figure 4). In contrast, BM was affected by the treatment, being significantly higher in 2 h/d than 6 h/d (P<0.05) but it was not responsive to the grazing day effect (P>0.05, Figure 4).

Intake of herbage and supplements and estimated diet composition

Herbage DM intake (HDMI) was significantly lower in 2 h/d than in the less restricted time access-groups ($P<0.001$, Table 7). Unexpectedly HDMI did not decrease linearly during grazing period with an increase on Day 4 as compared to Day 1 ($P<0.05$). Herbage intake per hour of access time was higher in the 2h/d group as a result of the feeding behaviour already described (585, 458, and 316 g DM/h for 2, 4 and 6 h/d groups, respectively, $P<0.001$ among groups).

The lucerne hay was almost completely consumed. Orts of ryegrass hay were on average higher than 20% in all groups. Total hay intake decreased with time access to pasture ($P<0.05$), and decreased also between Day 1 and Day 4, being Day 7 undifferentiated from either (Table 7). Concentrate intake was not affected by access time but tended to be affected by the grazing day ($P = 0.045$) with a slightly lower intake on Day 4 ($P< 0.06$,as compared to Day 1 and $P<0.09$ as compared to Day 7, Table 7). In contrast, total DM intake (TDMI, g/d) and DMI as percent of BW (TDMIBW, % of BW), both measured in the same day, were affected by the access time, with higher levels in 4 and 6 h/d groups than in 2 h/d groups (Table 7). No effect of grazing day or interaction was detected on this variable. The intake of CP and NE_L were similarly affected by the factors under study, with 2 h/d displaying lower values for both variables (Table 7).

Estimated diet composition was featured by lower levels of ash and CP and slightly higher levels of NDF ($P<0.08$) and ADF ($P<0.05$) in the ewes submitted to the most severe access time restriction (Table 8). The grazing day tended to affect all variables, with a decrease in dietary CP ($P <0.01$) and IVDMD ($P<0.07$) contents and an increase of fiber contents, although the latter was not linear, being Day 4 featured by a diet with lower fiber level than Day 1 and 7 (Table 8).

Milk yield and milk composition

Similarly to total intake, milk yield was affected by the access time to pasture with 2 h/d being lower than the other two treatments, and no significant effect of grazing day (Table 9). Fat normalized milk yield responded similarly to milk yield (not shown). Milk fat, protein and casein contents were higher in 2 h/d groups although for protein and casein differences were found only between 2 and 6 h/d ($P<0.05$, Table 9). For protein and casein an effect of the grazing day was detected, with a drop on the last day of grazing period (Day 7, $P<0.05$, Table 9). Milk lactose content was not affected by any of the factors under study. Milk urea was unaffected by access time, although a trend was detected ($P<0.08$) with lower value in 2 h/d than in the other treatment groups. Moreover milk urea trend during grazing period, mirrored the one of casein (Day 7 lower than the others, $P<0.05$, Table 9).

Body weight, BCS and energy balance.

Both average group BW and BCS did not differ among treatments at the beginning (P > 0.5) and the end of the experiment (P<0.17). Group average BW increased along with the experiment in all groups, with differences between 2h/d and the other groups (35 vs 70 and 93 g/day of average daily gain; P<0.05 for the comparison between extreme treatments, P<0.06 between 2 h/d and 4 h/d) corresponding to a total increase of BW amounting to 1.97 vs 3.91 and 5.19 kg, respectively. The same trend was found for the BCS, with lower increase in 2h/d than the other groups (0.05 vs. 0.11 and 0.11 BCS units, P = 0.05).

The estimated energy balance, based on intake and requirements computations, mirrored this trend: 0.56 Mcal/ewe day (2 h/d) vs. 1.02 Mcal/ewe day (4 h/d) and 0.93 Mcal/ewe day (6 h/d, P<0.001). This variable was neither affected by the grazing day nor by the interaction between the factors under study.

Energy and N utilization efficiencies

Overall the estimated ingested energy was used for milk production more efficiently in the most time restricted group (40.68 vs 35.20 and 36.18%, in order of raising access time, P < 0.05) whereas there was only a trend to a better utilization of N (19.03% vs 17.0% and 16.83, respectively, P = 0.10 between the extreme groups). None of the efficiency coefficients was affected by the grazing day or the interaction.

4. Discussion

Pasture characteristics

Berseem clover pasture was featured by high sward height, herbage mass, and allowance throughout the study period, with an expected fluctuation during the grazing period due to the rotational grazing (Table 3). The latter was applied according to a widespread scheme for Mediterranean forage crops, which is based on standard weather pattern and growth curves (e.g. Di Grigoli et al., 2012). The weather pattern was featured by a very wet February, with a low herbage mass up to March, which prompted a temporary reduction of stocking density in the first week of the experiment. This was followed by an increase of both variables (herbage mass and stocking densities), mirroring the increase of temperature up to the end of the first rotation. Herbage regrowth was not measured in this experiment but the herbage mass at the second rotation turn was below the expectations, suggesting a decrease of stocking density and projected stocking rate, which averaged 25 ewes/ha at the end of the experiment (Table 1). The adjustment of stocking density or "put and take" approach used in this experiment reflects quite well the tactics often implemented at farm scale, with the aim to keep herbage mass, sward height and proportion of leaves as close as possible to the optimal ranges (e.g. USDA, NRCS, 1997). According to Giambalvo et al., 2011, under cutting regimen, residual (stubble) sward height should be kept above 6 cm when rotation length is as short as 28 days, in order to optimize primary production. During the first rotation, the sward left behind the experimental grazers was often higher than 10 cm in some subplots. Thus, spare sheep were introduced as "followers" (after the experimental "leaders"). This technique has been recently re-named "first-last stocking" by Allen et al. (2010).

The implementation of the rotational grazing technique, coupled with put-and-take and first-last stocking resulted in pasture characteristics overall quite similar between access-time plots ($P > 0.4$, for the herbage mass on offer). Only one pasture variable (weed percentage) was affected, with lower levels in the intermediate access groups (4 h/d). This difference was numerically low (- 2.8 units in terms of proportion of total DM on offer, as compared with other treatments) and it is hardly explainable. We cannot rule out that in some subplots there were differences from the beginning of their grazing, not statistically detectable.

In contrast with access time, the grazing day within the grazing period affected the basic pasture variables (Table 3), although the effects were not evident on herbage mass on offer and herbage allowances. We cannot put forward a simple explanations for this since sward height did decrease significantly along pasture depletion. The possible trampling effect during the grazing period could be evoked to partially explain this mild discrepancy.

Pasture structure components and LAI were clearly influenced by the grazing day (Table 4), with a linear trend only on the number of stems per plant. Pasture depletion is not necessarily a linear process although, for sake of simplicity, it is often represented in this way (e.g. Gregorini et al., 2009).

The last grazing day was anyway the most limiting for the pasture variables, usually regarded as putative drivers of herbage intake, such as sward height, herbage mass (n.s.), herbage allowance (n.s.), leaf proportion, leaf to stem ratio and LAI. Literature regarding the optimal and limiting levels of these pasture characteristics for rotationally grazed berseem clover is poor (USDA, NRCS, 1997) and often not specifically referred to small ruminants. De Santis et al. (2004) suggested, as main criterium for the beginning of an optimal cutting regime, to use the number of elongated internodes (i.e. 6), which is however a measurement more easily applicable to experimental than commercial farm conditions. In general, based on the indications of Hodgson (1990), the residual levels of herbage mass and sward height, as well as herbage allowance and LAI, seemed to be reasonably high to suggest that they did not impose severe restrictions on sheep intake, although the Hodgson's guidelines refer to temperate grasslands rather than Mediterranean legumes and meat rather than dairy sheep. Results of this study are in partial compliance with those of a sister study on the rotational grazing of Italian ryegrass, which however had lower values of quality indexes, such as proportion of leaf lamina and LAI. Indeed, the latter was below 1 on Day 7 of the grazing period (Molle et al., 2014).

Herbage chemical composition and its nutritional value

Access time to the plots affected only the CP and EE levels of the herbage probably eaten by the grazing ewes, with a slightly higher value in the most time-restricted treatment groups (Table 5). This result is mirrored by the herbage IVDMD, which was numerically higher in 2 h/d groups. Overall these data could suggest that the ewes at pasture for only 2 h/d tried to improve the quality of their diet while grazing, in line with findings of Ginane and Petit (2005). In fact, their heifers, which had 5 h/d of time access to a mosaic of vegetative and reproductive strips of *Dactylis glomerata*, prioritised quality rather than quantity of ingested herbage during grazing, as compared with a time-unrestricted control.

Another possible reason is that the slighter higher (not significant) herbage allowance in the 2 h/d subplots, coupled with *de facto* lower grazing pressure, due to lower access time, could have possibly resulted in higher quality on offer, at least at the end of grazing period.

The depletion of pasture affected its chemical and nutritional value, with slightly lower, CP and IVDMD ($P<0.07$), higher ash and fiber fractions contents in the last grazing day, although trends were sometimes unclear (Table 5). For instance, it is unclear why Day 4 was sometimes featured by a better herbage composition than the other two. This could be possibly related to the grazing of younger leaves from the intermediate layer of grazing horizon, as observed on occasions during feeding behaviour monitoring sessions.

Overall these results suggest that the quality of the herbage was not very different among treatment groups and declined with pasture depletion without abrupt changes for the key variables (NDF, CP, IVDMD) usually considered as predictors of herbage intake in grazing ruminants.

Feeding behaviour at pasture and intake rate

There was a clear impact of the treatment and the blocking factor on ewes'feeding behaviour at pasture, as displayed in table 6 and Figure 1. Most of results were, to some extent, expected. In particular, the compensatory behaviour of sheep, which increased the proportion of time devoted to grazing along with the decrease of access time. Iason et al (1999) found similar results in continuously stocked lactating meat sheep, grazing a temperate perennial grassland maintained at 3 or 6 cm sward height. In their study the time devoted to grazing (eating) was around 87% of access time in the ewes whose access to pasture was restricted to about 9 h/day as compared to the 52% of access time in the unrestricted counterparts at pasture for 24 h/day.

Recent data on meat sheep (Chen et al., 2013) and lambs (Zhang et al., 2014) raised in desertic steppe and submitted to access time to pasture of 2, 4, 8 and 12 h/day confirm this general trend, although in both these studies the authors did not detect differences between 2 and 4 h access time for the percentage of time used to graze.

Data on grazing dairy sheep are scanty although tend to align with the literature on cattle and meat sheep (Perojo et al., 2003, Garcia Rodriguez et al., 2005, Molle et al., 2014). With reference to the last study, the ewes rotationally grazing Italian ryegrass reacted to time restriction very similarly in terms of proportion of time devoted to grazing, although no interaction between access time and grazing day was detected in that study. In the current experiment, the ewes grazing at the same access time a berseem clover pasture showed an increase of grazing time percentage along with the pasture depletion but only in the groups at pasture for either 4 or 6 h/day (Figure 1). The opposite trend was found for idling time proportion. This means that these groups reacted to the decrease of pasture accessibility and quality with a switch between eating and idling within their time budget, with the aim of curbing the reduction of nutrient intake. Rumination activity on the plot tended to mirror ITP, with a much higher percentage in the less restricted groups (6 h/d, Table 6, $P<0.001$). It is noteworthy that ewes at pasture for 6 h/d spent in total almost 30% of their access time for idling and ruminating, thus limiting their grazing to less than 4 h/day, which overlaps the time access to pasture of the mid-restricted groups (4 h/d).

Interestingly, similar to the UK experiment by Penning et al., (1994), in the current study there was a slight but not significant decrease of proportion of time devoted to grazing in the 2 h/d groups on the last grazing day (Day 7, Figure 1) which recall what Penning et al., (1994) named 'giving-up' behaviour. This could be a response of the lessening will of these ewes to strive for compensating the two constraints operating on the Day 7: i.e. time on the plot and the limited herbage accessibility and quality. However in our case these constraints operated at a mild level.

Herbage DM intake rate was affected only by the time access (Figure 2, P<0.001). Intake rate was also increased by the time restriction in the study by Iason et al. (1999) in meat ewes, although at levels much lower than in the present study (1.8 g DM/min grazing in the control group vs 2.4 g DM/min grazing in the time restricted group).

Results of studies on lambs in China (Zhang et al., 2014) show also an increase of intake rate along with the severity of time restriction (2.6 g DM/min grazing (12 h/d); 3.1 g DM/min grazing (8 h/day); 3.6 g DM/min grazing (4 h/day); and 5.4 g DM/min grazing (2 h/day)), with significant differences between treatment groups, except for the first two.

In our previous study on dairy sheep, the effect of time access was also evident on intake rate but, in that case, also the grazing day significantly affected it (Molle et al., 2014). In contrast, intake rate tended only to decline in this study along with the grazing period (P < 0.07), particularly on Day 7, without evidence of interaction between the two factors. The lower fibre content of the legume can be evoked to explain this result, in line with data by Orr et al., (2001) who found higher intake rates in sheep grazing white clover than perennial ryegrass, under time access to pasture limited to the first uninterrupted meal. The decrease of intake rate during grazing period was also found by Penning et al. (1994) in lactating meat ewes rotationally grazing a perennial ryegrass sward with unrestricted time access to pasture. In that case, however, the grazing period length was above 10 days and the herbage intake rate ranged on average between 9.7 to 1.5 g OM/min grazing.

Motivation to eat is a complex subject still undermined, despite some relevant advances (Gregorini, 2012, Allen, 2014). According to the above reviews and original papers such as Gregorini et al. (2009a), a significant role for hunger expression in grazing ruminants, particularly in the first meal corresponding to dawn hours, is played by ghrelin. This hormone is synthesized in the abomasus of ruminants. Its incretion peak usually anticipates the feeding of the first daily meal, as found by Sugino et al. (2004). In the study by Gregorini et al. (2009a), the higher were ghrelin and NEFA concentrations in cows' plasma, the higher was their herbage intake during the morning grazing session (4 hours), suggesting a strict relationship between these variables. In a subsequent study Gregorini et al. (2009b) found a weaker relationship between ghrelin and intake rate at the first meal but in the meanwhile highlighted a positive correlation between the plasma concentration of this hormone and the bite mass, which is the main driver of intake rate. We suppose that higher levels of ghrelin and NEFA in 2 h/d ewes, possibly associated with a food anticipatory activity, typical of time restriction to feeding (Verwey and Amir, 2009) possibly boosted intake rate. In fact, if we consider the IR measured simultaneously in the first grazing hour in all groups, this IR value is higher in the 2h/d ewes than in the counterparts: 10.2 g DM/min grazing (2 h/d), 8.9 g DM/min grazing (4 h/d) and 7.6 g DM/min grazing (6 h/d), P<0.01 among groups.

Was hunger stimulus more intense in the 2h/d than 4h/d and 6h/d groups? According to the energy balance (see below) it was probably the case. We do not know about the role played by the rumen fill in the regulation of herbage intake, although, despite the lower intake of herbage of 2 h/d ewes, their diets were proportionally richer in roughage than those of the other groups. Other hormones besides ghrelin such as insulin have probably contributed to the short-term expression of the compensatory feeding behaviour displayed by the most time restricted ewes (see Gregorini, 2012, and Allen, 2014).

In general, the pattern of the feeding behaviour by hour (Figure 3) suggests that ruminating and idling were concentrated mainly after the second hour of grazing. The access time also impacted on the hourly time budget, in line with average daily results. Bite rate (Figure 4), measured only in one week of April, increased with the grazing period in line with findings by Kennedy et al. (2009). BM was bigger in 2 h/d and 4 h/d than 6 h/d as also found in dairy cattle by Gregorini et al. (2009). The value of BM was on average slightly higher than that measured in lactating ewes exposed for 5 min to micro-swards of sulla (0.26 g DM, Giovanetti et al., 2011) and was also similar with average values of BM measured by Gong et al. (1996), in sheep grazing legume turves (white clover and red clover).

<u>Intake of herbage and nutrients and estimated diet composition</u>

Herbage DM intake was strikingly constrained in the 2 h/d groups as compared with the less time restricted groups, which reached levels undifferentiated between them (Table 7). This occurred also in the previous study on Italian ryegrass, but levels were much lower and differences between treatment groups were detected also between 4 and 6 h/d (648 g, 2 h/d; 1059 g, 4 h/d; and 1233 g , 6 h/d, Molle et al., 2014). This result is not surprising, because legume-based diets give usually higher intakes and better performance than grasses (Rochon et al., 2004; Lüscher et al, 2014). Despite the higher intake of hay (ryegrass component), the 2 h/d ewes showed the lowest total DM, CP and total NE_L intake (Table 7). The compensatory increase of roughage intake in the most time restricted groups is in line what found in the previous study by Molle et al. (2014) and backs the hypothesis that intake of hay (even roughage of low nutritive value) can be used in dairy sheep as a good gauge of constrained herbage intake at pasture (Molle et al., 2008). Providing access to a better hay than the ryegrass used in this study, could have sourced different results from the ones observed, as shown by De Renobales et al, (2012). They offered to three groups with the same access time (4 h/day) different levels of lucerne hay (300, 600 and 900 g/ewe day) finding a linear decrease of HDMI with the increase of the level of supplementation. A substitution rate close to 1 was also recently found by Zhang et al., (2014), who fed unreplicated groups of lambs different levels of concentrate and hay.

The rotational grazing scheme usually results in a pendulum-like effect on herbivores intake, which can brings about a drop of nutrient supply below requirements, as found by Molle et al. (2004b) in Sarda dry ewes rotationally grazing annual ryegrass (*Lolium rigidum* Gaudin). In the current study, effects of pasture depletion on intake were less consistent, with higher HDMI and lower hay and concentrate DM intakes on Day 4 than on Day 1, being intakes on Day 7 intermediate (Table 7). This is explainable with the lower NDF level of the ingested herbage (Table 5) and diet (Table 8) on Day 4. A reason underlying it could be the presence of a young leaf layer below the upper leaf horizon. However, the vertical distribution of dry matter and plant parts was beyond the scope of this study.

No effect of pasture depletion was found on total DM intake or CP and NE_L intake (Table 7). This confirms the lower impact of this factor in this than in the previous study, suggesting a lower quality constraint with a grazed legume (this study) than a grazed grass (Molle et al., 2014).

Overall TDMI and TDMIBW (Table 7) were very high as compared with other data of milked ewes grazing legume-based pastures (e.g. Molle et al., 2008). However in this study average milk was very high, particularly in the less time restricted groups.

The time access restriction caused some mild effects on the estimated nutritional composition of the diet, with a lower level of ash and, as expected, a slightly lower level of CP in the most restricted groups (2 h/d, Table 8). This CP level (17.3 % DM) is anyway regarded as non-limiting for average fat-normalized milk yield in the range between 1400 and 1700 g/ ewe day (Cannas, 2004). Dietary levels of NDF around 36% DM, with a slightly higher value in the diet of the 2 h/d groups (Table 8) were also in an optimal range for a target milk yield around 1700 g/ ewe day, according to the same author (Cannas, 2004).

Pasture depletion affected the estimated nutritional composition of diet although, likewise the intake of nutrients, the response was not linear for many variables. In fact dietary ash content increased and dietary CP content decreased from Day 1 to Day 7, although the absolute value of the differences was low (Table 8).

Milk yield and milk composition

Milk yield was markedly constrained in the ewes grazing berseem clover 2 h/day, differently from the previous study where the same performance of ewes grazing Italian ryegrass were already constrained at intermediate time allowance (i.e. 4 h/day, Table 9).

As a consequence, milk composition showed some typical dilution effects on milk fat and protein, which were anyway insufficient to offset the loss of yield, since fat-normalized milk yield was still higher by far in the less time restricted groups (4 and 6 h/d). Milk urea showed just a trend to a lower content in the groups receiving the diet with the lower CP content, in line with the relationship between milk urea and dietary CP content found by Cannas et al. (1998).

To the best of our knowledge, comparable studies on the response of dairy sheep grazing a pure legume pasture for restricted time are lacking. In a previous study of our laboratory, when dairy ewes had access to sulla and annual ryegrass monocultures for 22 h/d or to the legume for 3 or 6 h/d and the remaining time to the grass, milk yield in spring was higher for the sulla monoculture, followed by 3 and 6 h/d of sulla grazing (Molle et al., 2003). No difference was found in the time-restricted groups in terms of intake and performance, suggesting that tannins contained in the sulla forage probably limited intake and performance of the group on sulla plots for 6 h/d, as found by Rutter et al., 2004. This should not be a problem with berseem clover, whose content of tannins is regarded as low if any (data of our laboratory).

In this study, unlikely the previous one (Molle et al., 2014), effects of pasture depletion in terms of quantity and quality were basically nil on milk performance. This is in line with the mild and, sometimes, inconsistent impact of this factor on intake of nutrients and ewes'diet composition above shown. Therefore, under the experimental conditions of these two studies, their results overall suggest that the oscillating flow of nutrients during the grazing period did affect dairy sheep performance more when grazing a grass -namely Italian ryegrass- than a legume –i.e. berseem clover. This outcome fits quite well to a vast literature sustaining the higher feeding value of legume pastures for milk production, even when accessibility and quality are limited (Rochon et al.,2004). The role of grazing legume-based pastures in spring to sustain milk performance in dairy ewes grazing Mediterranean pastures has been reviewed by Molle et al. (2008) and this concept is widely implemented in many dairy sheep farms across Mediterranean Countries.

<u>Body weight, BCS and energy balance.</u>

The results on BW, BCS and energy balance all convey the concept that the less time restricted ewes (4 and 6 h/d) were overall in a better energy status than the most time restricted groups. However, we regard the estimate of energy balance as a rough proxy of energy status of sheep, because we know that energy expenditures could have been different in the sheep submitted to different time restrictions. In fact, Chen et al. (2013) in lambs with time access to pasture of 12, 8, 4 and 2 h/d, found a decrease of walking distance but an increase of grazing velocity in terms of m/min of grazing in sheep with access time progressively restricted from 12 to 2 h/day. In particular walked distance almost doubled from 4 to 8 h/day and grazing velocity increased from 4 to 2 h/day by 50% and then slightly dropped at lower time restrictions.

Gregorini et al. (2011) found a similar compensatory behaviour in grazing cows, whose access time to pasture ranged between 24 and 8 h/day. In that case, the velocity of walking was higher in the first part of grazing session in the cows with restricted access time.

Thermo-regulation, distance and walking velocity all affect energy expenditures of grazing ewes. In our case herbage accessibility was overall high and plot size small, therefore we can suppose that thermo-regulation and walking distance could have probably played a dominant role, since feeding stations were probably too close to stimulate an increase of walking velocity in the most time-restricted ewes.

The reason for the similar body weight and BCS accretion between 4 and 6 h/d could be related to the lower numerical intake of energy in the latter than the former group, and probably the higher energy expenditures related to both higher thermo-regulation and dynamic activities in the group with the longest access time to pasture.

Energy balance was possibly overestimated in our study due the lack of data on the actual energy expenditures but also the unavailability of *in vivo* digestibility data. We cannot in fact exclude the occurrence of negative associative effects, which sometimes impair rumen functions. Cannas et al. (2013) found for instance that increasing the level of NFC in the diet by replacing digestible fibre with starch reduced *in vivo* NDF digestibility and milk yield in mid-lactating dairy ewes. The level of starch in this experiment was low; nevertheless the substitution of the maize with a source of digestible fiber such as dehydrated sugarbeet pulps or soybean hulls, would have possibly improved digestibility and performance of the sheep, particularly for the ones with 2 h/d access to pasture.

<u>Energy and protein utilization efficiencies and on-farm application</u>

Efficiencies were all in favour of the most time restricted groups, showing that marginal effect of grazing is higher when herbage intake is limited, which is in line with the general law of decreasing marginal response from allocation of a limiting resource. The high sensitivity of the system to the input of the first “quantum” of grazed forage is probably a consequence of the big discrepancy between the nutritive value of grazed herbage at growing phase and that of conserved forages, quite commonly found in Mediterranean sheep farms. Despite the high amount of supplements offered, and the relative consistency between CP intake and requirements, the N utilization efficiency was relatively low in all treatments. This can support the hypothesis that true digestibility of diets was lower than the estimated value. Similar N efficiency levels were found during three subsequent springs, in sheep rotationally grazing a burr medic-based pasture without supplementation (17.62, 18.93 and 19.28% in years 1, 2 and 3; Molle et al., 2007). However, in that study, intake level and milk yield were on average lower than in this experiment.

Our study was not strictly designed to provide guidelines for the management of dairy sheep grazing berseem clover, however some practical considerations can be put forward. In the light of our results, to get optimal *individual* herbage intake and milk yield, 4 h/d access is the best option. It allows for a recovery of fat depot (BCS) and does not significantly lower the N utilization efficiency, as compared with 2 h/d access to pasture. Using this approach in spring, it could be probably advisable to decrease the supplement level and possibly change the quality of the concentrate (less starch) along with the advancement of the grazing season. Further studies are however needed to refine the above tactics.

A different timing of the access time is also prone to marginally improve intake and performance of part-time grazing dairy ewes: for instance 4 h/day allocated in the afternoon instead of morning could result in better intake and performance according to the review by Gregorini (2012) thanks to an increase of intake rate around dusk and better hormonal profile. This timing however is not always implementable due to the short daylight time in winter and early spring, which can impair sheep husbandry, particularly when grazed paddocks and milking parlour are not very close. Also the partitioning of the optimal access time into two periods could be effective to improve the part-time grazing management, although results from literature are partially inconsistent (e.g. Kennedy et al., 2009) and on-farm application of it could be difficult and, possibly, unfeasible.

We cannot draw conclusions on the best management for optimizing milk production *per unit area* since we did not take into account the animal production of the follower (last) spare sheep which grazed the subplots after the experimental groups. Furthermore, a longer experiment would be advisable to better evaluate at system level how part-time grazing animal response are modulated by pasture variables across the grazing season.

5. Conclusions

The restriction of time access to a pasture of berseem clover, rotationally grazed by dairy ewes in spring, markedly constrained their intake and milk performance when access time was below 4 h/day. This happened despite the compensatory feeding behaviour of sheep, such as the increase of time devoted to grazing and of intake rate, and the increase of hay intake.

In contrast, the pasture depletion as gauged by the grazing day, while affecting the feeding behaviour, with a trend to a higher proportion of time devoted to grazing in the less time-restricted ewes (4 and 6 h/d), does not impact in a consistent way on herbage and nutrient intake and, as a consequence, on milk performance.

Overall, this study suggests that efficacy of foraging and milk production in lactating ewes rotationally grazing berseem clover tends to an optimum at 4 h/day access time. Below that access time intake and performance are constrained and above the surplus of ingested energy is directed more to fat depots or lost as energy expenditures. Finally, 6 h/ d tends to be less efficient in terms of N utilization, under the conditions of this study.

Further studies are warranted to better quantify and explain the energy partitioning in mid-lactating dairy ewes submitted to restricted access to pasture and to assess the effect of restricted grazing in a more holistic way.

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Table 1. Number of ewes per group, stocking density and projected stocking rate during the experiment.

Period	Ewes per group	Stocking density	Stocking rate#
	n. ewes	n. ewes/ha	n. ewes/ha
11 March - 17 March	4	64	16
18 March - 24 March	6	96	24
25 March -7 April	8	128	32
8 April - 29 April	6	96	24
Weighted means	6	101	25

Projected value computed considering a rotation of 4 subplots.

Table 2. Chemical and nutritional composition of supplements.

Feed		DM	Ash	CP	EE	NDF#	ADF	ADL	IVDMD
		%	% DM	% DM	% DM	% DM	% DM	% DM	%
Italian ryegrass hay	mean	86.46	7.33	4.57	1.58	73.92	44.73	5.30	43.82
	sd	1.91	1.00	0.77	0.17	1.95	2.74	0.26	2.61
Lucerne hay	mean	87.82	8.96	16.08	1.71	56.01	38.90	8.11	60.51
	sd	1.30	0.96	2.24	0.28	2.38	3.93	0.93	2.37
Pelletted concentrate	mean	88.85	12.66	15.45	2.64	33.93	18.54	3.06	77.27
	sd	0.52	0.09	0.08	0.02	0.81	0.31	0.10	0.43
Maize grain	mean	87.86	1.41	10.53	4.52	9.83	2.62	0.07	91.41
	sd	0.20	0.04	0.35	0.25	0.30	0.10	0.02	0.74

aNDFom for concentrates and NDFom for hays;

Table 3. Effect of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7 day grazing period (Day 1, first, Day 4, intermediate, and Day 7 last day) on pasture sward height, herbage mass, weed percentage, and herbage allowance.

	Sward height cm	Herbage mass t DM/ha	Weed % DM	Herbage allowance kg DM/ewe day	Green Herbage allowance kg DM/ewe day
Access time h/day					
2	20.5	2.24	9.3a	3.47	3.27
4	19.7	2.18	6.4b	3.37	3.14
6	19.1	2.05	9.4a	3.17	2.98
Grazing day					
Day 1	24.7a	2.30	8.0	3.49	3.37
Day 4	19.7b	2.17	9.5	3.36	3.15
Day 7	15.1c	2.00	7.6	3.16	2.87
P level <					
Access time	ns	ns	0.02	ns	ns
Grazing day	0.001	0.12	ns	ns	ns
AT x GD	ns	ns	ns	ns	ns
RMSE[A]	6.69	0.10	4.1	0.98	1.04

Within column and effect, means followed by the same letter are not significantly different (P = 0.05)

[A] Root mean square error

Table 4. Effect of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7 day grazing period (Day 1, first, Day 4, intermediate, and Day 7 last day) on pasture composition (expressed as % of herbage mass), stems per plant, specific leaf area and LAI.

	Green leaves % DM	Green stems % DM	Leaf stem ratio	Dead matter % DM	Stems per plant N	Specific leaf area cm^2/g	LAI
Access time h/day							
2	46.7	47.1	1.07	6.2	4.4	39.5	2.7
4	45.1	47.1	1.08	7.7	4.4	40.8#	2.6
6	43.3	49.7	1.06	7.0	4.0	38.4#	2.3
Grazing day							
Day 1	52.8 a	43.7 b	1.31a	3.4 b	5.1 a	39.7	3.0 a
Day 4	47.5 a	46.2 b	1.15a	6.3 b	4.3 b	39.4	2.7 a
Day 7	34.8 b	54.0 a	0.75b	11.2 a	3.4 c	39.7	1.9 b
P level <							
Access time	ns	ns	ns	ns	ns	0.06	ns
Grazing day	0.001	0.001	0.05	0.01	0.001	ns	0.001
AT x GD	ns	ns	ns	ns	ns	0.09	ns
RMSE[A]	13.5	9.5	0.55	7.5	0.88	3.45	0.91

Day 4 and Day 7 differ at P<0.05.

Within column and effect, means followed by the same letter are not significantly different (P = 0.05)

[A] Root mean square error

Table 5. Effect of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7 day grazing period (Day 1, first, Day 4, intermediate, and Day 7 last day) on the chemical composition of herbage (berseem clover) hand-plucked samples.

	DM	Ash	CP	EE	NDF	ADF	ADL	WSC	IVDMD
	%	% DM	% DM	% DM	%DM	% DM	% DM	% DM	%
Access time h/day									
2	15.20	12.69	22.68a	5.52a	32.04	20.40	3.34	10.38	84.80
4	15.33	13.29	21.53b	5.10b	32.34	20.65	3.31	10.45	84.00
6	15.34	12.94	21.98ab	5.20b	32.91	20.82	3.43	10.08	84.23
Grazing day									
Day 1	14.74b	12.71b	23.08a	5.61a	32.83 ab	20.67ab	3.31ab	10.19ab	85.03
Day 4	15.69a	12.67b	21.78b	5.22b	31.22 b	19.80b	3.13b	11.42a	84.44
Day 7	15.43b	13.53a	21.34b	4.99b	33.26 a	21.39a	3.65a	9.31b	83.57
P level <									
Access time	ns	0.08	0.05	0.05	ns	ns	ns	ns	ns
Grazing day	0.05	0.01	0.001	0.01	0.01	0.01	0.05	0.01	0.07
AT x GD	ns	ns	ns	ns	ns	ns	ns	ns	ns
RMSE [A]	1.16	0.92	0.32	0.56	0.46	1.74	0.61	1.99	2.17

Within column and effect, means followed by the same letter are not significantly different (*P* = 0.05)

[A] Root mean square error

Table 6. Effects of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on the feeding behaviour on the plots of dairy sheep rotationally grazing berseem clover

Effects	Total access time (min)	Grazing time % of access time	Ruminating time % of access time	Idling time % access time
Access time on the plots (h/day)				
2	118c	95.5a	0.6b	3.9c
4	233b	86.3b	2.8b	10.9b
6	328a	71.3c	9.0a	19.6a
Grazing day				
1	221	79.6b	4.2	16.1a
4	231	85.5a	4.3	10.2b
7	229	88.0a	3.9	8.1b
Level of probability (*P*<)				
Access time	0.001	0.001	0.001	0.001
Grazing day	ns	0.001	ns	0.001
AT x GD	ns	0.01	ns	0.001
RMSE[A]	18	6.6	3.1	5.8

Within column and effect, means followed by the same letter are not significantly different (*P* = 0.05)

[A] Root mean square error

Table 7. Effects of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on intake of dairy sheep rotationally grazing berseem clover.

Effects	Herbage DM intake (g)	Hay DM intake (g)	Concentrate [A] DM intake (g)	Total DM intake (g)	Total DM intake (% BW)	CP intake (g)	NE_L Intake (Mcal/d)
Access time (h/day)							
2	1168b	540a	612	2320b	5.53b	396b	3.60b
4	1782a	525ab	612	2918a	6.58a	515a	4.62a
6	1723a	439b	609	2771a	6.20a	506a	4.50a
Grazing day							
1	1465b	538a	615#	2619	5.99	472	4.15
4	1663a	446b	602#	2710	6.18	485	4.41
7	1545ab	521ab	615#	2681	6.15	459	4.15
Level of probability (*P<)*							
Access time	0.001	0.05	n.s.	0.001	0.001	0.001	0.001
Grazing day	0.05	0.05	0.05	ns	ns	ns	0.14
AT x GD	ns	ns	ns	ns	ns	ns	ns
RMSE[B]	270	125	20	288	0.63	57	0.52

Within column and effect, means followed by the same letter are not significantly different (*P* = 0.05); # P = 0.07 in the comparison between Day 1 and 4 and P<0.09 in the comparison between Day 4 and Day 7.

[A]Sum of pelleted commercial concentrate and maize seed intake. Maize was totally consumed.

[B] Root mean square error

Table 8. Effect of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7 day grazing period (Day 1, first, Day 4, intermediate, and Day 7 last day) on diet chemical composition of dairy ewes rotationally grazing bersee

	Ash	CP	EE	NDF	ADF	ADL	IVDMD
	% DM	% DM	% DM	%DM	% DM	% DM	%
Access time h/day							
2	10.22b	17.10b	4.06	37.43	22.66a	3.51	76.66
4	11.12a	17.63ab	4.12	36.40	22.21ab	3.43	78.00
6	10.93a	18.26a	4.25	35.57	21.56b	3.44	79.12
Grazing day							
Day 1	10.53b	18.03a	4.24	37.10a	22.30a	3.44ab	77.85
Day 4	10.69ab	17.88a	4.22	34.93b	21.28b	3.28b	78.84
Day 7	11.06a	17.07b	3.96	37.36a	22.85a	3.67a	77.08
P level <							
Access time	0.001	0.001	ns	0.08	0.03	ns	ns
Grazing day	0.05	0.01	0.07	0.01	0.001	0.05	0.07
AT x GD	ns	ns	ns	ns	ns	ns	ns
RMSE[A]	0.68	1.06	0.48	2.83	1.47	0.39	2.57

Within column and effect, means followed by the same letter are not significantly different (*P* = 0.05)

m clover. [A] Root mean square error

Table 9. Effects of access time (AT, 2, 4 and 6 h/day) and grazing day (GD) within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on milk yield and composition of dairy sheep rotationally grazing berseem clover

	Milk yield	Milk composition				
		Fat	Protein	Casein	Lactose	Urea
Effects	(g/ewe day)	%	%	%	%	%
Access time on the plots (h/day)						
2	1435b	6.28a	5.09a	4.05a	4.80	45.87
4	1706a	5.63b	5.04ab	3.98ab	4.82	47.50
6	1700a	5.84b	4.98b	3.93b	4.78	48.92
Grazing day						
1	1624	5.90	5.05ab	4.00a	4.78	49.08a
4	1662	5.90	5.09a	4.04a	4.83	48.43a
7	1555	5.94	4.96b	3.91b	4.78	44.77b
Level of probability (*P*<)						
Access time	0.001	0.001	0.06	0.01	ns	0.10
Grazing day	ns	ns	0.05	0.001	ns	0.05
AT x GD	ns	ns	ns	ns	ns	ns
RMSE[A]	260	0.51	0.16	0.14	0.12	4.8

Within column and effect, means followed by the same letter are not significantly different ($P = 0.05$)

[A] Root mean square error

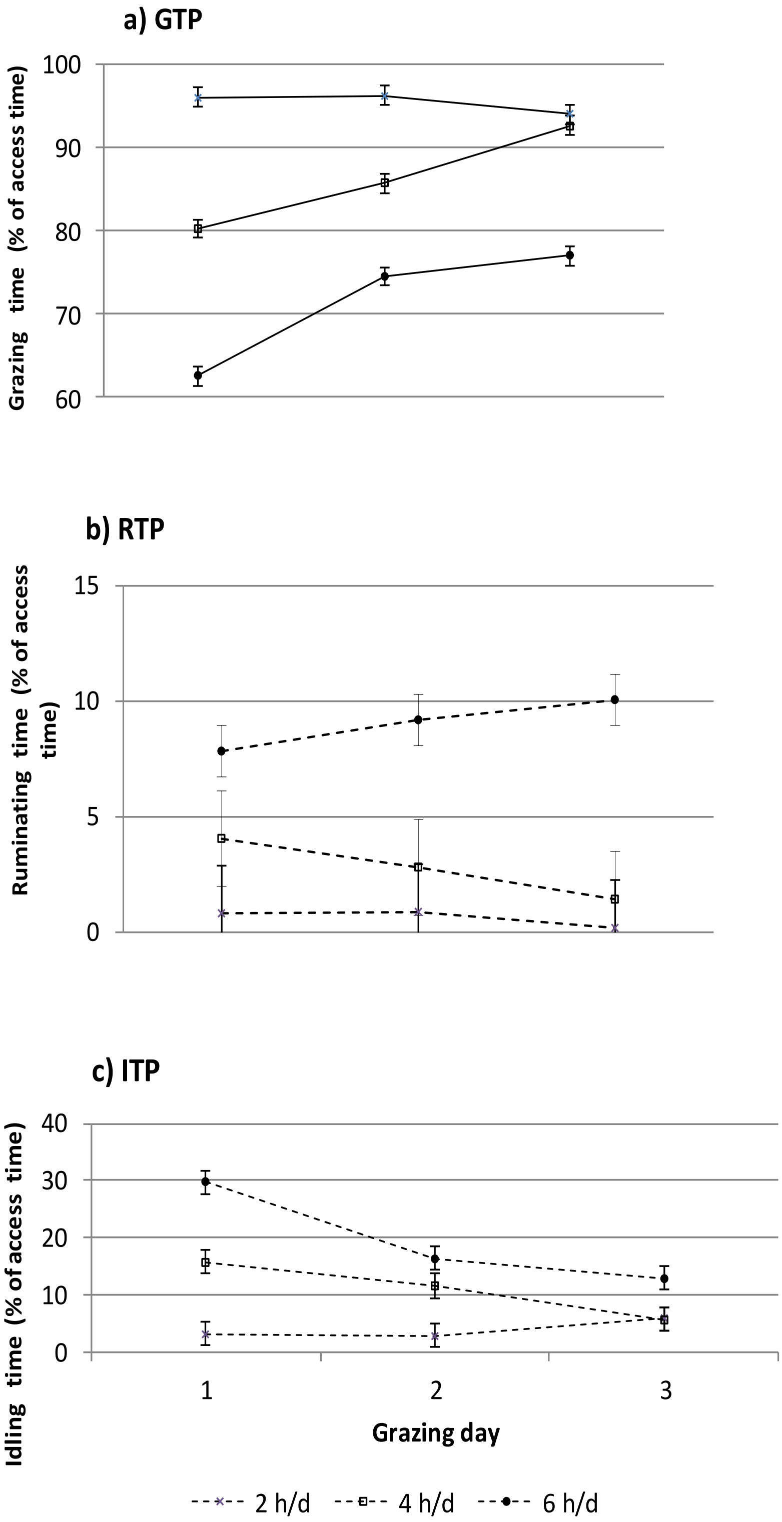


Fig. 1. Effects of access time (2, 4 and 6 h/day) and grazing day within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on: a) grazing time (GTP), b) ruminating time (RTP) and c) idling time (ITP). as proportion of access time, in dairy sheep rotationally grazing berseem clover. Means ± s.e. Interaction effect between access time and grazing time, significant at a level of P< 0.01 for GTP and P <0.001 for ITP.

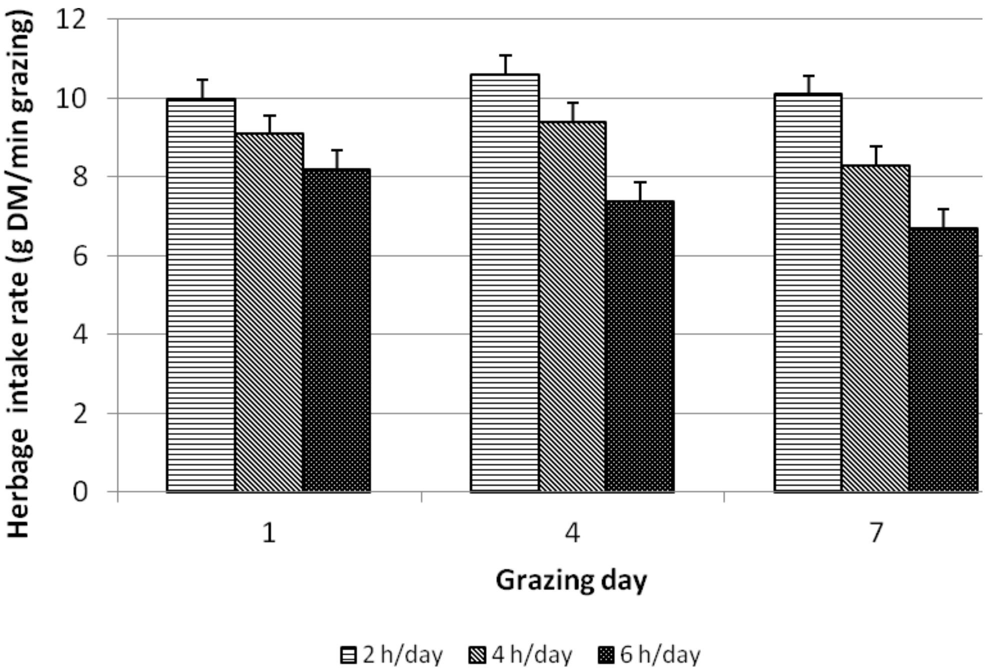
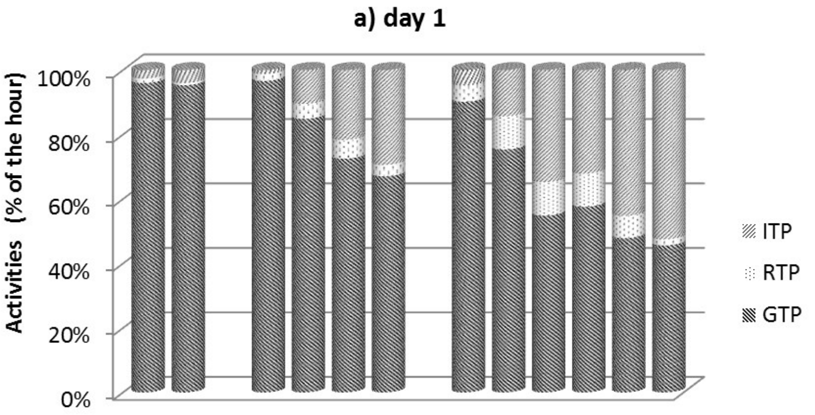
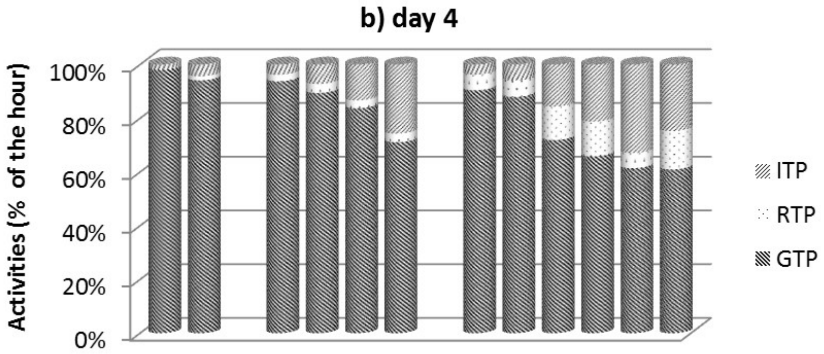


Fig. 2. Effects of access time (2, 4 and 6 h/day) and grazing day within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on intake rate in dairy sheep rotationally berseem clover. Mean ± s.e. Treatment means are different at $P < 0.001$. Details in the text.





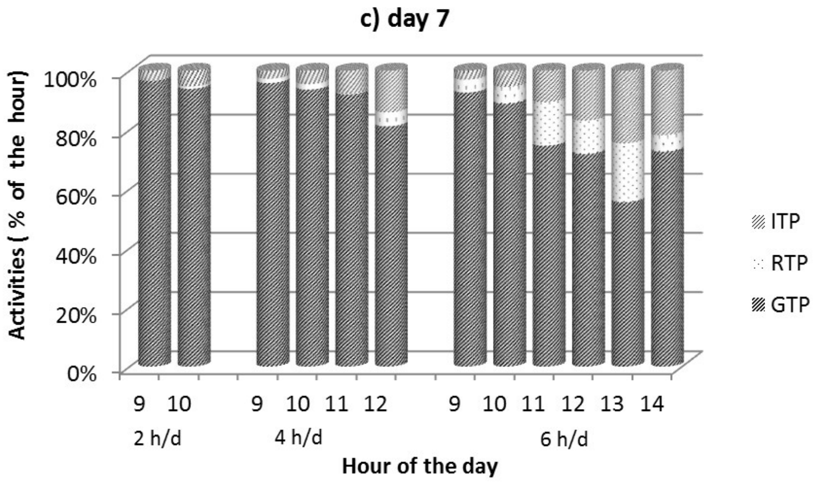


Fig. 3. Effects of access time (2, 4 and 6 h/day) and grazing day within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on the hourly pattern of feeding behaviour intake rate in dairy sheep rotationally berseem clover. Treatment means are different at P < 0.001.

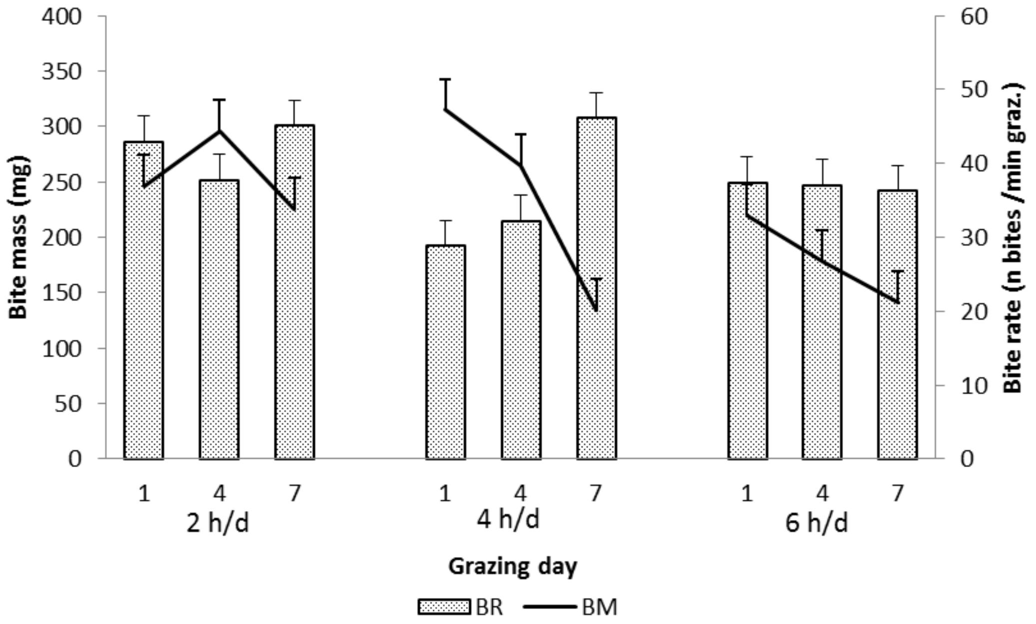


Fig. 4. Effects of access time (2, 4 and 6 h/day) and grazing day within a 7-day grazing period (Day 1, first, Day 4, intermediate, and Day 7, last day) on bite rate and bite mass of dairy sheep rotationally berseem clover, as measured on one occasion per target day in the last intake measurement week (April 15 (Day 1), 18 (Day 4) and 22 (Day 7)). Treatment means are different at P < 0.001.

CHAPTER 4

Modelling herbage intake of part-time grazing dairy sheep

Modelling herbage intake of part-time grazing dairy sheep

Abstract

Current models for predicting herbage intake of dairy sheep do not account for the effect of time of access to pasture. The restriction of time access to pasture (TA, h/day) to 2, 4 or 6 h/d was the focus of two experiments (E1, and E2), in which intake and performance response of lactating dairy ewes were measured while rotationally grazing Italian ryegrass (*Lolium multiflorum* Lam, E1) or berseem clover (*Trifolium alexandrinum* L, E2) pastures. The objective of this study was to model the results gathered in E1 and E2 with the aim of highlighting the main predictive variables of herbage intake (g DM/ewe day) when time access to pasture is restricted. To this end, the database of N=144 records was split randomly in a training (N=124) and an evaluation dataset (N=20). Both ordinary least square stepwise regression (OLSR) and partial least square regressions (PLSR) analyses were applied to the training datasets and a subset inclusive of 15 variables previously selected on the basis of a conceptual framework model and a preliminary screening by correlation analysis. The best-fitting model sourced from OLSR included 6 regressors (milk yield (g/ewe day) the content of DM and NDF in grazed herbage (%DM), TA and its quadrat). It showed a R^2 and RMSE of 0.86 and 178 g DM in calibration and 0.66 and 253 g DM in evaluation steps. Evaluation models based on PLSR provided better precision than those based on OLSR, (R^2= 0.74 and RMSE = 231 g DM in the best case). The advantage of using PLSR was more evident when all variables were used.

1. Introduction

Modelling the herbage intake (HDMI, g DM/head day) of grazing herbivores is notoriously a challenging task.

In fact, firstly the process itself is complex due to the simultaneous interplays of different factors, namely: i) animal factors (body weight, physiological state, production level), which are bound to the level of animal requirements); ii) diet quality factors, with particular reference to the fiber content which putatively affects rumen fill; iii) herbage accessibility factors (sward height, herbage mass, sward structure); iv) managerial factors (such as supplementation level and type, and grazing management); v) environmental conditions (weather and topography in particular).

Secondly, despite the advancements of methodologies and techniques, HDMI data of grazing herbivores can be more appropriately regarded as estimates than measures, particularly under heterogeneous pasture conditions. Actually, proper validation of intake estimates of grazing ruminants is impossible, since no measured data can be regarded as reference ("true values").

Thirdly, herbage intake is dynamically affected by the factors above quoted, hence even when HDMI estimates are sourced from sound experiments and updated methods, the inference resulting from empirical and mechanistic models can capture only snapshots of this complex and ever-changing process.

These and other reasons make the literature on the modelling of HDMI in grazing ruminants relatively sparse, but this is particularly the case for small ruminant grazing Mediterranean forages. As a matter of fact, the number of papers devoted to modelling exercises in dairy cows and beef cattle has abruptly increased in the last decade, thanks to a re-evaluation of grazing in many production systems across Europe, America and in the Newest World (New Zealand and Australia) (e.g. Faverdin et al., 2010, Baudracco et al., 2011, Gregorini et al., 2013). These models represent a significant progress in the prediction of cow response under grazing but refer mainly to temperate grasslands based on *Lolium perenne*, possibly including some legumes (e.g. *Trifolium repens*).

Models focused on the prediction of intake in small ruminants have been recently reviewed by Pulina et al. (2013). In the above review the main characteristics of models relevant to grazing sheep are evaluated and they are briefly reported in the following section.

Baumont et al. (2004) developed a mechanistic model aimed at predicting HDMI of meat-type sheep grazing grasslands typical of temperate climate, i.e. based on perennial grasses. This model assumes that sheep defoliate the sward by horizons (two dimension grazing), which is mainly the case of homogeneous pastures (swards). This model was successfully challenged with experimental data of sheep grazing under continuous and rotational grazing, although, on occasions, absolute discrepancies were relevant.

Deterministic models (e.g. Small Ruminant Nutrition System (SRNS) by Cannas et al., 2004, recently updated by Tedeschi et al. 2010), do not incorporate any specific sub-model to estimate the intake of grazing ruminants. Few deterministic models consider algorithm to address grazing conditions. Freer et al.(1997) developed a mixed empirical-mechanistic model (GrazFeed) to predict both potential and actual intake of sheep grazing temperate perennial grasses, Mediterranean annual forages and sub-tropical C4 grasses and legumes. In this model, HDMI is predicted on the basis of sheep requirements, pasture availability and ingestibility; the latter in its turn estimated on the basis of forage digestibility. Pasture availability depends basically upon the herbage mass on offer (HM), measured as kg DM/ha.

GrazFeed model by Freer et al. (1997) has robust mathematical and biological basis, with exception of the selection process algorithm (Pittroff and Kothmann, 2001) and has proven to be sensitive to changes of animal production levels, pasture characteristics and supplementation levels and types (Dove et al., 2010). Unfortunately, although well built, Grazfeed does not take into account dairy sheep distinctive traits, such as milk yield of milked ewes, and their different body composition as compared with meat breeds, as well as managerial factors, such as the restriction of time access to pasture (part-time grazing).

Conversely, Avondo et al. (2002, 2005) developed a simple empirical model for estimating the intake of supplemented milked ewes, grazing Mediterranean pastures for restricted times (5-6 hours daily). This model consists of multiple regressions calculated plotting individual estimates of herbage intake against animal (body weight, milk yield) and pasture variables (herbage mass per unit area and pasture height).

Unfortunately, the precision of some equations is low. Nevertheless, the model is useful to get a first-approximation estimates of intakes under the conditions of its setting. Other empirical models have been put forward for the estimation of intake of dairy sheep under non restricted (22 h/d) time allocation to pasture. For instance, Molle et al. (2008) after meta-analysis of HDMI of unsupplemented sheep grazing different grass-legume mixtures, found a strict relationship between HDMI intake scaled by metabolic weight and *in vivo* dry matter digestibility DMD, measured on the same animals by the n-alkane method.

To summarise, although progress has been made in developing models of the ingestive behaviour of sheep and goats, their prediction ability is constrained for several facets. Among them, current models do not account for the effect of time of access to pasture (TA, h/d) when it is lower than 5-6 h/d (Pulina et al., 2013). Severe restrictions of time allocation to pasture (below 5-6 h/d) are frequently practised in sheep farms, particularly when pasture is young and too rich in protein or if there is shortage of herbage. The TA to pasture limited to 2, 4 or 6 h/d was the focus of two experiments (E1, and E2) recently undertaken in Sardinia (Italy) to measure intake and performance response of lactating dairy ewes rotationally grazing Italian ryegrass and berseem clover pastures.

The objective of the present study was to model the HDMI estimates gathered in E1 and E2 with the aims of: assessing the main explanatory and predictive variables of HDMI, and developing empirical models for the prediction of HDMI in dairy ewes part-time grazing grass and legume forage crops during the vegetative phase (winter- spring) of pastures.

2. Materials and methods

Database and exploratory analyses

A database was set up using mean data of pasture plots (625 m^2 each) and animal groups (4 to 8 milked ewes per group, 3 core animals being used to measure intake and performance). The experiments were set to compare three daily time access to pasture (TA, 2, 4 and 6 h/d) of Italian ryegrass (*Lolium multiflorum* Lam, experiment 1, E1) and berseem clover (*Trifolium alexandrinum,* experiment 2, E2) Methods are detailed in Molle et al. (2014; see also chapter 2 and 3 of this thesis). Pastures were in vegetative conditions and ewes in mid-lactation, with body weight (BW) ranging between 49 and 36 kg and milk yield (MY) ranging between 2200 and 700 g/ewe day. The ewes were supplemented with 200 g/ewe of a pelleted concentrate (CP = 15% DM , net energy = 0.90 Feed unit (UFL)/kg DM) for each of the 2 daily milkings, and at turn out from pasture with 300 g/ewe of lupin seed (E1) or maize (E2) and 700 g/ewe of Italian ryegrass-based hay.

The database consisted of 144 records inclusive all the variables (34 in total), except for some pasture variables (such as pasture structure leaf, stem and dead matter proportions, LAI, green herbage mass and green herbage allowance), available for 96 records only.

The main features of the database are summarized in Table 1.

Before mining of the database, a conceptual mechanistic model was used for classifying the regressors of interest for the prediction of HDMI (Figure 1). According to this model , based on Hodgson (1990) and updated by a recent review on the mechanics of grazing (Chilibroste et al., in press), five regressor classes were identified (Table 1):

- Related to animal (e.g. BW, MY and so forth);
- Related to diet quality, with particular reference to the fibre (NDF) component which is evoked as major determinant of rumen fill (e.g., Mertens, 1987);
- Related to herbage accessibility, which markedly affects bite mass and, through it, herbage intake rate;
- Related to animal and grazing management, such as supplementation level and daily access time to pasture;
- Related to environmental conditions (weather), as measured in each experimental day.

The database variables were then submitted to exploratory data analysis to assess the normality of their distribution and subsequently examined for their covariance by mean of correlation analysis.

After this step, two complementary statistical approaches were used: ordinary least square stepwise regression analysis (OLSR) and partial least square regression analysis (PLSR).

The first statistic technique is able to disentangle the regressors which play the most significant role in explaining the variability of response variables. Moreover it highlights the strength and direction of the relationships, lending support to the explanation of the phenomenon under scrutiny, based on the current knowledge of its physiological basis.

However, ordinary regression analysis of multifactorial response variables, such as the herbage intake often results in poor prediction performance due to multicollinearity and non-linearity effects of predictors. These shortcomings can be partially tackled by other methods such as model reduction, ridge regression, principal component regression analysis, and partial least square regression analysis, which was used in this study as univariate statistic.

Correlation analysis

Within each class, an *ex ante* screening of variables was based on the preliminary exploratory correlation analysis, assessing the Spearman correlation coefficients among response and explanatory variables. Fifteen variables were chosen for further analysis (variable with asterisk superscript in Table 1 plus the quadrat of TA).

Then the database was divided into two subset:

a) a model development (training) dataset inclusive of 124 records;

b) an evaluation dataset inclusive of 20 records, chosen at random within experiment and treatment in order to adequately represent the source-database.

Stepwise regression analysis

A stepwise regression analysis was then conducted on the training dataset in order to select the most explanatory variables of HDMI among the variables *ex-ante* screened from each class. The "entry" and "stay in" thresholds were set at $P<0.15$.

The goodness of fit was evaluated on the basis of R^2, root mean square error (RMSE) and predicted residual sum of square (Press). Residuals of all the selected models were evaluated for their normality using the Anderson and Darling test. In no occasion the test was significant at $P<0.05$.

The best models were then evaluated for the presence of extreme points which can influence in an inordinate fashion, without possible explanation, the relationship between regressors and response variable. After running a preliminary stepwise OLSR, using the studentized deleted residue test, n. 9 points were identified and deleted from the calibration dataset and 1 from the validation dataset, using the same model for both steps.

Further analysis were run on both the full data (FD) dataset and the outlier-free (OF) dataset .

The best model sourced from the stepwise analysis was then submitted to a step by step reduction of the regressors, in order to evaluate the changes of goodness of fit along with the increase of parsimony. To this end, variance inflation factor of each regressor (VIF) was considered as multicollinearity criterium, as suggested by Rook et al. (1990b).

Finally, the best models were evaluated using the evaluation subsets, according to Tedeschi (2006), implementing the Model Evaluation System (MES, release 3.1.13.). At this stage, model evaluation was based, besides the already quoted parameters, on the mean bias, means square error of prediction (MSEP) and its components, bias, line, and random errors expressed as MSEP percentages. Furthermore, the difference between the intercept and 0 and the slope and 1 of the regression of the measured (Y) upon predicted values (X) were evaluated by both independent and simultaneous tests (Dent and Blackie, 1979), the latter resuming the overall accuracy of the models with respect to mean and slope bias.

Partial Least Square Regression (PLSR)

This method was implemented to accommodate the unavoidable multicollinearity usually entailed by OLSR when inclusive of several regressors (Hubert and Branden, 2003). This method, while extracting the latent factors which contain most of the information of the original regressors without their redundancy, is able to take into account the variance-covariance matrix of predictors but also of response variables, when implemented as multivariate technique. Thanks to this distinctive property, this technique can provide better precision performance than regressions based on principal component analysis or OLSR, as recently shown, by Dimauro et al. (2013).

The PLSR was implemented for model development and their evaluation in four data scenarios:

- all variables (34) - all data (N=124 in calibration and N = 20 in validation datasets);
- all variables– outlier-free data (N = 115 in calibration and N=19 in validation datasets);
- selected variables (15, the same used in the previous stepwise regression analysis) – all data;

- selected variables - outlier-free data.

The adjusted R^2 was used as criterion to optimize the number of latent variables to be included in the model. The performance of PLSR in the evaluation step was evaluated as previously described for the stepwise regression analysis. To this end, it was also compared to evaluation results of full model OLSR stepwise, run on the same databases. In this case no model reduction was performed, mimicking an euristic approach, which is usually associated with the analysis of laboratory data, usually fully available (e.g. spectra of NIRS).

Sensitivity analysis

Finally, a brief sensitivity analysis was run to assess the effects on HDMI of increasing proportionally by 1.5 and 2.0 MY and NDFH from a previously set basal level.

3. Results

Correlation analysis

The correlation coefficients show that HDMI was linearly correlated with several variates, belonging to all the factorial classes already quoted (Table 2). Namely, MY was ranked as first among the animal related variables for its correlation coefficient (0.70), which was also numerically the highest for the relationships between HDMI and putative regressors. A negative strong relationship was found between HDMI and the content of NDF in the grazed herbage (NDFH, -0.63), which overcame in absolute value the positive coefficients of other herbage quality components such as EE, CP, NFC contents or the net energy content measured as feed units (UFL/kg DM).

Variates related to feeding management were moderately correlated with HDMI, with reference to the intake of supplemented maize and lupins (GCDMI, -0.51) or the time access to pasture (TA, 0.50).

Lower - although significant - correlation coefficients linked the response variable to pasture characteristics (leaf area index, (LAI), in particular) and some weather features, namely mean air temperature (TMEAN, -0.26) and minimum relative humidity (RHMIN, 0.24).

The correlations among the explanatory variables were sometimes moderate or strong. For instance, MY was negatively correlated with NDFH (-0.77) but displayed also relatively high correlation coefficients with other herbage quality variates (e.g. CP, 0.77), pasture quality variates (LAI, 0.48) and weather variables (maximum daily temperature (TMAX) and TMEAN, -0.46).

Stepwise regression analysis

Stepwise regression analysis was based on the *ex-ante* selected putative regressors, which included, besides the variates indicated in Table 1, the quadrat of the time access to pasture (TA^2). In fact, this variate had been proven significantly correlated to HDMI in previous literature review (see Chapter 1). The preliminary exclusion of some pasture variables, such as LAI, was due to the smaller dataset available for these variables (96 vs 144 records) but also the moderate to high correlation coefficients between these variables and some of the pasture putative regressors selected for the stepwise (Table 2).

The stepwise process was run in four steps, progressively excluding from the model the variables non significantly ($P<0.05$) related to HDMI. However, at step 4, the number of significant regressors (8) was still deemed too high to avoid marked multicollinearity and probable overfitting. Therefore, two variables were discarded from the model (RHMAX and WINDMEAN). This reduction showed a limited effect on model precision (c.a. 0.03 unit from R^2).

The resulted model sourced from the complete database and other derived models are described in Table 3. The 6 regressor model (model 6, m6FD) included only five significant predictors ($P<0.05$), being sward height coefficient only close to significance level ($P<0.1$). The model explained ~ 0.80 of HDMI variance, with a RMSE of 217 g DM but with some moderate and some high VIF for TA and TA^2in particular.

This model was then reduced by the omission of the non-significant regressor (SH, see m5FD) without any marked drop of R^2or increase of RMSE. In this model HDMI depended on: MY, increasing by 0.38 kg DM for kg of MY, NDF in the grazed herbage, decreasing by 0.037 kg DM for any percent unit of NDFH. Furthermore, HDMI was quadratically related to TA with an increase by 0.519 kg DM per hour of access to pasture, partially compensated by a decrease of 0.049 kg DM per TA^2.

Subsequent reduction steps (m4) was aimed at assessing the effect of the exclusion of one of the pasture quality regressors, which were correlated each other (NDFH and DMH, see Table 2). The best model sourced by this reduction (m4FD, Table 3 and Figure 2a) showed only a slight decrease in terms of $R^2$and increase of RMSE. Finally, to account for the correlation between MY and NDFH, the first variable was omitted (m3FD), with minor changes in model calibration performance (Table 3).

Evaluation of these models is also depicted in table 3. Evaluation performances were, as expected, lower than calibration performances, with R^2 close to 0.60 and RMSEP ~281 g DM. Overall all evaluation models showed a slight mean bias towards under prediction and a relative inaccuracy, being the simultaneous test of the intercept and regression slope different from the targets ($P<0.001$). However, the percentage of MSEP random component was close or above 90%, with an increasing trend with model parsimony. The value of Press was slightly lower in m6FD validation model, followed by m4FD (Figure 2b).

Results of stepwise regression analysis of the outlier-free databases are shown in Table 4.

In this case, only two steps were needed to reach the 8 regressor stage. Again the above quoted weather variates (RHMAX and WINDMEAN) were significant but they were omitted from the model due to parsimony purpose. Likewise for the full data dataset, calibration performance was overall unchanged after this omission. The outlier-free dataset training model with 6 variables (m6OF, Figure 3) included five out of the six variables kept by the m6FD training model: namely MY, NDFH, SH, TA and TA^2 , being DMH replaced by UFLH (Table 4, Figure 3). The model explained 0.86 of the total variance of HDMI with a RMSE of 178 g DM, and a relatively low VIF for all the variates with exception of NDFH, TA and TA^2. In this case all regressors were significant.

Subsequent steps were model reductions based on the contribution of the variates to the explanation of the response variance (not shown), taking into account also the collinearity of regressors, as indicated by high VIF.

Hence UFLH was first excluded (m5OF), being negatively associated with NDFH. This did not badly affect the overall model performance (Table 4). The following step (m4OF, Table 4 and Figure 4a) was aimed at evaluating the effect of the omission of SH due to its lower impact on variance explanation. A further step in model simplification was operated in m3OF, discarding the effect of MY, as was the case of m3FD in Table 3.

As expected, model reduction overall tended to decrease R^2 and inflate RMSE but these changes were minimum comparing m6OF with m4OF and mild even when comparing m6OF to m3OF.

The evaluation of the OF regressions models (Table 4) showed overall a better performance than that resulting from the analysis of the full database. In fact, R^2 ranged between 0.66 and 0.68 with RMSEP around 250 g DM. Mean bias was ~30-40 g but, and apart from m6OF, all the models showed a good accuracy, being intercept and slope not different from targets (P>0.05). Random error share was between 95-85%, again with higher levels in the most parsimonious model (m3OF). The best (lowest) Press value was detected in m5OF model,followed by m4OF (Figure 4b).

Partial Least Square Regression (PLSR)

Training and evaluation models sourced from partial least square regression analyses are depicted in Table 5 for the four scenarios and compared with the outcome of stepwise OLSR performed on the same data. In this case no reduction of model regressors was operated.

This table show a general a trend to better performance of PLSR as compared with OLSR, particularly when all variables and all data were used. In contrast, the selection of variables and data tended to favour the OLSR performance, although even in these conditions PLSR performed very closely to OLSR. Interestingly, all models were overall acceptably accurate, although only in the selected variables all data, and selected variables outlier-free data scenarios the random error proportion accounted almost 90% of the MSEP.

Sensitivity analysis

Herbage intake, while affected by the overriding quadratic effect of time access to pasture showed to be quite sensitive (as average values) to MY (Figure 5) but NDFH had numerically much bigger impact on HDMI (Figure 6).

4. Discussion

Ruminant intake modelling is the subject of a wide literature, although only recently the interest for estimating intake in grazing ruminants has resumed, thanks to upgrading in the estimation methods and modelling theory and applications. As often occurs, small ruminant lag behind dairy cattle and this is certainly the case of dairy sheep, despite the role of pasture in their feeding management, in EU and non EU Mediterranean Countries.

Problems with modelling the intake of grazers were brilliantly reviewed by Dove (1996). Unavoidable errors of estimates, absence of reference data for proper on-field validation, low reproducibility of the environmental conditions all play a role in decreasing the robustness of any prediction, even when model development and evaluation is done within precise boundaries of local conditions with reference to animal, pasture, management and environment features.

This modelling exercise has the above characteristics, since it refers to models which are calibrated and “validated” within specific local conditions. However, this paper, unlike others (e.g., Avondo et al., 2002) while explicitly addresses the problem of low precision of intake models, implements updated statistical tools to tackle it.

Correlation analysis

The results of the correlation analysis confirm the multifactorial nature of intake and, in a sense, the rationale of the conceptual model depicted in Figure 1. In fact positive drivers of intake associated to animal requirements (MY and BW) are counteracted by factors related to rumen fill (NDFH). Herbage quality factors (NDFH, CPH, EEH, UFLH) were more strongly related to intake than regressors associated with herbage accessibility such as SH, HM or HA. This was probably due to stocking density and the rotational scheme implemented in the experiments from which the database was sourced. In fact, stocking density was adjusted in order to avoid any marked deficit of herbage mass or allowance. However, this did not prevent the effect of pasture depletion on herbage quality as shown by Molle et al. (2014) and in chapter 3 of this thesis.

Interestingly, in this database the substitution effects of supplementation when herbage mass and SH are not limiting was confirmed, although the negative relationship with HDMI was evident only for the grain or pulses components of supplementation (whole lupin or maize). These concentrates can elicit rumination activity which can conflict with the grazing and hence with intake, as previously shown by Molle et al. (1996) in ryegrass grazing sheep, supplemented with whole maize. Other aspects can be evoked, such as the effect of starchy concentrates on rumen environment and hormone profiles which can favour a lower forage intake (Cannas et al., 2013).

The part-time grazing management did affect herbage intake, as shown by the correlation between intake and TA. This regressor tended to have a prominent effect, although not linear (see regression analysis and discussion of it below).

Herbage Intake was weakly related to weather conditions, which can be understood considering that the experiments were carried in spring, in a period when extreme conditions affecting intake or performance are rare. Despite this there were some variates significantly associated with HDMI, such as air temperature parameters (all negatively) and RHMIN (positively). Temperature correlation coefficients are probably explainable with the trend to lower intakes shown in both experiments along with the decay of pasture quality and the advancement of lactation.

Overall the strength of the associations between herbage intake and the explanatory variables under scrutiny was numerically higher than those put in evidence by Avondo et al. (2002) for many variables except for the indicators of herbage accessibility, such as HM or SH. This could be due to the use of individual instead of average data (with exception of pasture variables) and wider range of experimental conditions encompassed by Avondo et al. (2002) in their database.

Stepwise regression analysis

The focussed analysis of data based on *ex-ante* variable selection and subsequent stepwise OLSR, resulted in models with a maximum of 6 regressors using both the complete database (Table 3) and the outlier-free database (Table 4). In both cases the reduction of the model with the omission of the sixth variate did not result in much poorer performance, in both calibration and evaluation models, particularly in the outler-free database . The reduction to three variates (m3OF, Table 4, NDFH, TA and TA^2) was quite effective in maintaining the levels of precision acceptable as compared with the full rank model (m6OF). All reductions were associated with an increase of the intercept, which is expected, because it resumes the effect of all the unexplained factors. This could increase the bias of the model, which was not so evident, as shown. In general, at the same level of imprecision, the lower is the MSEP proportion associated with the bias and the line (slope), the higher is the accuracy of the model. In all models the random MSEP share prevailed, but in the validation regressions of full database (with outliers) intercept and slope differed from the ideal 0 and 1, respectively. In general, precision and accuracy was better when regressions were applied to the outlier-free database.

In dairy cows, a meta-analytical modelling exercise was run by Vasquez and Smith (2000) using OLSR analyses of a rather heterogeneous database , including *ex-post* intake estimates (based on animal requirements). The best calibration equations explained between 0.91 and 0.78 of HDMI variance with low CV (8% and 11%, respectively) but these results could be possibly affected by multicollinearity and overfitting since the number of regressors were 8 and 7, respectively. No validation was performed, although a comparison between predictions and other model sourced data did show marked differences among estimates, particularly at low herbage allowance.

Rook and Yarrow (2002), while modelling the herbage intake of dairy cows grazing perennial pastures in UK, using both OLSR and ridge regression found that HDMI was mainly dependent upon MY, concentrate DM intake and days in milk, although in some regression also sward height (SH), milk fat (MF) and the rate of ruminating chew (chews/min) were explanatory to some extent. The calibration exercise gave however moderate to poor R^2 and RMSE as high as 40-50% of average observed HDMI.

More recently, reviewing the precision of nine empirical (multiple regressions) and mechanistic models for the estimation of HDMI in cows grazing temperate grassland, Delagarde and O'Donovan (2005) found that R^2 of validation equations ranged between 0.14 and 0.68 depending on model and dataset, with MSEP around 10-15% of the mean in the best models. Among the best models there were Grazein and a simple model based on OLSR.

In dairy sheep, some of the most important regressors in our study were common to those found by Avondo et al. (2002) and subsequently reviewed by Avondo (2005). Among them MY, although in that case normalized for fat and protein content (FPNMY), and pasture SH. Interestingly the value of regression coefficients of FPNMY were relatively close to the ones of MY in this study (0.28, and 0.33 in two out of three equations, Avondo, 2005). This author however considered individual rather than mean group intake and did not account for the effect of time access to pasture which was as steady as 5-6 h/d.

Conversely, supplementation level and quality changed probably more in the database by Avondo et al. (2002) than in ours. Hence it is quite understandable that in this modelling exercise, unlikely that by Avondo et al. (2002), the supplementation-related regressors were basically overlooked by the stepwise selection.

Keeping all the above in mind, it is quite understandable that calibration performance of the OLSR herein described are overall better than those quoted in the papers by Avondo et al. (2002) and Avondo (2005). Furthermore no validation was performed to our knowledge of the equations suggested in those papers.

An integration of different locally-based databases, could be a way to overcome the limitations of local empirical modelling, allowing for wider and possibly more robust inference, provided that HDMI estimates are sound and proper data screening is applied.

<u>Partial Least Square Regression analysis</u>

Table 5 displays the output of analysis when it proceeds as "one step".

PLSR confirmed to be an effective statistical technique to overcome collinearity problems by substituting the original variates with orthogonal latent components. This was proven useful to improve the stability of the prediction models as shown in Table 5. In fact, in the evaluation models, PLSR reached much higher coefficients of determination and lower RMSEP than the OLSR evaluation models based on the all variable all data and all variables outlier-free scenarios. (Table 5). PLSR outperformed OLSR even when only the selected variables were used (selected variables all data scenario). In contrast, no advantages were detected when data and variable were selected (selected variables outlier-free scenario, Table 5).

Overall, these results point out that PLSR can successfully handle modelling of datasets containing extreme and influential points, providing "validation" results better than those sourced by running stepwise OLSR, unless the database is reduced in terms of variables and devoid of outliers.

This is fundamental especially when the calibration dataset is properly external and may contain extreme points which can possibly indicate meaningful deviations, although so far undermined. This was not probably the case in our database, since outliers were sourced from different treatment groups, grazing different forages and with either low or high measured intakes. Elimination of outliers is an accepted step in the processing of data before modelling (e.g. Rook et al., 1990a).

Although in this study PLSR did not prove to outperform OLSR when the database was reduced in size and devoid of extreme points, this does not allows to conclude that OLSR was better, since multicollinearity was not addressed by OLSR and stability of predictions could be at risk, particularly if no sound model reduction is implemented.

Overall the validation performance of PLSR was more stable and this confirms its adequacy in modelling multifactorial response variables. However, the putative advantage of PLSR was not fully challenged in this study since it was used as an univariate rather than a multivariate technique. For instance, a multivariate PSLR approach can be envisaged using two instead of one response variable such as considering the two components of herbage intake (Allden and Whittaker, 1970) i.e. grazing time and intake rate instead of their product (herbage intake).

Other statistical techniques can be used to upgrade empirical modelling of intake and may deserve further consideration in future. Ridge regression, a statistic technique aimed at controlling the multicollinearity and stability of predictions has been successfully applied by Rook et al. (1990b) to predict silage intake in cattle. In that case ridge outperformed many OLS models, even after model reductions (Rook et al., 1990b and 1990c).

Although PLSR and multivariate approach can be useful to advance in intake empirical modelling, their use is possibly more suitable to conditions were many variables are easily detectable, e.g., to process thousands of data recorded by automatic feeding behaviour recorders. In fact, PLSR need quite big full-rank database (no missing data). In grazing studies many variables are difficult or heavy to measure, so this condition cannot be easily fulfilled.

<u>Sensitivity analysis</u>

Results of the sensitivity analysis showed that in part-time grazing sheep, increasing the NDF content of the grazed herbage by 50% and 100% (from 30% to 45% and 60% on DM basis) had an overwhelming negative effect on HDMI (Figure 6), much more evident than the positive effect of MY when it was increased by the same proportions (from 1000 to 1500 and 2000 g/ewe day, Figure 5).

Interestingly, when HDMI was divided by the time of access (hours), a higher sensitivity was displayed at short than long time access to pasture for both MY and NDFH (Figures 7 and Figure 8, respectively). This provides evidence that, when time access is severely restricted, it is fundamental to offer high quality (low NDF) forages to dairy sheep, particularly if at high milk production level.

5. Conclusions

The data analysis carried out so far is the first step of a process aimed at advancing our knowledge on the mechanisms underlying the foraging of part-time grazing dairy sheep.

According to the above results, under the conditions of the studies included in our database, HDMI was positively related to animal factors (MY in particular), managerial factors (time access to pasture) and negatively with the content of herbage NDF, which is a putative proxy of rumen fill.

The predictive equations obtained using OLSR procedure as implemented on the full or outlier-screened database showed that the best models with 6 regressors explain at best 0.86 of the variance of herbage intake, with a CV between 15% and 20%, depending on models.

Reduced models set to curb multicollinearity, performed as well as the full rank ones, particularly under outlier-free conditions. The "validation" based on an external database sourced from the same data pool, provided the best performances when the most relevant variates were preliminarily selected and outliers screened out.

The equations obtained using the PLSR were more stable to extreme points and devoid of multicollinearity problems but their evaluation performance was markedly better than that of OLSR models only when all regressors were used in both training and evaluation databases.

A preliminary sensitivity analysis of one of the simplest model provides encouraging and interesting results but warrants further delving.

Overall this paper represents an advancement in the prediction of herbage intake in dairy sheep, increasing its predictability within the conditions set by the database: supplemented milked sheep part-time grazing grass or legume pastures in spring, without marked constraints of herbage accessibility.

Further advancements in the empirical modelling of intake in grazing ruminants could source from the integration of local experiment-based datasets, and the use of advanced statistical methods to analyse them, accounting for the influence of extreme points and multicollinearity. PLSR is among these statistical techniques but other approaches can be envisaged.

Mechanistic and mechanistic-dynamic modelling can provide further biologically-sound progress towards a better estimation of herbage intake in grazing ruminants. However, these forefront approaches need a well-rooted body of knowledge on the sub-models they are based on (grazer, pasture, management and environmental conditions) which has still to be built for Mediterranean grazing systems.

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Table 1. Database characteristics. Asterisks indicates the regressors selected for modelling.

Variable	Acronym	Unit	N	Mean	SE	Max	Min
Response variables (Y)							
Herbage DM intake	HDMI	g/ewe d	144	1269	39	2452	326
Regressors (X)							
Milk yield*	MY	g/ewe d	144	1321	31	2236	737
Fat normalized MY	FNMY	g/ewe d	144	1254	27	2020	711
Milk fat*	MF	%	144	6.05	0.04	7.51	5.01
Milk protein	MP	%	144	5.03	0.02	5.61	4.52
Body weight*	BW	Kg/ewe	144	43.3	0.2	49.2	36.4
Body condition score	BCS		144	2.48	0.01	2.74	2.28
Hay intake	HADMI	g DM/ewe d	144	526	8	775	202
Grain and pulse intake*	GCDMI	g DM/ewe d	144	264	0.1	263	265
Pelleted concentrate intake	PCDMI	g DM/ewe d	144	347	2	357	209
Total concentrate intake	TCDMI	g DM/ewe d	144	612	2	622	474
Total supplement DM intake	TSDMI	g DM/ewe d	144	1137	9	1397	758
Access time*	TA	Hours/d	144	4.0	0.0	6.0	2.0
Dry matter*	DMH	%	144	17.12	0.21	24.35	13.14
Ash	ASHH	% DM	144	12.88	0.14	8.67	18.78
Ether extract	EEH	% DM	144	4.40	0.09	6.31	2.28
Crude protein	CPH	% DM	144	18.13	0.37	25.27	9.10
NFC	NFCH	% DM	144	25.05	0.38	33.93	15.38
NDF*	NDFH	% DM	144	39.54	0.66	60.25	26.91
ADF	ADFH	% DM	144	21.90	0.22	31.84	16.86
ADL	ADLH	% DM	144	2.21	0.11	5.21	0.10
Net energy (UFL)*	UFLH	UFL/kg DM	144	0.96	0.01	1.14	0.49
Sward height*	SH	cm	144	18.7	0.7	43.6	6.3
Herbage mass*	HM	t DM/ha	144	1.93	0.06	3.83	0.55
Herbage allowance*	HA	kg DM/ewe d	144	2.9	0.8	6.1	0.1
Green herbage allowance	GHA	kg DM/ewe d	96	2.8	0.1	6.1	0.7
Leaf Area Index	LAI		96	2.2	0.1	4.7	0.3
Air temperature mean*	TMEAN	C°	144	13.0	0.2	19.9	9.5
Air temperature min	TMIN	C°	144	6.4	0..2	14.4	1.9
Air temperature max	TMAX	C°	144	18.9	0.3	29.8	15.1
Relative humidity mean	RHMEAN	%	144	84.5	0.4	92.0	74.0
Relative humidity min	RHMIN	%	144	95.8	1.0	98.0	89.0
Relative humidity max*	RHMAX	%	144	67.9	1.0	90.0	32.0
Rainfall	RAIN	mm/d	144	0.41	0.06	3.00	0.00
Wind speed mean*	WINDMEAN	m/s	144	1.65	0.05	3.00	0.60
Wind speed max	WINDMAX	m/s	144	7.79	0.23	16.20	3.40

Table 2 – Correlation coefficient. In italics P<0.05; italics and bold P<0.01; Italics, bold and underlined P<0.001. For acronyms see Table1.

	HDMI	HADMI	PCDMI	GCDMI	TCDMI	TSDMI	MY	MF	MP	FNMY	BW	BCS	DMH	EEH	CPH	ASHH	NFCH	NDFH	ADFH	ADLH	UFLH	SH	HM	LAI	HA	GHA	Tmax	T min	Tmean	RHmax	RHmin	Rhrmean	Rain	Windmean	Windmax	TR
HDMI	1.00																																			
HADMI	-0.35	1.00																																		
PCDMI	-0.07	0.14	1.00																																	
GCDMI	-0.51	0.24	0.04	1.00																																
TCDMI	-0.09	0.14	1.00	0.08	1.00																															
TSDMI	-0.35	0.98	0.34	0.25	0.35	1.00																														
MY	0.70	-0.49	-0.01	-0.64	-0.03	-0.47	1.00																													
MF	-0.40	0.31	0.07	0.12	0.07	0.31	-0.54	1.00																												
MP	-0.11	-0.01	-0.08	-0.11	-0.08	-0.02	-0.09	0.36	1.00																											
FNMY	0.68	-0.46	0.01	-0.68	-0.02	-0.44	0.98	-0.39	-0.03	1.00																										
BW	0.44	0.04	0.09	-0.15	0.09	0.05	0.20	-0.18	-0.17	0.19	1.00																									
BCS	-0.11	0.18	0.11	0.14	0.11	0.20	-0.22	0.02	-0.13	-0.23	0.17	1.00																								
DMH	-0.37	0.23	-0.04	0.63	-0.02	0.22	-0.68	0.19	0.01	-0.72	0.03	0.23	1.00																							
EEH	0.52	-0.30	0.02	-0.71	0.00	-0.28	0.73	-0.22	0.09	0.75	-0.01	-0.22	-0.74	1.00																						
CPH	0.51	-0.20	0.06	-0.72	0.03	-0.19	0.77	-0.27	-0.01	0.79	0.11	-0.23	-0.82	0.89	1.00																					
ASHH	0.00	0.06	0.20	-0.11	0.19	0.10	0.19	-0.12	-0.22	0.20	0.01	-0.16	-0.47	-0.04	0.18	1.00																				
NFCH	0.48	-0.35	-0.24	-0.40	-0.25	-0.39	0.36	-0.12	0.25	0.36	0.03	-0.03	-0.13	0.42	0.26	-0.44	1.00																			
NDFH	-0.63	0.34	0.06	0.75	0.08	0.34	-0.77	0.28	-0.10	-0.79	-0.08	0.21	0.73	-0.87	-0.87	-0.05	-0.68	1.00																		
ADFH	-0.46	0.39	0.09	0.40	0.10	0.39	-0.57	0.26	-0.11	-0.57	0.04	0.20	0.50	-0.62	-0.60	-0.11	-0.62	0.79	1.00																	
ADLH	0.42	-0.03	0.10	-0.68	0.07	-0.02	0.64	-0.27	-0.11	0.65	0.16	-0.16	-0.68	0.66	0.80	0.26	0.09	-0.64	-0.18	1.00																
UFLH	0.50	-0.35	-0.06	-0.50	-0.07	-0.35	0.54	-0.16	0.15	0.56	-0.05	-0.15	-0.55	0.79	0.67	-0.15	0.67	-0.83	-0.84	0.25	1.00															
SH	0.11	-0.07	-0.18	-0.05	-0.18	-0.10	0.00	0.14	0.16	0.02	0.10	0.10	0.12	0.10	-0.04	-0.48	0.28	-0.05	0.14	-0.02	-0.03	1.00														
HM	0.18	0.04	-0.17	-0.33	-0.18	0.00	0.09	0.10	0.19	0.12	0.16	0.09	0.06	0.20	0.10	-0.40	0.32	-0.18	0.09	0.19	0.01	0.80	1.00													
LAI	0.28	-0.33	-0.05	-0.41	-0.07	-0.33	0.48	0.01	0.17	0.53	-0.22	-0.30	-0.55	0.64	0.49	0.00	0.29	-0.60	-0.55	0.24	0.59	0.54	0.59	1.00												
HA	0.17	-0.22	-0.21	-0.32	-0.22	-0.25	0.25	-0.06	0.19	0.25	0.05	-0.03	0.03	0.32	0.19	-0.46	0.34	-0.25	-0.02	0.21	0.11	0.63	0.86	0.58	1.00											
GHA	0.19	-0.38	-0.07	-0.41	-0.08	-0.38	0.46	-0.05	0.19	0.49	-0.21	-0.21	-0.39	0.68	0.48	-0.21	0.26	-0.54	-0.37	0.29	0.60	0.36	0.65	0.69	0.97	1.00										
Tmax	-0.19	0.33	0.08	0.34	0.09	0.34	-0.42	0.11	-0.16	-0.43	0.11	0.23	0.48	-0.34	-0.39	-0.41	-0.16	0.44	0.57	-0.18	-0.44	0.44	0.32	-0.16	0.15	-0.29	1.00									
T min	-0.33	0.40	0.15	0.31	0.16	0.41	-0.46	0.28	-0.18	-0.45	0.01	0.23	0.34	-0.47	-0.40	-0.09	-0.46	0.57	0.73	-0.15	-0.61	0.11	0.04	-0.42	-0.11	-0.38	0.64	1.00								
Tmean	-0.26	0.46	0.10	0.30	0.11	0.46	-0.46	0.20	-0.18	-0.47	0.11	0.28	0.39	-0.37	-0.33	-0.29	-0.33	0.48	0.69	-0.09	-0.52	0.28	0.22	-0.36	0.06	-0.25	0.87	0.87	1.00							
RHmax	0.07	0.40	0.13	0.16	0.13	0.40	-0.29	0.22	-0.11	-0.26	0.07	0.16	0.17	-0.20	-0.17	-0.04	0.02	0.12	0.08	-0.08	-0.03	-0.03	-0.05	-0.28	-0.31	-0.47	0.16	0.14	0.09	1.00						
RHmin	0.29	-0.06	0.00	-0.41	-0.01	-0.06	0.35	-0.02	0.04	0.38	-0.02	-0.08	-0.55	0.39	0.49	0.34	0.13	-0.47	-0.36	0.43	0.39	-0.32	-0.23	-0.08	-0.19	0.13	-0.71	-0.25	-0.41	0.17	1.00					
Rhrmean	0.24	0.17	0.14	-0.27	0.13	0.18	0.13	0.09	-0.06	0.17	0.03	0.01	-0.32	0.18	0.28	0.32	0.01	-0.26	-0.14	0.37	0.17	-0.26	-0.18	-0.22	-0.27	-0.17	-0.45	-0.06	-0.25	0.59	0.84	1.00				
Rain	-0.21	0.23	0.02	-0.02	0.01	0.22	-0.28	0.22	0.00	-0.27	0.12	0.15	0.28	-0.28	-0.24	-0.24	-0.24	0.36	0.68	0.08	-0.54	0.28	0.31	-0.36	0.24	-0.17	0.50	0.70	0.64	-0.01	-0.23	-0.10	1.00			
Windmean	-0.17	0.00	0.03	0.22	0.04	0.01	-0.18	0.19	0.03	-0.17	-0.15	0.05	0.27	-0.25	-0.27	-0.08	-0.33	0.39	0.35	-0.25	-0.34	0.14	0.09	0.45	0.11	0.47	0.11	0.30	0.24	-0.16	-0.10	-0.10	0.05	1.00		
Windmax	-0.21	0.02	-0.02	0.20	-0.01	0.02	-0.21	0.15	0.03	-0.22	-0.07	0.08	0.44	-0.36	-0.36	-0.22	-0.29	0.46	0.50	-0.22	-0.52	0.22	0.27	0.27	0.32	0.44	0.28	0.42	0.37	-0.18	-0.28	-0.22	0.30	0.89	1.00	
TRT	0.50	-0.28	-0.10	0.00	-0.10	-0.28	0.24	-0.27	-0.33	0.21	0.47	0.02	0.05	-0.08	-0.03	0.07	-0.04	0.04	0.05	0.02	-0.07	-0.02	-0.04	-0.13	-0.04	-0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	HDMI	HADMI	PCDMI	GCDMI	TCDMI	TSDMI	MY	MF	MP	FNMY	BW	BCS	DMH	EEH	CPH	ASHH	NFCH	NDFH	ADFH	ADLH	UFLH	SH	HM	LAI	HA	GHA	Tmax	T min	Tmean	RHmax	RHmin	Rhrmean	Rain	Windmean	Windmax	TR

Table 3. Estimation of herbage DM intake. Calibration and evaluation of models including different regressors. Full database (FD).

Model	Calibration m6FD		Calibration m5FD		Calibration m4FD		Calibration m3FD	
	N = 124		*N = 124*		*N = 124*		*N= 124*	
Intercept	197.84±322.86		217.33±325.00*		920.05±302.9**		1536.50±198.15***	
Regressors	Regressors coefficient±SE	VIF	Regressors coefficient±SE	VIF	Regressors coefficient±SE	VIF	Regressors coefficient±SE	VIF
X1	+ 0.37±0.09 MY***	3.1	+0.38±0.09 MY***	3.18	+0.26±0.10 MY**	2.9		
X2	-36.87±4.44 NDFH***	3.2	-37.66±4.45 NDFH***	3.2	-30.01±4.39 NDFH***	2.7	-39.23±2.74 NDFH***	1.0
X3	46.71±12.19 DMH***	2.5	+51.85±11.91 DMH***	2.3				
X4	+4.38±2.57 SH	1.1						
X5	+524.20±84.67 TA***	50.5	519.18±85.29 TA***	50.5	+536.47±91.4 TA***	50.4	567.97±92.86 TA***	49.5
X6	-49.70±10.40 TA^2****	49.9	-49.13±10.47 TA^2***	49.8	-50.44±11.23 TA^2 ***	49.8	-52.61±11.48 TA^2 ***	49.5
R^2	0.796		0.791		0.757		0.744	
Adj. R^2	0.786		0.782		0.750		0.737	
Mean	1273		1273		1273		1273	
RMSE	217		219		235		240	
	Evaluation m6FD		Evaluation m5FD		Evaluation m4FD		Evaluation m3FD	
	N=20		*N=20*		*N=20*		*N=20*	
Pred. R^2	0.600		0.596		0.602		0.599	
Pred. adj. R^2	0.578		0.573		0.580		0.576	
RMSEP	281		283		281		282	
P < a = 0	0.231		0.332		0.551		0.761	
P < b = 1	0.240		0.223		0.374		0.600	
P < a = 0, b = 1	0.001		0.001		0.001		0.001	
Mean bias	-33		-43		-59		-48	
MSEP partition								
Mean bias %	1.42		2.32		4.46		3.11	
Systematic bias %	8.10		7.94		4.22		1.51	
Random errors %	90.48		89.74		91.32		95.38	
Press/1000	1695		1711		1702		1727	

Table 4. Estimation of herbage DM intake. Calibration and evaluation of models including different regressors. No extreme points in calibration and evaluation datasets, excluded on the basis of studentized deleted residue (Database OF).

Model	Calibration m6OF		Calibration m5OF		Calibration m4OF		Calibration m3OF	
	N = 115		*N = 115*		*N = 115*		*N= 115*	
Intercept	-556.87±488.94		585.02±245.89*		802.46±302.9**		1558.21±176.51***	
Regressors	Regressors coefficient±SE	VIF	Regressors coefficient±SE	VIF	Regressors coefficient±SE	VIF	Regressors coefficient±SE	VIF
X1	+ 0.37±0.08 MY***	3.0	+0.33±0.08 MY***	2.8	+0.32±0.08 MY***	2.8		
X2	-17.92±5.23 NDFH***	6.1	-28.46±3.54 NDFH***	2.7	-28.98±3.80 NDFH***	2.7	-40.48±2.47 NDFH***	1.0
X3	+655.02±244.60 UFLH**	3.5						
X4	+10.17±2.19 SH***	1.03	+9.42±2.23 SH***	1.0				
X5	+564.62±73.38 TA***	55.0	552.11±75.28 TA***	54.8	+545.53±80.81 TA***	50.7	+587.15±84.87 TA***	53.8
X6	-54.40±9.01 TA^2****	54.3	-52.85±9.25 TA^2***	54.1	-51.74±9.93 TA^2 ***	54.1	-54.64±10.49 TA^2 ***	53.8
R^2	0.8596		0.850		0.825		0.802	
Adj. R^2			0.8430		0.819		0.797	
Mean	1281		1281		1281		1281	
RMSE	178		181		195		207	
	Evaluation m6OF		Evaluation m5OF		Evaluation m4OF		Evaluation m3OF	
	N=19		*N=19*		*N=19*		*N=19*	
Pred. R^2	0.663		0.683		0.670		0.670	
Pred. adj. R^2	0.643		0.665		0.650		0.651	
RMSEP	253		245		250		250	
P < a = 0	0.233		0.241		0.339		0.572	
P < b = 1	0.154		0.149		0.223		0.465	
P < a = 0, b = 1	0.001		0.310		0.398		0.362	
Mean bias	-34		-41		-44		-29	
MSEP partition								
Mean bias %	1.80		2.62		3.00		1.40	
Systematic bias %	11.37		11.55		8.35		3.13	
Random errors %	86.83		85.83		88.65		95.46	
Press/1000	1349		1269		1319		1332	

Table 5. Description and evaluation of models sourced from partial least square (PLSR) and ordinary least square stepwise regressions (OLSR) as implemented to estimate herbage intake using four databases: all variables (34) all data (N=124 in calibration and N=20 in validation databases); all variables outlier-free data (N=115 in calibration and N=19 in validation databases); selected variables (15) all data; and selected variables outlier-free data.

Parameter	All variables				Selected variables			
	All data		Outlier-free data		All data		Outlier-free data	
	OLSR	PLSR	OLSR	PLSR	OLSR	PLSR	OLSR	PLSR
N. regressors	16	34	20	34	12	15	10	15
Variables in the model	PCDMI TCDMI TSDMI MY FNMY BW DMH EEH ASHH NFCH ADFH TMEAN RHMAX WINDMEAN TA TA^2	ALL		ALL	GCDMI MY MF BW DMH NDFH UFLH SH RHMAX WINDMEAN TA TA^2	SEL	MY BW DMH NDFH UFLH SH RHMAX WINDMEAN TA TA^2	SEL
Model evaluation								
R^2	0.606	0.642	0.617	0.727	0.681	0.719	0.772	0.736
RMSEP	305	299	303	246	262	248	211	231
P < a = 0	0.139	0.097	0.094	0.091	0.120	0.091	0.189	0.146
P < b = 1	0.053	0.037	0.033	0.001	0.074	0.061	0.135	0.096
P < a = 0, b = 1	0.101	0.088	0.070	0.082	0.210	0.190	0.336	0.257
Mean bias	-77	-62	-79	-59	-25	-14	-18	-21
MSEP partition								
Mean bias %	6.39	4.74	6.75	5.26	0.93	0.30	0.71	0.95
Systematic bias %	17.95	20.88	22.37	22.37	16.46	18.09	12.55	15.26
Random errors %	75.66	74.38	70.88	72.37	82.61	81.61	86.74	83.79

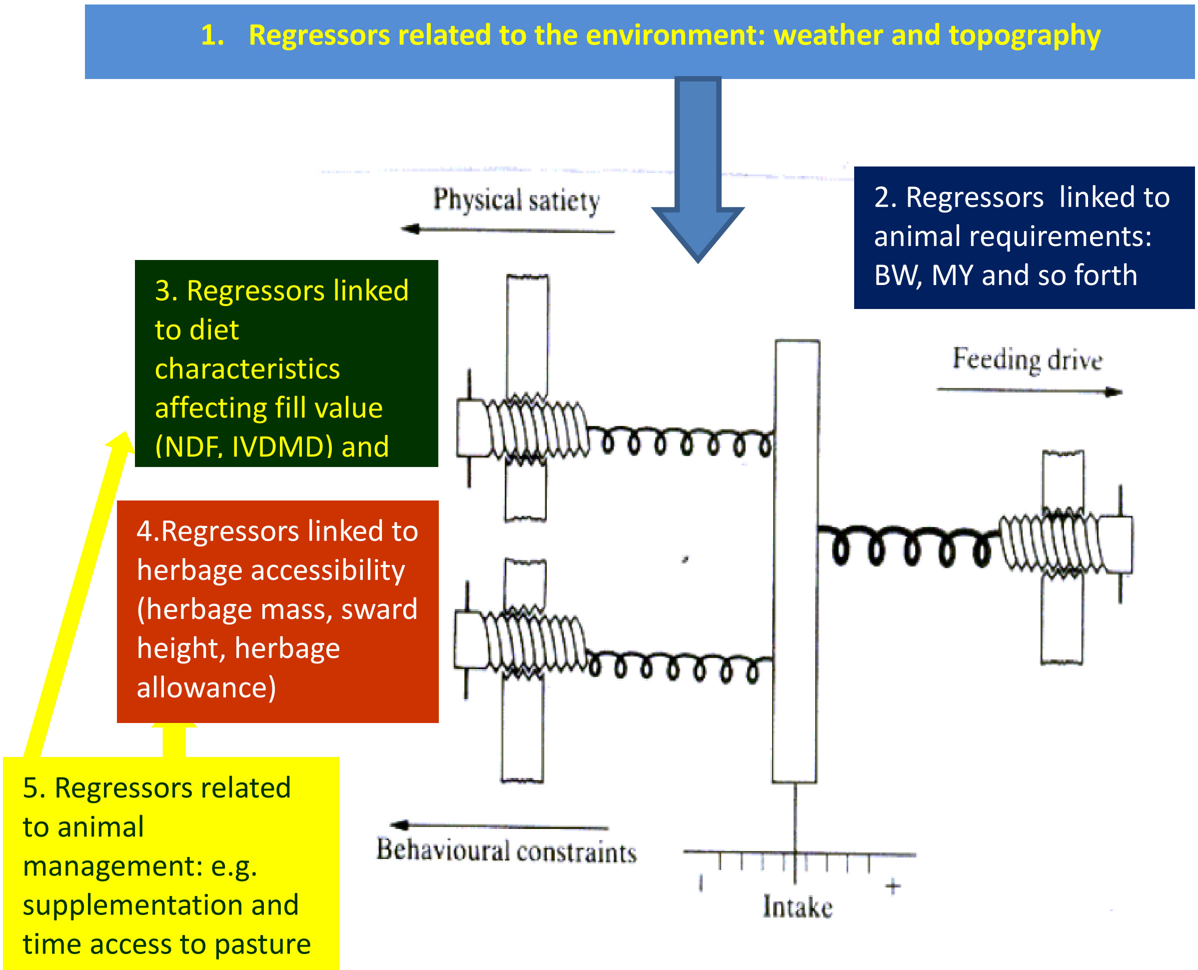


Figure 1. Conceptual model of the drivers (regressors) of herbage intake in grazing ruminants.

From top: 1) environmental factors that exert an overriding effect on the grazing ecosystem; 2) the nutrient requirements which set the animal potential intake; 3) the factors associated with diet composition and quality that impinge on rumen fill and metabolic intake control; 4) those related to herbage accessibility; and finally; 5) the factors related to animal management, inclusive of grazing management and supplementation. Among them the restriction of time access, which can act directly on herbage accessibility, but also indirectly modifying the selected diet. Modified after Hodgson, 1990.

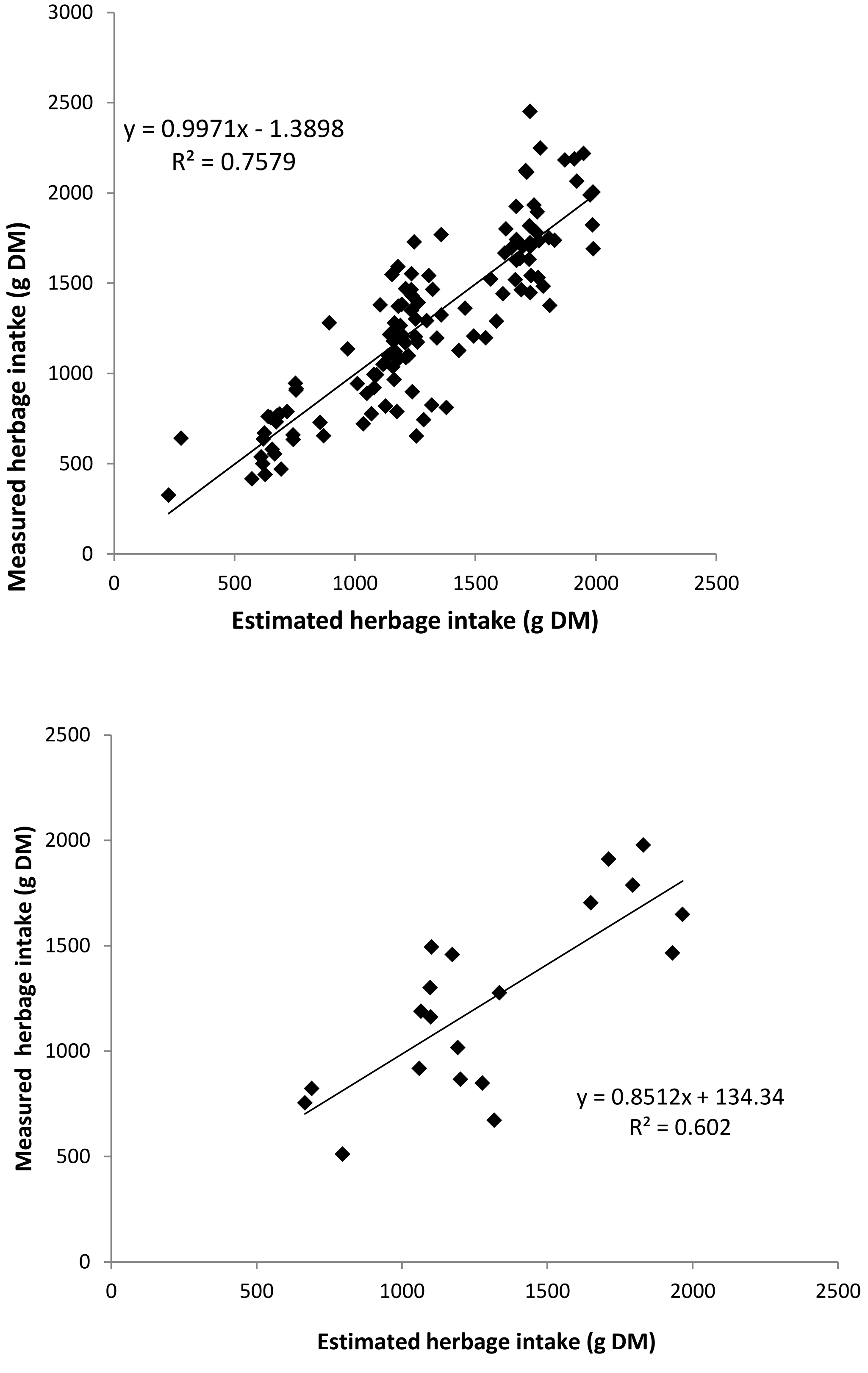
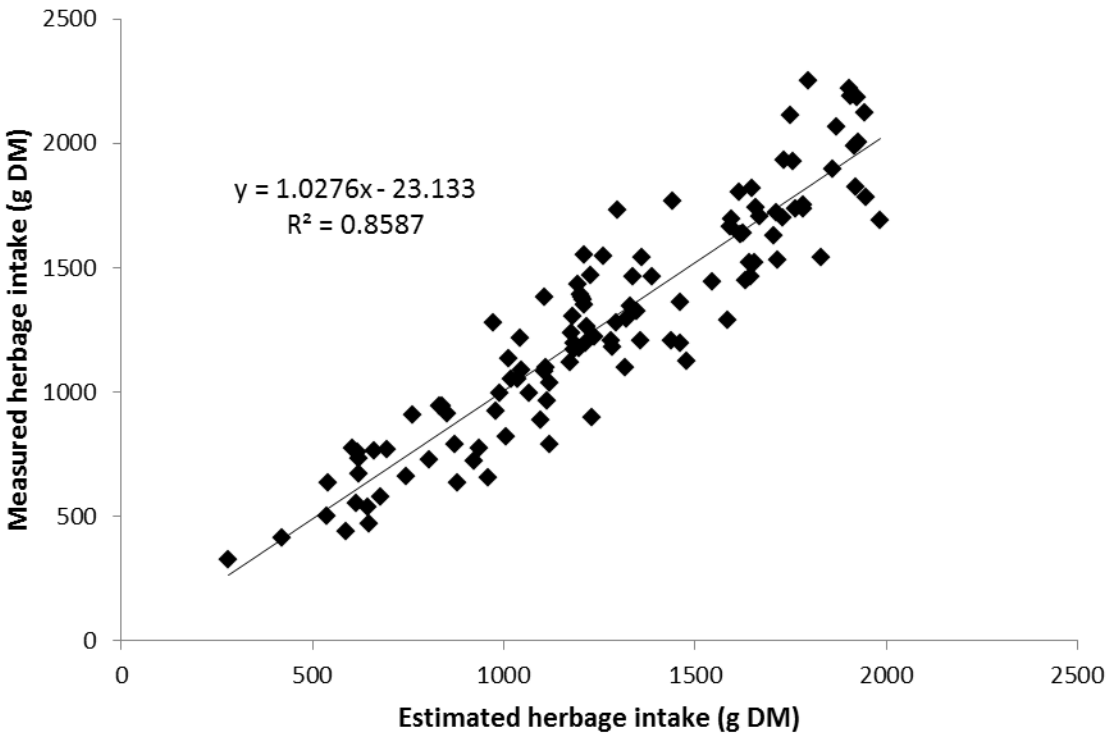


Figure 2. Model evaluation – model m4FD, top graph calibration, bottom graph evaluation



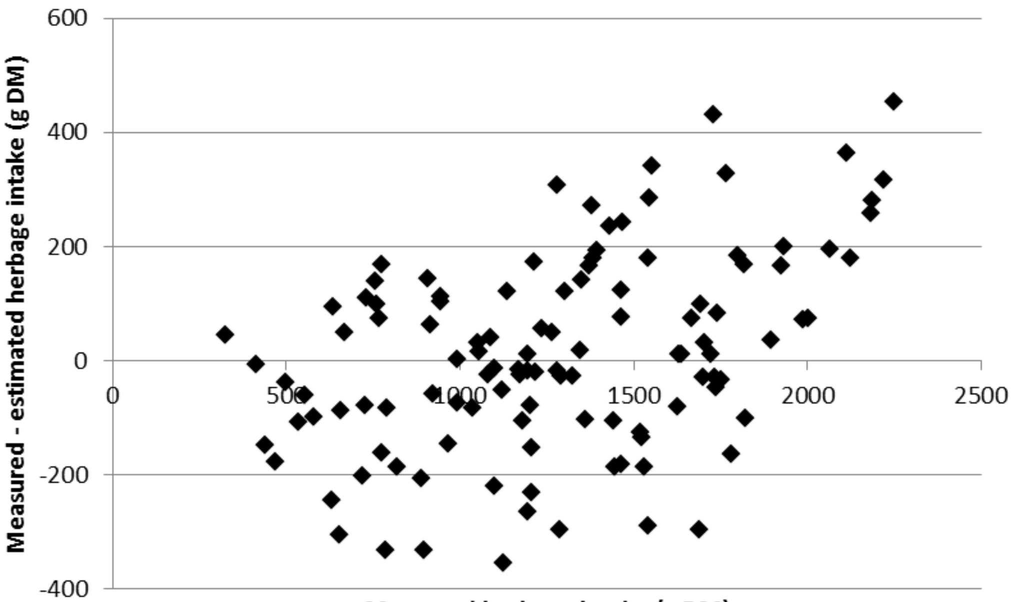
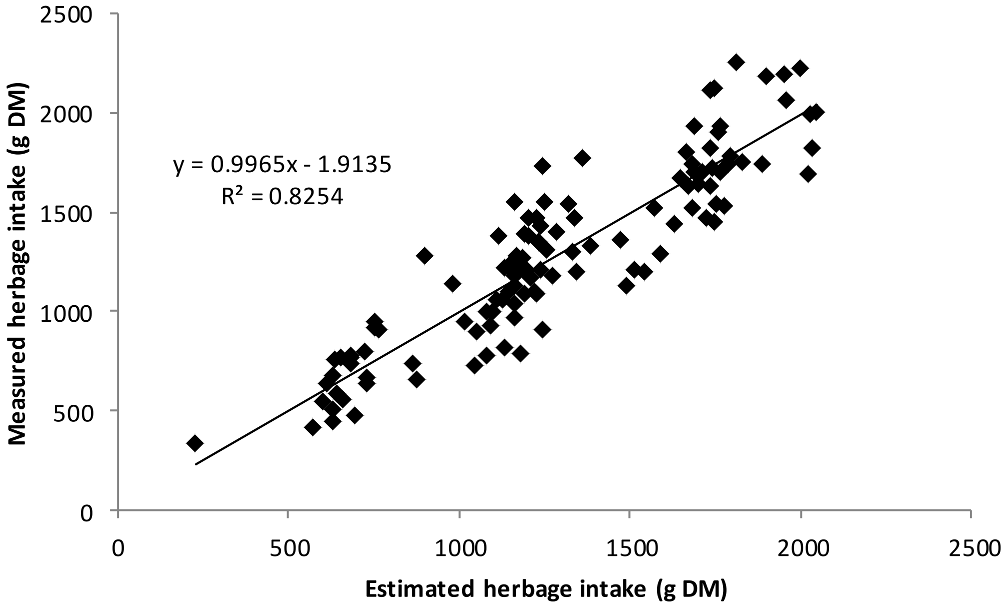


Figure 3. Model evaluation – model m6OF: top graph, model calibration, bottom graph residuals



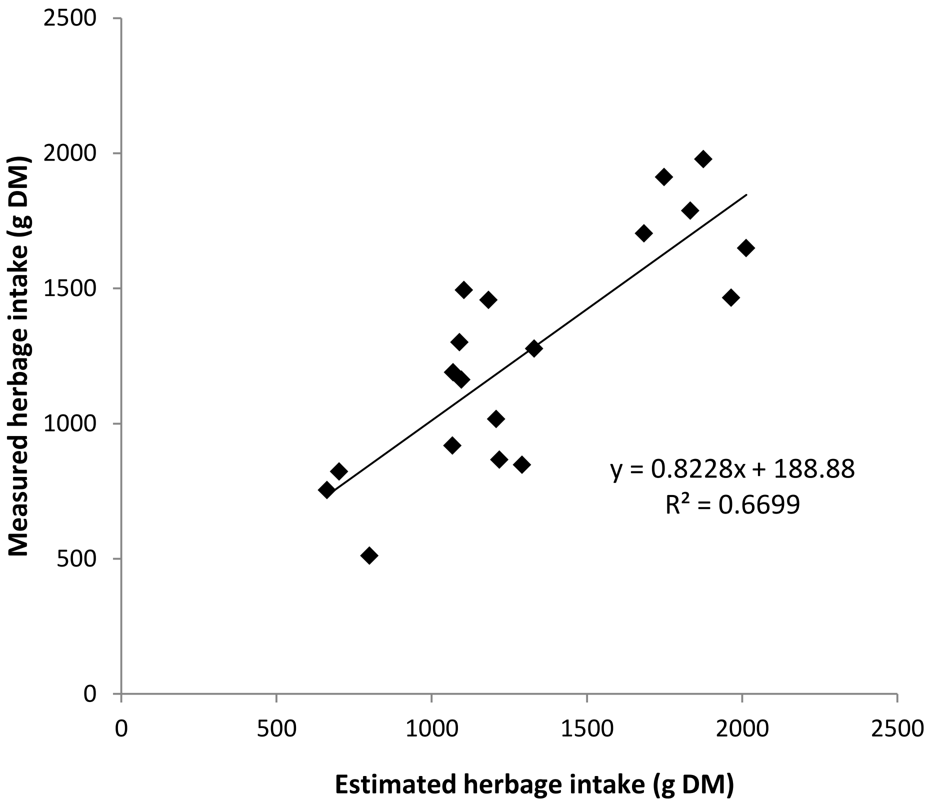


Figure 4. Model evaluation – model m4OF: top: graph calibration, bottom graph evaluation.

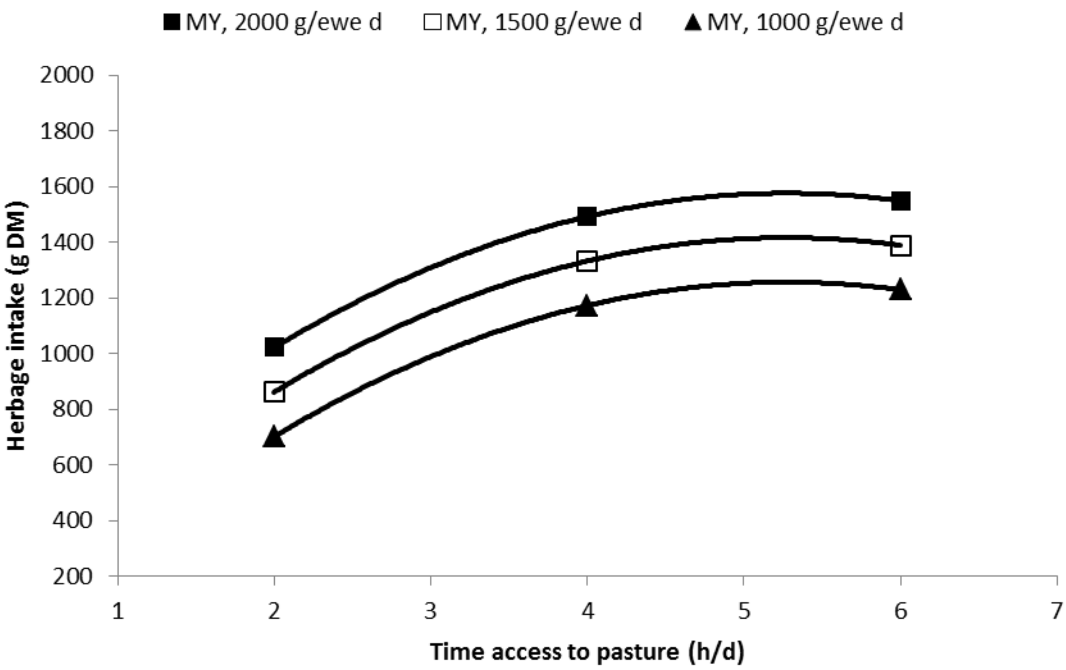


Figure 5. Sensitivity analysis of model m4OF: effect of milk yield (g/ewe day) on herbage intake in dairy ewes part-time grazing Mediterranean pastures containing 45% of NDF on DM basis.

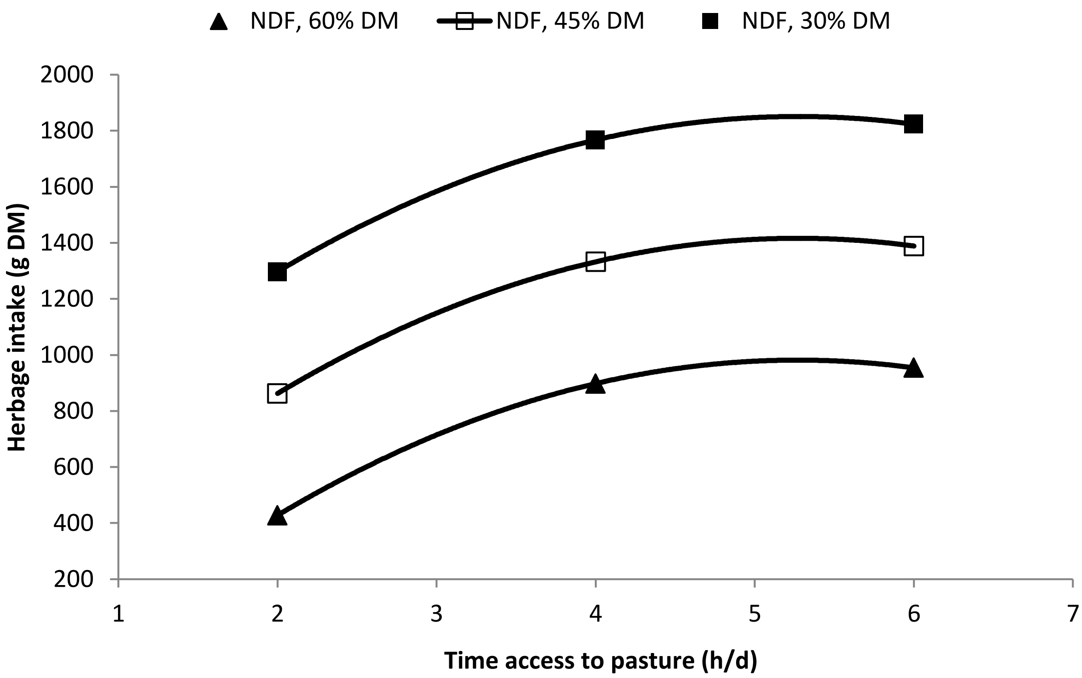


Figure 6. Sensitivity analysis of model m4OF: effect of the content of NDF (% DM basis) in the grazed herbage on herbage intake in dairy ewes part-time grazing Mediterranean pastures with production level of 1500 g/ewe day.

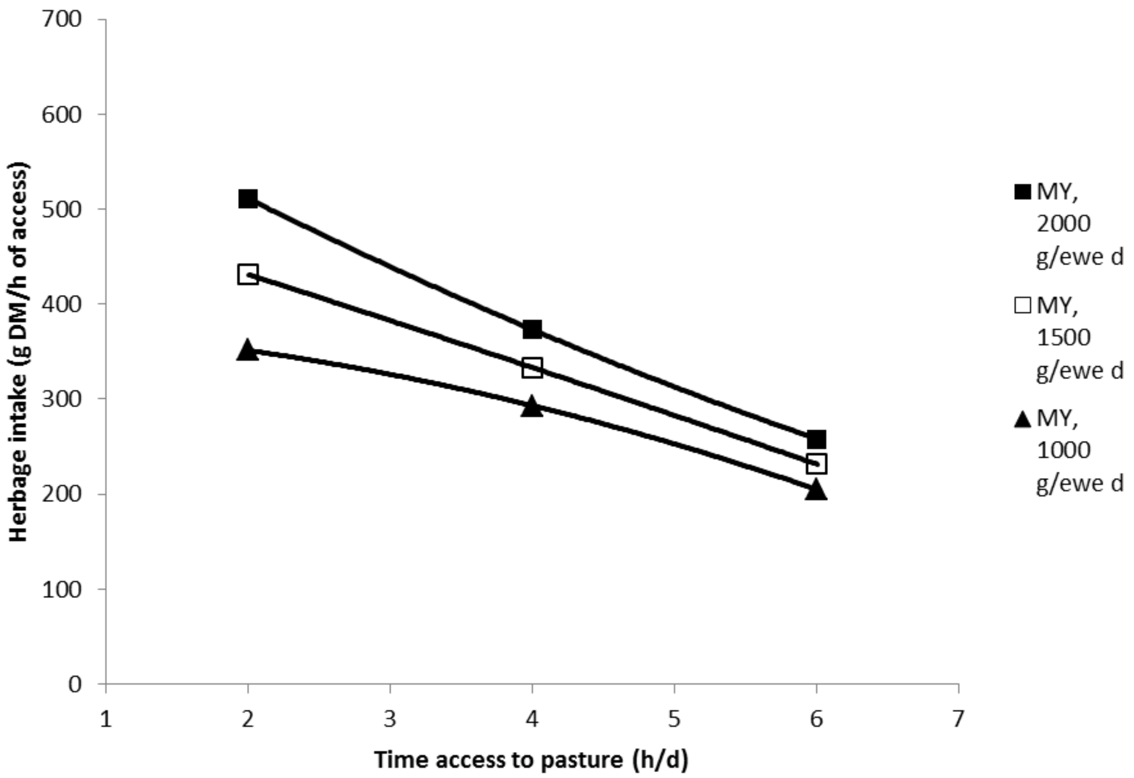


Figure 7. Sensitivity analysis of model m4OF: effect of milk yield (g/ewe day) on herbage intake per access hour to pasture in dairy ewes part-time grazing Mediterranean pastures containing 45% of NDF on DM basis.

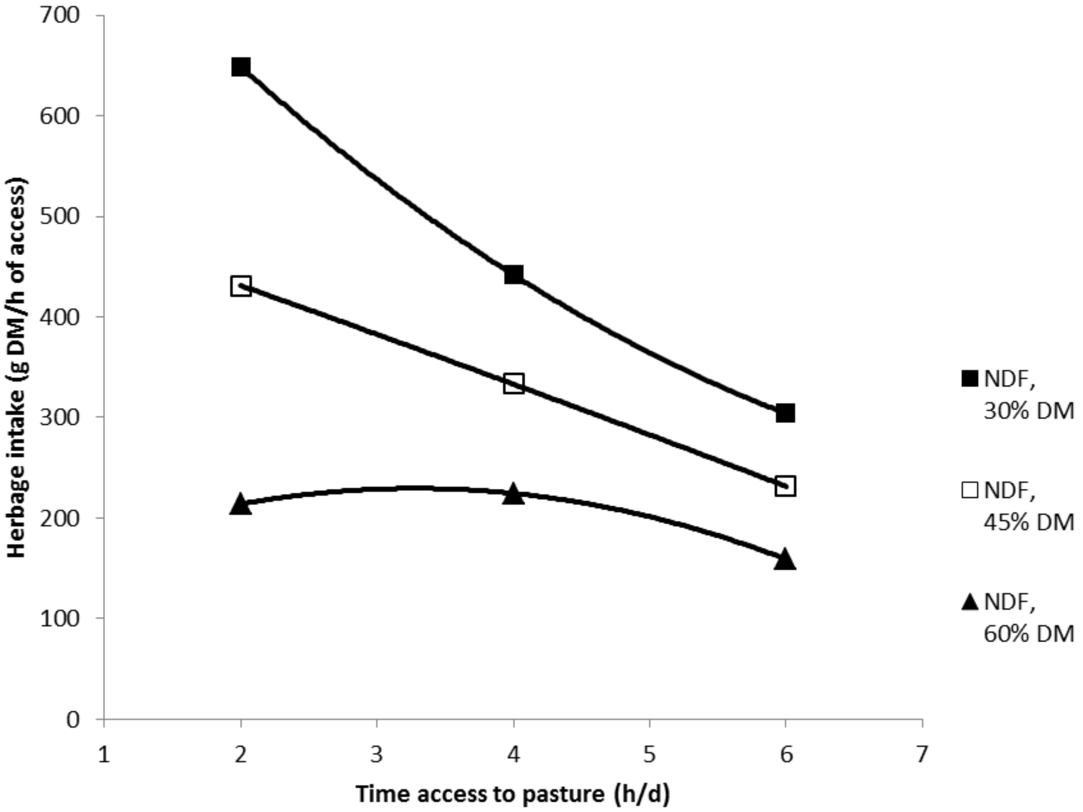


Figure 8. Sensitivity analysis of model m4OF: effect of the content of NDF (% DM basis) in the grazed herbage on herbage intake per access hour to pasture in dairy ewes part-time grazing Mediterranean pastures with production level of 1500 g/ewe day.

Appendix 1

Database of the review

Appendix 1 – Publications on dairy cows - experimental settting

Npub	Animtype	TRT	n	PT	PYSTAGE	DIM	InBW	InMY	EXPD	PASTSP	GP	GM	HM	SHM	preSH	posSW	HA	OFFAREA	TSUP	CSUP	FSUP
1	dc	S5-HA11 8h	12	Mi	Lact	166	599	29.6	60	LP	GR	SG	1.4		106		10.7	87	5	0.7	4.4
1	dc	S10-HA7 8h	12	Mi	Lact	166	599	29.6	60	LP	GR	SG	1.3		103		6.7	58	10	1.3	8.7
1	dc	S5-HA11 4h	12	Mi	Lact	166	599	29.6	60	LP	GR	SG	1.5		111		11.2	85	5	0.7	4.4
1	dc	S10-HA7 4h	12	Mi	Lact	166	599	29.6	60	LP	GR	SG	1.5		112		6.6	46	10	1.3	8.7
2	dc	4h	20	Mi	Lact	96	592	31	42	LPTR	GR	CS	1.8	115				35	ADL	ADL	ADL
2	dc	6.5h	20	Mi	Lact	96	592	32	42	LPTR	GR	CS	1.6	110				37	ADL	ADL	ADL
2	dc	9h	20	Mi	Lact	96	592	33	42	LPTR	GR	CS	1.5	103				43	ADL	ADL	ADL
3	dc	22h	13	Mi	Lact	202	591	23.8	31	LPB	GR	SG	1.2		86	35	15.5	151	3	3	
3	dc	9h	13	Mi	Lact	202	591	23.8	31	LPB	GR	SG	1.2		86	38	15.5	160	3	3	
3	dc	4.5h + 4.5h	13	Mi	Lact	202	591	23.8	31	LPB	GR	SG	1.3		90	36	15.4	140	3	3	
3	dc	3h + 3h	13	Mi	Lact	202	591	23.8	31	LPB	GR	SG	1.3		89	39	15.4	140	3	3	
4	dc	8h	16	Mi	Lact	35	470	24.2	37	LPB	GR	SG	3.2				32.9				
4	dc	4h + 4h	16	Mi	Lact	35	470	24.2	37	LPB	GR	SG	3.2				32.9				
4	dc	24 h	16	Mi	Lact	35	470	24.2	37	LPB	GR	SG	3.2				32.9				
5	dc	22h 13 HA	22	Mi	Lact	211	626	28.7	42	LP	GR	SG	2.7		172		13.1	50	5	0.7	4.4
5	dc	9h 13 HA	24	Mi	Lact	211	626	28.7	42	LP	GR	SG	2.8		176		13.2	47	10	1.3	8.7
5	dc	2.75h + 2.75h 13 HA	26	Mi	Lact	211	626	28.7	42	LP	GR	SG	3.0		177		13.2	44	5	0.7	4.4
5	dc	22h 24 HA	27	Mi	Lact	211	626	28.7	42	LP	GR	SG	3.1		183		23.7	75	10	1.3	8.7
5	dc	9h 24 HA	29	Mi	Lact	211	626	28.7	42	LPTR	GR	CS	3.2		182		23.8	76	ADL	ADL	ADL
5	dc	2.75h + 2.75h 24 HA	31	Mi	Lact	211	626	28.7	42	LPTR	GR	CS	3.3		177		23.9	80	ADL	ADL	ADL
6	dc	T7-15	33	Mi	Lact	60	550	25.3	63	LPTR	GR	CS	3.4		65		20.3		ADL	ADL	ADL
6	dc	T7-11	13	Mi	Lact	60	550	25.3	63	LPB	GR	SG	3.6		70		21.4		3	3	
6	dc	T11-15	13	Mi	Lact	60	550	25.3	63	LPB	GR	SG	3.7		66		20.4		3	3	
13	dc	1 h 10 cm	13	Mi	Lact				3	LPB	GR	SG	3.8		100				3	3	
13	dc	2 h 10 cm	13	Mi	Lact				3	LPB	GR	SG	3.9		100				3	3	
13	dc	4 h 10 cm	16	Mi	Lact				3	LPB	GR	SG	4.0		100						
13	dc	8 h 10 cm	16	Mi	Lact				3	LPB	GR	SG	4.2		100						
13	dc	15 h 10 cm	16	Mi	Lact				3	LPB	GR	SG	4.3		100						
13	dc	1 h 13 cm	34	Mi	Lact				3	LP	GR	SG	4.4		130				5	0.7	4.4
13	dc	2 h 13 cm	36	Mi	Lact				3	LP	GR	SG	4.5		130				10	1.3	8.7
13	dc	4 h 13 cm	38	Mi	Lact				3	LP	GR	SG	4.7		130				5	0.7	4.4
13	dc	8 h 13 cm	39	Mi	Lact				3	LP	GR	SG	4.8		130				10	1.3	8.7
13	dc	15 h 13 cm	41	Mi	Lact				3	LPTR	GR	CS	4.9		130				ADL	ADL	ADL
8	dc	4 h	43	Mi	Lact	149	643	37	35	LPTR	GR	CS	5.0		143	85	12		ADL	ADL	ADL
8	dc	8 h	45	Mi	Lact	149	643	37	35	LPTR	GR	CS	5.1		150	69	12		ADL	ADL	ADL
8	dc	21 h	13	Mi	Lact	149	643	37	35	LPB	GR	SG	5.3		145	61	12		3	3	
9	dc	EL - 22h	13	Mi	Lact	35	470		20	LPB	GR	SG	5.4		98	52			3	3	
9	dc	EL - 8 h	13	Mi	Lact	35	470		20	LPB	GR	SG	5.5		94	60			3	3	
9	dc	EL 4 h + 4h	13	Mi	Lact	35	470		20	LPB	GR	SG	5.6		97	52			3	3	
9	dc	LL - 22h	16	Mi	Lact	225	498		15	LPB	GR	SG	5.8		71	44					
9	dc	LL - 8 h	16	Mi	Lact	225	498		15	LPB	GR	SG	5.9		70	46					
9	dc	LL 4 h + 4h	16	Mi	Lact	225	498		15	LPB	GR	SG	6.0		70	45					
14	dc	0 h	46	Mi	Lact	107		33	90	LP	GR	SG	6.1				13		5	0.7	4.4
14	dc	12 h night	48	Mi	Lact	107		33	90	LP	GR	SG	6.2				13		10	1.3	8.7
14	dc	0 h	50	Mi	Lact	131				LP	GR	SG	6.4				13		5	0.7	4.4
14	dc	6 h day	51	Mi	Lact	131				LP	GR	SG	6.5				13		10	1.3	8.7
7	dc	5 h	53	Mi	Lact	143	568	32.3	41	LPTR	GR	CS	6.6	7					ADL	ADL	ADL
7	dc	10 h	55	Mi	Lact	143	568	32.3	41	LPTR	GR	CS	6.7	7					ADL	ADL	ADL
7	dc	19 h	57	Mi	Lact	143	568	32.3	41	LPTR	GR	CS	6.8	7					ADL	ADL	ADL
7	dc	20 h	13	Mi	Lact	143	568	32.3	41	LPB	GR	SG	7.0	7.4					3	3	
7	dc	5 h	13	Mi	Lact	149	580	24.1	42	LPB	GR	SG	7.1	7.1					3	3	
7	dc	10 h	13	Mi	Lact	149	580	24.1	42	LPB	GR	SG	7.2	7.1					3	3	
7	dc	19 h	13	Mi	Lact	149	580	24.1	42	LPB	GR	SG	7.3	7.1					3	3	
15	dc	8 h	16	Mi	Lact	33	488	16.4	35	LPB	GR	SG	7.5				15				
15	dc	4 h + 4 h -m	16	Mi	Lact	33	488	16.4	35	LPB	GR	SG	7.6				15				
15	dc	4 h + 4 h -e	16	Mi	Lact	33	488	16.4	35	LPB	GR	SG	7.7				15				
11	dc	6S	58	Mi	Lact	20			56	LP	GR	SG	7.8				8.5		5	0.7	4.4
11	dc	6SG	60	Mi	Lact	20			56	LP	GR	SG	7.9				8.5		10	1.3	8.7
11	dc	4SG	62	Mi	Lact	20			56	LP	GR	SG	8.1				8.5		5	0.7	4.4
11	dc	2SG	63	Mi	Lact	20			56	LP	GR	SG	8.2				8.5		10	1.3	8.7
11	dc	6S	65	Mi	Lact	20			49	LPTR	GR	CS	8.3				14		ADL	ADL	ADL
11	dc	6SG	67	Mi	Lact	20			49	LPTR	GR	CS	8.4				14		ADL	ADL	ADL
11	dc	4SG	69	Mi	Lact	20			49	LPTR	GR	CS	8.5				14		ADL	ADL	ADL
11	dc	2SG	13	Mi	Lact	20			49	LPB	GR	SG	8.7				14		3	3	
10	dc	22 h	13	Mi	Lact	25	509	24.5	30	LPB	GR	SG	8.8		10	4	14.6	85.9	3	3	
10	dc	4.5h + 4.5h	13	Mi	Lact	25	509	24.5	30	LPB	GR	SG	8.9		10	4	14	93.4	3	3	
10	dc	3h + 3h	13	Mi	Lact	25	509	24.5	30	LPB	GR	SG	9.0		10	4	14.6	90	3	3	
10	dc	3h + 3h S	16	Mi	Lact	25	509	24.5	30	LPB	GR	SG	9.2		9.9	4.8	14.5	89.3			
12	dc	PC - 20 h + corn	16	Mi	Lact	145	547	24	85	LPB	GR	SG	9.3				28				
12	dc	PCSB - 20 h + corn+SBM	16	Mi	Lact	145	547	24	85	LPB	GR	SG	9.4				28				
12	dc	SC- 7 h + corn	70	Mi	Lact	145	547	24	85	LP	GR	SG	9.5				28		5	0.7	4.4
12	dc	SCSB - 7 h + corn+SBM	72	Mi	Lact	145	547	24	85	LP	GR	SG	9.6				28		10	1.3	8.7
16	dc	8 h	74	Mi	Lact	35	470		37	LP	GR	SG	9.8				33	100	5	0.7	4.4
16	dc	4 h + 4 h	75	Mi	Lact	35	470		37	LP	GR	SG	9.9				33	100	10	1.3	8.7
16	dc	22 h	77	Mi	Lact	35	470		37	LPTR	GR	CS	10.0				33	100	ADL	ADL	ADL
17	dc	8 h	79	Mi	Lact	35	470		37	LPTR	GR	CS	10.1				33	100	ADL	ADL	ADL
17	dc	4 h + 4 h	81	Mi	Lact	35	470		37	LPTR	GR	CS	10.3				33	100	ADL	ADL	ADL
17	dc	22 h	13	Mi	Lact	35	470		37	LPB	GR	SG	10.4				33	100	3	3	

Appendix 1 – Publications on dairy cows - experimental results

Npub	Animtype	TA	NAD	HIN	HOUT	GT	RT	IT	WT	GTP	RTP	ITP	WTP	BM	BR	HDMIR	HDMI	HDMIH	HDMIHPV	TOTSDMI	CONDMI	FSDMI	TDMI	HCP	HNDF	HOMD	HDMD	TDOMD	TDMD	Mean BW	BW change	MY	MF	MP	MUREA
1	dc	8.0	1.0	09:00	17:00	352				0.73						33	11.5	1.44	0.25	4.90	0.60	4.20	16.3	227	578	771				576		21.9	39.4	29.8	
1	dc	8.0	1.0	09:00	17:00	301				0.63						28.2	8.4	1.05	0.18	9.70	1.20	8.50	18.1	237	564	774				591		22.6	39.5	30.5	
1	dc	4.0	1.0	09:00	13:00	219				0.91						43.7	9.5	2.38	0.42	4.90	0.60	4.30	14.4	219	588	767				560		20.5	40.3	29.3	
1	dc	4.0	1.0	09:00	13:00	199				0.83						33.8	6.7	1.68	0.29	9.90	1.20	8.70	16.6	215	585	769				583		21.8	39.5	29.9	
2	dc	4.0	1.0	06:30	10:30	211				0.86		0.15	0.04	0.900	56.6	49	10.4	2.55	0.44	9.30	4.93	4.37	19.7	175	345	754				579	0.164	30.3	41.1	32.0	15.3
2	dc	6.5	1.0	06:30	13:00	308				0.78		0.22	0.03	0.660	59.2	37	11.4	1.73	0.30	9.30	4.93	4.37	20.7	180	362	750				583	0.293	31.3	42.6	32.9	18.8
2	dc	9.0	1.0	06:30	15:30	349				0.64		0.36	0.02	0.660	57.9	36	12.7	1.40	0.24	8.80	4.66	4.14	21.5	181	356	752				594	0.483	32.4	40.5	33.2	18.2
3	dc	22.0	1.0			549	401	490		0.42				0.470	57.1	25.9	13.8	0.63	0.12	3.00			16.8	243	349	862				540	-1.310	21.8	41.0	35.1	
3	dc	9.0	1.0			437	363	640		0.81				0.480	57.7	27.9	12.1	1.34	0.25	3.00			15.1	228	368	866				531	-1.260	22.4	42.0	34.1	
3	dc	9.0	2.0			436	438	566		0.81				0.520	58.7	30.1	12.9	1.43	0.27	3.00			15.9	227	358	859				535	-1.180	21.5	40.1	34.1	
3	dc	6.0	2.0			346	344	750		0.96				0.690	55.9	37.6	13.0	2.17	0.41	3.00			16.0	242	339	870				531	-1.270	20.9	41.4	33.4	
4	dc	8.0	1.0	08:00	16:00					0.81	0.01	0.13	0.02	1.280	52.1	*29.6*	12.5	1.56	0.33	0.00			12.5	204	419					470					
4	dc	8.0	2.0	08:00-12:00	16:00-20:00					0.68	0.01	0.21	0.05	1.100	54.9	*28.2*	13.9	1.74	0.37	0.00			13.9	204	419					470					
4	dc	22.0	2.0	08:00	06:00					0.58	0.11	0.21	0.07	1.460	46.5	*12.3*	13.7	0.62	0.13	0.00			13.7	204	419					470					
5	dc	22.0	1.0	09:00	17:00	446				0.77						28.5	14.5	0.66	0.11	0.00			14.5	187	548	797				613		19.8	41.2	30.8	
5	dc	9.0	1.0	09:00	17:00	464				0.77						28	12.1	1.34	0.23	0.00			12.1	186	533	823				586		19.2	43.4	30.6	
5	dc	5.5	2.0	09:00	13:00	483				0.76						40.6	12.5	2.27	0.39	0.00			12.5	187	527	823				582		19.2	41.7	29.5	
5	dc	22.0	1.0	09:00	13:00	501				0.76						31.1	17.0	0.77	0.12	0.00			17.0	179	540	825				621		22.6	39.4	32.5	
5	dc	9.0	1.0	06:30	10:30	519				0.76		0.15	0.05	1.219	50.6	35.3	13.9	1.54	0.26	0.00			13.9	188	532	835				590		20.4	41.9	31.0	
5	dc	5.5	2.0	06:30	13:00	537				0.76		0.22	0.06	1.292	49.6	43.6	13.1	2.38	0.40	0.00			13.1	192	524	828				590		20.6	41.8	31.1	
6	dc	8.0	1.0	06:30	15:30	555				0.76		0.36	0.06	1.364	48.7		8.3	1.04	0.19	10.80	6.10	4.70	19.1	195	366					538		25.4	39.6	30.3	
6	dc	4.0	1.0			574	363	788		2.39			0.06	1.436	47.8	28.8	6.6	1.65	0.31	10.40	6.10	4.30	17.0	201	353					536		23.6	37.1	29.8	
6	dc	4.0	1.0			592	353	859		2.47			0.06	1.508	46.9	36	6.7	1.68	0.31	10.50	6.10	4.70	17.2	212	368					535		24.6	36.6	29.9	
13	dc	1.0	1.0			610	343	929		10.17			0.07	1.581	45.9		7.7	7.70						207	602	607									
13	dc	2.0	1.0			628	334	1000		5.24			0.07	1.653	45.0		7.6	3.80						207	602	607									
13	dc	4.0	1.0	08:00	16:00					0.46	0.15	0.26	0.07	1.725	44.1		14.2	3.55						207	602	607									
13	dc	8.0	1.0	08:00-12:01	16:00-20:01					0.35	0.20	0.30	0.07	1.797	43.1		17.1	2.14						207	602	607									
13	dc	15.0	1.0	08:00	06:00					0.23	0.25	0.34	0.08	1.870	42.2		14.1	0.94						207	602	607									
13	dc	1.0	1.0	09:00	17:00	646				0.76							16.4	16.40						207	602	607									
13	dc	2.0	1.0	09:00	17:00	665				0.76							17.9	8.95						207	602	607									
13	dc	4.0	1.0	09:00	13:00	683				0.76							17.3	4.33						207	602	607									
13	dc	8.0	1.0	09:00	13:00	701				0.76							24.6	3.08						207	602	607									
13	dc	15.0	1.0	06:30	10:30	719				0.76		0.15	0.08	1.942	41.3		20.3	1.35						207	602	607									
8	dc	4.0	1.0	06:30	13:00	737				0.76		0.22	0.08	2.014	40.4		7.6	1.90	0.32											587		24.4	39.4	27.4	
8	dc	8.0	1.0	06:30	15:30	756				0.76		0.36	0.08	2.086	39.4		9.7	1.21	0.20											610		27.0	38.7	28.6	
8	dc	21.0	1.0			774	324	1070		0.61			0.09	2.158	38.5		10.7	0.51	0.08											609		28.6	36.6	28.4	
9	dc	22.0	1.0			792	315	1141		0.60			0.09	2.231	37.6																	24.0	41.0	35.0	43.9
9	dc	8.0	1.0			810	305	1212		1.69			0.09	2.303	36.7																	20.8	43.0	34.0	49.3
9	dc	8.0	2.0			828	295	1282		1.73			0.10	2.375	35.7																	22.1	41.0	34.0	46.9
9	dc	22.0	1.0	08:00	16:00					0.12	0.30	0.38	0.10	2.447	34.8																	10.3	55.0	41.0	
9	dc	8.0	1.0	08:00-12:02	16:00-20:02					0.00	0.35	0.42	0.10	2.520	33.9																	10.3	55.0	43.0	
9	dc	8.0	2.0	08:00	06:00					-0.12	0.40	0.46	0.10	2.592	33.0																	10.4	58.0	41.0	
14	dc	0.0	0.0	09:00	17:00	847				0.76											7.80	10.53	18.3							596		27.5	40.7	31.7	34.9
14	dc	12.0	1.0	09:00	17:00	865				0.76							6.8	0.56	0.09	13.27	7.77	5.50	20.1	229		729				600		31.4	38.2	32.8	35.5
14	dc	0.0	0.0	09:00	13:00	883				0.75													18.6							607		27.0	41.1	33.4	32.2
14	dc	6.0	0.0	09:00	13:00	901				0.75							4.2	0.70	0.12	14.60	7.50	7.03	18.8	214		715				599		28.5	38.7	33.3	32.8
7	dc	5.0	1.0	06:30	10:30	919				0.75		0.15	0.11	2.664	32.0	27.1	5.5	1.10	0.18	13.80	4.60	9.20	19.3							598	1.290	27.1	35.8	32.3	
7	dc	10.0	1.0	06:30	13:00	938				0.75		0.22	0.11	2.736	31.1	33.3	12.2	1.22	0.21	6.50	3.30	3.20	18.7							589	0.700	27.4	37.6	33.1	
7	dc	19.0	1.0	06:30	15:30	956				0.75		0.36	0.11	2.809	30.2	26.5	12.8	0.67	0.12	5.80	3.20	2.60	18.6							584	0.480	27.5	39.2	32.7	
7	dc	20.0	1.0			974	286	1353		0.81			0.11	2.881	29.3	30.5	15.4	0.77	0.13	2.60	2.60	0.00	18.0							578	0.170	27.6	37.1	32.3	
7	dc	5.0	1.0			992	276	1423		3.31			0.12	2.953	28.3	5.4	1.0	0.20	0.03	16.70	5.00	11.70	17.7							577	0.670	25.0	41.5	34.1	
7	dc	10.0	1.0			1010	267	1494		1.68			0.12	3.025	27.4	9.2	3.4	0.34	0.06	14.30	4.60	9.70	17.7							583	0.680	26.1	40.2	35.4	
7	dc	19.0	1.0			1029	257	1565		0.90			0.12	3.098	26.5	16	7.5	0.39	0.07	8.80	3.70	5.10	16.3							577	0.490	25.3	39.0	34.0	
15	dc	8.0	1.0	08:00	16:00					-0.23	0.46	0.50	0.12	3.170	25.5					10.50	6.30	4.20										17.7	34.8	30.7	
15	dc	6.0	2.0	08:00-12:03	16:00-20:03					-0.35	0.51	0.54	0.13	3.242	24.6					10.50	6.30	4.20										18.2	32.5	30.2	
15	dc	6.0	2.0	08:00	06:00					-0.46	0.56	0.58	0.13	3.314	23.7					10.50	6.30	4.20										20.2	31.5	30.1	
11	dc	0.0		09:00	17:00	1047				0.75							0.0			13.65	5.25	8.40	13.7						721.5			22.0	36.3	30.6	
11	dc	6.0	1.0	09:00	17:00	1065				0.75							5.5	0.92		11.10	5.25	5.85	16.6	237	369	850	821		693			24.9	36.0	31.7	
11	dc	6.0	1.0	09:00	13:00	1083				0.75							5.6	0.93		9.70	3.50	6.20	15.3	237	369	850	821		698			23.4	37.5	31.5	
11	dc	6.0	1.0	09:00	13:00	1101				0.75							5.4	0.89		8.60	1.75	6.85	14.0	237	369	850	821		696			21.9	36.9	31.2	
11	dc	0.0		06:30	10:30	1120				0.75		0.15	0.13	3.387	22.8		0.0			14.55	5.30	9.25	14.6						718			23.5	36.3	30.6	
11	dc	12.0	1.0	06:30	13:00	1138				0.75		0.22	0.13	3.459	21.8		9.1	0.75		8.70	5.35	3.35	17.8	217	375	832	854		732			27.1	36.0	31.7	
11	dc	12.0	1.0	06:30	15:30	1156				0.75		0.36	0.14	3.531	20.9		10.6	0.88		7.15	3.50	3.65	17.7	217	375	832	854		727			27.5	37.5	31.5	
11	dc	12.0	1.0			1174	247	1635		1.63			0.14	3.603	20.0		10.5	0.88		5.65	1.80	3.85	16.2	217	375	832	854		724.5			25.5	36.9	31.2	
10	dc	22.0	2.0			1192	238	1706		0.90			0.14	3.676	19.1	24.8	11.8	0.54	0.00	3.00	3.00	0.00	14.8	237	375	843				495		28.1	41.4	33.7	
10	dc	9.0	2.0			1211	228	1776		2.24			0.15	3.748	18.1	29.1	11.7	1.30	0.26	3.00	3.00	0.00	14.7	256	375	849				493		28.3	41.3	32.5	
10	dc	6.0	2.0			1229	219	1847		3.41			0.15	3.820	17.2	34.4	12.2	2.03	0.41	3.00	3.00	0.00	15.2	250	374	845				494		28.2	41.4	32.6	
10	dc	6.0	2.0	08:00	16:00					-0.58	0.61	0.62	0.15	3.892	16.3	*34.1*	9.6	1.60	0.31	7.00	3.00	4.00	16.6	249	367	856				509		28.6	42.6	32.2	
12	dc	20.0	1.0	08:00-12:04	16:00-20:04					-0.69	0.66	0.66	0.15	3.965	15.4		14.7	0.74	0.13	5.30	5.30		20.0	228	498		782			569		25.3	36.9	32.6	35.2
12	dc	20.0	1.0	08:00	06:00					-0.81	0.71	0.70	0.16	4.037	14.4		14.7	0.74	0.13	5.30	5.30		20.0	228	498		782			566		24.7	37.9	32.4	40.4
12	dc	7.0	1.0	09:00	17:00	1247				0.75							6.8	0.97	0.17	13.00	5.30	7.70	19.8	228	498		782			576		24.7	36.0	31.3	25.4
12	dc	7.0	1.0	09:00	17:00	1265				0.75							7.4	1.06	0.18	13.20	5.30	7.90	20.6	228	498		782			580		25.9	37.8	31.5	31.1
16	dc	8.0	1.0	09:00	13:00	1283				0.74																									
16	dc	8.0	2.0	09:00	13:00	1302				0.74																									
16	dc	22.0	1.0	06:30	10:30	1320				0.74		0.15	0.16	4.109	13.5																				
17	dc	8.0	1.0	06:30	13:00	1338				0.74		0.22	0.16	4.181	12.6																				
17	dc	8.0	2.0	06:30	15:30	1356				0.74		0.36	0.16	4.254	11.6																				
17	dc	22.0	1.0			1374	209	1918		1.04			0.17	4.326	10.7																				

Appendix 1 – Publications on other animal classes - experimental setting

Npub	Animtype	TRT	n	PT	PYSTAGE	DIM	InBW	InMY	EXPD	PASTSP	GP	GM	HM	SHM	preSH	posSW	HA	OFFAREA	TSUPPOFF	CONCOFF	FORSUPOFF
1	sh	4 h	24	Mi	Lact	39	63	1.5		PP	GR	CS			70					0.52	0.25
1	sh	7 h	24	Mi	Lact	39	63	1.5		PP	GR	CS			70					0.52	0.25
10	sh	4 h	24	Mi	Lact	44	58	1.818	42	PP	GR	CS			70					0.53	0.26
10	sh	7 h	24	Mi	Lact	44	58	1.818	42	PP	GR	CS			70					0.53	0.26
6	sh	4 h	24	Mi	Lact		65.45	1.32	56	LPTR	GR	CS			70					0.53	0.40
6	sh	7 h	24	Mi	Lact		65.45	1.32	56	LPTR	GR	CS			70					0.53	0.38
7	sh	0 h + conc and hays	12	Mi	Lact	50	59.5	1.45	60	LPB	GR	CS								0.50	1.60
7	sh	4 h + con + 0.3 kg hay	12	Mi	Lact	50	59.5	1.45	60	LPB	GR	CS								0.5	0.3
7	sh	5 h + con + 0.6 kg hay	12	Mi	Lact	50	59.5	1.45	60	LPB	GR	CS								0.5	0.6
7	sh	6 h + con + 0.9 kg hay	12	Mi	Lact	50	59.5	1.45	60	LPB	GR	CS								0.5	0.9
8	sh	22 h	12	Mi	Lact	60	44.6	2.321	120	LM	GR	RG								0	
8	sh	3 h + conc sup NF	12	Mi	Lact	60	44.6	2.321	120	LM	GR	RG							1.13	0.81	0.32
8	sh	3 h + conc sup C182	12	Mi	Lact	60	44.6	2.321	120	LM	GR	RG							1.13	0.81	0.32
8	sh	4 h + conc sup C183	12	Mi	Lact	60	44.6	2.321	120	LM	GR	RG							1.13	0.81	0.32
2	sh	2 h	12	Mi	Lact	90	42.5	1.449	60	LM	GR	RG					2.8			0.616	0.595
2	sh	4 h	12	Mi	Lact	90	42.5	1.449	60	LM	GR	RG					2.9			0.616	0.595
2	sh	6 h	12	Mi	Lact	90	42.5	1.449	60	LM	GR	RG					3.0			0.616	0.595
3	sh	Rest- HSW	6	Me	Lact		53.8		49	LPB	GR	CS			5.0						
3	sh	Rest- LSW	6	Me	Lact		53.8		49	LPB	GR	CS			3.0						
3	sh	Unrest- HSH	6	Me	Lact		53.8		49	LPB	GR	CS			5.0						
3	sh	Unrest-LSH	6	Me	Lact		53.8		49	LPB	GR	CS			3.0						
4	sh	2 h	10	Me	Grow		15.62		120	Steppe		CS	1.9							0.36	
4	sh	4 h	10	Me	Grow		15.62		120	Steppe		CS	1.9							0.20	
4	sh	8 h	10	Me	Grow		15.62		120	Steppe		CS	1.9							0.20	
4	sh	12 h	10	Me	Grow		15.62		120	Steppe		CS	1.9							0.00	
5	sh	2 h	8	Me	Grow		21.86		99	Steppe										0.397	ADL
5	sh	4 h	8	Me	Grow		21.86		99	Steppe										0.267	ADL
5	sh	8 h	8	Me	Grow		21.86		99	Steppe										0.228	ADL
5	sh	12 h	8	Me	Grow		21.86		99	Steppe										0.123	ADL
9	sh	Rat-GR - 8 h + conc.	12	Me	Lact		45.2		160	PP		CS									
9	sh	Gr - 24 h	12	Me	Lact		45.2		160	PP		CS									
9	sh	Gr-S as Gr but sup. lambs	12	Me	Lact		45.2		160	PP		CS									
9	sh	Rat-GR - 8 h + conc.	12	Me	Grow				160	PP		CS									
9	sh	Gr - 24 h	12	Me	Grow				160	PP		CS									
9	sh	Gr-S as Gr but sup. lambs	12	Me	Grow				160	PP		CS									
1	go	AM- 4 h	8	Mi	Lact	135	37	1.108	45	LM		SS									
1	go	PM - 4 h	8	Mi	Lact	135	37	1.108	45	LM		SS									
2	go	Night -locked	12	Me	Allst				330	MIX	All	RG	2.1								
2	go	24 h	12	Me	Allst				330	MIX	All	RG	2.1								
3	go	4 h	6	Me	Grow		21		63	MIX	GR		1.7								
3	go	8 h	6	Me	Grow		21		63	MIX	GR		1.7								
3	go	24 h	6	Me	Grow		21		63	MIX	GR		1.7								
1	bc	24 h - HSH	5	Me	Grow		380		30	DG	All	SG			13.5						
1	bc	24 h - LSH	5	Me	Grow		380		30	DG	All	SG			7.5						
1	bc	5 h - HSH	5	Me	Grow		380		30	DG	All	SG			13.5						
1	bc	5 h - LSH	5	Me	Grow		380		30	DG	All	SG			7.5						
2	bc	12 h	16	Me	Lact		632		45	PP	All	SS	2.2	7.95							
2	bc	24 h	16	Me	Lact		632		45	PP	All	SS	2.2	7.95							
3	bc	MHA - 11 h m	4	Me	Grow		279		120	TA	All	SG	3.0								
3	bc	AHA - 4h + 7 h a	4	Me	Grow		279		120	TA	All	SG	3.0								
3	bc	MHAF - 4 h m	4	Me	Grow		279		120	TA	All	SG	3.0								
3	bc	AHAF - 4h a	4	Me	Grow		279		120	TA	All	SG	3.0								
1	hs	Period 1 October	4				576		60	FA	All										
1	hs	Period 2 February	4				576			FA	All										
1	hs	Period 3 May	4				576			FA	All										
2	hs	3 h	2				549														ADL
2	hs	6 h	2				549														ADL
2	hs	9 h	2				549														
2	hs	24 h	2				549														

Appendix 1 – Publications on other animal classes - experimental results

Npub	Animtype	TA	NAD	HIN	HOUT	GT	RT	IT	WT	GTP	RTP	ITP	WTP	BM	BR	HDMIR	HDMI	HDMIH	HDMIHPV	TOTSDMI	CONDMI	FSDMI	TDMI	HCP	HNDF	HOMD	HDMD	TDOMD	TDMD	Mean BW	BW change	MY	MF	MP	MUREA
1	sh	4.0	1.0			196				0.82																									
1	sh	7.0	1.0			246				0.60																									
10	sh	4.0	1.0																											59		1.35	63.4	51.0	
10	sh	7.0	1.0																											61		1.49	60.4	53.4	
6	sh	4.0	1.0			184				0.78						5.30	1.0	0.24	0.385	0.8	0.5	0.4	1.8	195		573			622	63		0.92	82.0	56.0	
6	sh	7.0	1.0			222				0.53						5.22	1.2	0.17	0.256	0.8	0.4	0.3	1.9	195		573			670	65		0.94	79.0	55.0	
7	sh	0.0	0.0																											60		1.03	69.8	50.5	
7	sh	4.0	1.0														1.5	0.37	0.613											60		1.32	61.2	48.8	
7	sh	4.0	1.0														1.1	0.27	0.458											60		1.36	63.4	50.5	
7	sh	4.0	1.0														0.5	0.12	0.202											60		1.46	63.0	49.6	
8	sh	22.0	1.0	08:30	06:30												2.3	0.10	0.229				2.3	215	379				730	46		1.63	62.1	51.2	
8	sh	3.0	1.0	08:30	11:30												0.9	0.31	0.685	0.9	0.8	0.2	1.8	215	379				653	45		1.75	52.3	47.2	
8	sh	3.0	1.0	08:30	11:30												0.9	0.31	0.658	1.0	0.7	0.2	1.9	215	379				632	46		1.48	58.6	52.0	
8	sh	3.0	1.0	08:30	11:30												1.0	0.35	0.779	1.0	0.7	0.2	2.0	215	379				639	45		1.71	63.3	50.5	
2	sh	2.0	1.0	08:30	10:30	107	8	5		0.90	0.06	0.04				6.51	0.6	0.32		1.2	0.6	0.6	1.8									0.96	62.3	50.8	
2	sh	4.0	1.0	08:30	12:30	189	31	9		0.83	0.13	0.04				5.72	1.1	0.26		1.2	0.5	0.6	2.2									1.00	63.3	51.2	
2	sh	6.0	1.0	08:30	14:30	256	58	20		0.77	0.18	0.06				4.97	1.2	0.21		1.1	0.5	0.6	2.4									1.13	60.2	48.7	
3	sh	9.5	1.0	09:30	18:30	485				0.85				0.030	72.1	2.22	1.1	0.11	0.207								640		640	54					
3	sh	9.5	1.0	09:30	18:30	512				0.90				0.038	69.6	2.532	1.3	0.14	0.258								710		710	54					
3	sh	24.0	1.0			775				0.54				0.024	68.5	1.668	1.2	0.05	0.093								690		690	54					
3	sh	24.0	1.0			726				0.50				0.028	66.4	1.85	1.4	0.06	0.105								690		690	54					
4	sh	2.0	1.0	16:00	18:00	106	0	5	6	0.88	0.00	0.04	0.05		53.6																				
4	sh	4.0	1.0	06:30	10:30	209	6	10	10	0.87	0.02	0.04	0.04		46.9																				
4	sh	8.0	1.0	08:30	16:30	376	46	29	17	0.78	0.10	0.06	0.04		45.7																				
4	sh	12.0	1.0	06:30	18:30	480	101	81	37	0.67	0.14	0.11	0.05		48.5																				
5	sh	2.0	1.0	06:00	08:00				12	0.95		0.00	0.10	0.109	50.0	5.43	0.6	0.31	1.151											27	0.150				
5	sh	4.0	1.0	06:00	10:00				18	0.96		0.00	0.07	0.070	50.5	3.56	0.9	0.22	0.829											27	0.147				
5	sh	8.0	1.0	06:00	14:00				28	0.69		0.11	0.06	0.065	47.5	3.11	1.0	0.12	0.332											37	0.138				
5	sh	12.0	1.0	06:00	18:00				26	0.68		0.13	0.04	0.055	46.2	2.55	1.2	0.10	0.394											26	0.136				
9	sh	8.0	1.0	08:00	16:00					0.51		0.50			50.0																	1.08	48.7	48.8	
9	sh	24.0	1.0							0.55		0.45			62.0																	1.19	45.7	47.2	
9	sh	24.0	1.0							0.51		0.48			53.0																	1.53	44.0	45.5	
9	sh	8.0	1.0	08:00	16:00																										0.299				
9	sh	24.0	1.0																												0.261				
9	sh	24.0	1.0																												0.313				
1	go	4.0	1.0	09:00	13:00												0.8	0.20														0.98	40.6	34.2	38.1
1	go	4.0	1.0	12:00	16:00												0.8	0.21														1.03	39.4	35.6	35.8
2	go	12.0	1.0	07:00	19:00	268		1112	59	0.37							1.6	0.14	0.260											52					
2	go	24.0	1.0			345		1000	95	0.24							2.0	0.08	0.149											56					
3	go	4.0	1.0	12:00	16:00	226	265	949		0.94							0.4	0.10	0.427											23	0.069				
3	go	8.0	1.0	08:00	16:00	381	380	679		0.79							0.5	0.07	0.283											24	0.085				
3	go	24.0	1.0			434	437	569		0.30							0.5	0.02	0.091											24	0.084				
1	bc	24.0	1.0							0.67							9.2	0.38								765									
1	bc	24.0	1.0							0.42							9.1	0.38								760									
1	bc	5.0	2.0	08.00-10:30	15:00-17:30					0.67							7.7	1.54								756									
1	bc	5.0	2.0	08.00-10:30	15:00-17:30					0.41							8.3	1.66								749									
2	bc	12.0	1.0			816											8.4	0.70	0.110	4.1			12.5	145	621	654				632					
2	bc	24.0	1.0			915											8.3	0.35	0.055	4.1			12.4	145	621	660				632					
3	bc	11.0	1.0	08:00	19:00	321	51	122		0.49	0.08	0.18					4.7	0.43	0.155					130	579					279					
3	bc	11.0	2.0	15:00-19:00	08:00-15:00	322	70	122		0.49	0.11	0.18					5.0	0.46	0.164					130	579					279					
3	bc	4.0	1.0	08:00	12:00	188	2	29		0.78	0.01	0.12					4.2	1.05	0.375					130	579					279					
3	bc	4.0	1.0	15:00	19:00	196	2	24		0.82	0.01	0.10					3.6	0.91	0.325					130	579					279					
1	hs	8.0	1.0	08:00	16:00												7.4	0.92	0.170											541					
1	hs	8.0	1.0	08:00	16:00												3.0	0.37	0.090											415					
1	hs	8.0	1.0	08:00	16:00												3.9	0.49	0.110											446					
2	hs	3.0	1.0	07:00	10:00												3.2	1.08	0.196	2.6		2.6	5.9							552					
2	hs	6.0	1.0	07:00	13:00												4.9	0.82	0.152	1.8		1.8	6.7							542					
2	hs	9.0	1.0	07:00	16:00												5.5	0.61	0.112	0.0			5.5							543					
2	hs	24.0	1.0	07:00													7.6	0.32	0.057	0.0			7.6							558					

List of acronyms in Appendix 1 (a).

Acronym	Explanation	Unit
dc	Dairy cows	
sh	Sheep	
Go	Goats	
bc	Beef cattle	
hs	Horses	
n	Number of animals per treatment group	
PT	Production type	
Mi	Milk	
Me	Meat	
PSTAGE	Physiological stage	
Lact	Lactation	
Grow	Growth	
DIM	Days in milk at the beginning of the experiment	Days
InBW	Body weight at the beginning of the experiment	kg
InBCS	Body condition score at the beginning of the experiment	BCS units 1-5
EXPD	Duration of the experiment	Days
PASTSP	Pasture dominant forage	
PP	Permanent pasture	
LPTR	Mix of perennial ryegrass and white clover	
LPB	Perennial ryegrass dominant >70 % DM on offer	
LM	Italian ryegrass	
MIX	Miscellanea of species	
DG	Cocksfoot	
TE	Wheat	
FA	Tall fescue	
GP	Phenology stage of pasture	
GR	Growth	
All	Various stages	
GM	Grazing method	
SG	Strip-grazing	
CS	Continuous stocking	
RG	Rotational grazing	
SS	Set stocking	
HM	Herbage mass	t DM/ha
SHM	Mean of pasture sward height	mm
preSH	Pre-grazing sward height	mm
posSW	Post-grazing sward height	mm
HA	Herbage allowance	Kg DM/head day
OFFAREA	Offered area of pasture	m^2/cow day
TSUPPOFF	Total supplement offered	Kg/head day
CONCOFF	Total concentrate offered	Kg/head day
FORSUPOFF	Total forage offered	Kg/head day
adlib	Ad libitum	
TA	Time of access to pasture	h/day
NAD	Number of daily access	hh:min

List of acronyms in Appendix 1 (b).

Acronym	**Explanation**	**Unit**
HIN	Hour of entry	hh:min
HOUT	Hour of exit	min
GT	Grazing time	min
RT	Ruminating time	min
IT	Idling time	min
WT	Walking time	min
GTP	Grazing time as proportion of access time	
RTP	Ruminating time as proportion of access time	
ITP	Idling time as proportion of access time	
WTP	Walking time as proportion of access time	
BM	Bite mass	g/bite
BR	Biting rate	n/min graz.
HDMIR	Herbage intake rate	g DM/min graz.
HDMI	Herbage intake	Kg DM/head day
HDMIH	Herbage intake per access hour	Kg DM/head h of TA
HDMIHPV	Herbage intake per access hour scaled to 100 kg body weight	Kg DM/head h of TA/100 kg BW
TOTSDMI	Total intake of supplement	Kg DM/head day
CONCDMI	Total intake of concentrates	Kg DM/head day
FSDMI	Total intake of supplemented forages	Kg DM/head day
TDMI	Total intake	Kg DM/head day
HCP	Herbage CP content	g/kg DM
HNDF	Herbage NDF content	g/kg DM
HOMD	Herbage organic matter digestibility	g/kg OM
HDMD	Herbage DM digestibility	g/kg DM
TDOMD	Total diey OMD	g/kg OM
TDMD	Total diet DMD	g/kg DM
Mean BW	Mean body weigt during the experiment	kg
BW change	BW change during the experiment (BWfin – Bwin, if applicable)	Kg
MY	Milk yield	Kg/head day
MF	Milk fat	g/kg of milk
MP	Milk protein	g/kg of milk
MUREA	Milk urea	mg/100 ml of milk

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