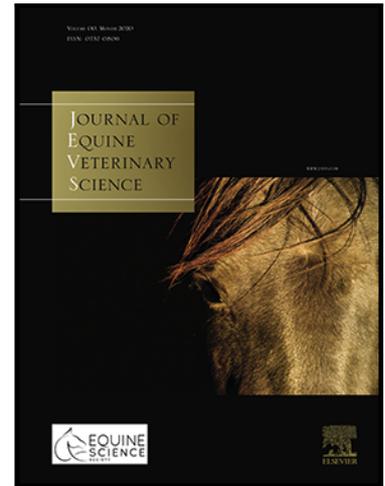


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Title: The effect of strip grazing on physical activity and behaviour in ponies

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Highlights

- Strip grazing did not alter the time ponies spent standing, grazing or locomoting.
- Strip grazed ponies grazed most when the fences had been recently moved.
- Strip grazed ponies preferred to graze the grass made available by fence moving.

Abstract

This work aimed to determine the effect of strip grazing on physical activity in ponies using behavioural observations alongside accelerometers positioned at the poll. In study one, ten British native breed ponies were randomly assigned to paddock A (50x110 m) or B (50x110 m divided into seven equal strips with access to one additional strip per day) for seven days (n=5/paddock). In study two, ten different British native breed ponies were randomly assigned for 14 days individually to 1) a control field where the animal was allowed complete access to their allotted area (n=4); 2) a field

that increased in size daily by moving a lead fence (n=2); and 3) a field that was strip grazed using lead and back fences moved the same distance daily (n=4). Accelerometer data were sorted into twenty-four-hour periods; each ten-second epoch was categorised as standing, grazing or locomoting using previously validated cut-off points; and time spent in each category for each day calculated. Behavioural monitoring was undertaken by direct observation on days 12-14 (study two only). Accelerometer and behavioural data were compared between grazing methods within each study. Strip grazing had no significant effect on the time spent in each physical activity category in either study. Behavioural observation revealed all ponies spent most time grazing ≤ 4 hours after fence moving and strip grazed ponies spent significantly more time grazing the newly available grass than elsewhere. Thus, strip grazing did not alter physical activity in ponies, but did result in preferential grazing of new grass.

Key words

strip, grazing, activity, pony, behaviour

1. Introduction

Strip grazing is used for various reasons in horses. For example, it is used to reduce the paddock area and therefore the amount of pasture available as part of a weight management strategy and/or to reduce the risk of endocrinopathic laminitis [1]. It is also used to protect the pasture as only a small area of pasture is grazed at a time, while the ungrazed areas are rested and allowed to recover [2]. Finally, it can be used when moving a horse to a new field with more grass to allow acclimatisation. Most of the paddock is sectioned off, typically using electric fencing, and additional grass is introduced utilising a lead fence which is progressively moved into the ungrazed area, thereby gradually increasing the paddock area. Alternatively, the strip size can remain constant by progressively moving a lead and a back fence the same distance. Frequently moving the electric

fencing short distances rather than larger distances less frequently results in more consistent forage intake [3]. If the strip is large enough, horses can be kept in groups allowing for social interaction. One potential negative impact is that the reduced paddock size associated with strip grazing could decrease the amount of time spent by the animals undertaking physical activity; however, this has yet to be investigated.

Direct behavioural observation is a common method of determining activity and time budgets in horses [4, 5]. Previous studies have examined the impact of varying the paddock size on locomotion through intermittent observation of behaviour. In one study, locomotion was decreased in smaller paddocks [6], whilst in another females spent more time locomoting in a small yard, whilst males spent more time locomoting in a larger yard [7]. In a third study, horses were more active in larger paddocks and locomotion was increased when the horses were not receiving any form of controlled exercise [8].

Accelerometers are small, wearable sensors that measure acceleration in up to three orthogonal axes [9]; these measurements can be used to estimate the intensity of physical activity (PA) [10]. They have been validated as a reliable means of quantifying PA in humans [11-13], dogs [14-19] and horses [20-22], demonstrating that horses spend significantly more time standing and less time locomoting when kept in smaller paddocks [20].

The use of direct behavioural observations alongside accelerometers can provide a more accurate picture of the percentage of time animals spend performing different activities. Additionally, where there is agreement between these two methods, behavioural observations will support the validity of the accelerometer findings. Thus, the aim of this work was to use validated accelerometers supported by behavioural observations to determine the effect of strip grazing on physical activity and behaviour in ponies kept at pasture either in groups or individually, using either just a lead fence or using a lead and a back fence.

2. Materials and Methods

Two studies were undertaken. Study one was approved by the Royal Veterinary College Clinical Research Ethical Review Board (URN 2017 U109) and study two was approved by the Royal College of Veterinary Surgeons Ethical Review Panel (2019-32-Harris). For both studies, each pony wore an Actigraph wGT3X-BT accelerometer (ActiGraph LLC, Pensacola, Florida, USA) attached to the poll using a headcollar continuously for the duration of the study. Study one was performed in September, whilst study two was performed in July. Prior to commencing study one, ponies were grazed as a single group in a paddock immediately adjacent to the study paddocks. During the 5 weeks prior to starting study two, ponies were individually grazed in electric fenced paddocks of similar pasture type and dimensions to those used in the study. They grazed these paddocks for 3 hours per day and were stabled for the rest of the day. While stabled they received 1% of body weight (BW) as dry matter (DM) of meadow hay per day in two equal meals and a forage balancer (SPILLERS Lite and Lean) at 1g/kg BW/day. In the two weeks prior to the start of study two, ponies were grazed in their non-study paddocks for 3 hours per day with their future neighbours on either side of them for 3 consecutive days. Ponies were kept in this manner to prevent their gaining too much weight before the commencement of the study [23].

2.1 Study one

Ten healthy British native breed ponies (mean \pm standard deviation [SD] age 17.8 ± 5.5 years; weight 355 ± 85.9 Kg; body condition score [24] [BCS] median 6 [range 5-7]; all mares) were randomly assigned to either paddock A or B for seven days (n=5 ponies per paddock). The two paddocks were adjacent, had been managed identically over the last 20 years, had the same total area (110 m long x 50 m wide) and subjectively contained the same amount of herbage; however, paddock B was

divided into seven equal sized strips using electric fencing. The ponies in paddock B were allowed access to one additional strip of the paddock each day whilst the ponies in paddock A had access to the entire area for the duration of the study. As the aim of this study was to determine the effect of a reduction in grazing area on physical activity only, the area of the paddock A and the size of the strips in paddock B were subjectively decided upon based on the usual grazing requirements of the animals used in the study to maintain a constant weight. Herbage dry matter yield, provision for individual animal requirements and dry matter intake were not assessed.

2.2 Study two

This study formed part of a larger 28-day study which investigated the effect of three restricted grazing practices on pasture intake and weight control [23]. Ten accelerometers were available for use, therefore ten (mean \pm SD age 12 ± 5.7 years; weight 370 ± 87 Kg; BCS 5.4 ± 0.7 ; 4 geldings, 6 mares) out of the twelve healthy ponies included in the larger 28-day study were included in this study. The battery life the accelerometers only reliably lasted 14 days, thus this study focused only on physical activity and behaviour during days 2-15 of the larger 28-day.

Each pony was allocated to an individual paddock and the herbage dry matter yield for that paddock was determined directly by clipping, drying [25] and weighing all of the herbage collected from within 15x15 cm quadrats (12 sites per paddock). Each paddock was 10 metres wide, but the length was determined based on the body weight of the individual animal and the initial herbage yield in that paddock, so that each paddock would provide sufficient herbage for the individual pony at 1.5% of body weight (BW) per day in dry matter [26] (as determined on day 1) for a 28 day period.

Each paddock was then randomly assigned to one of three grazing methods: 1) full sized field in which there was sufficient herbage for the duration of the larger 28-day study (control; n=4 ponies); 2) a field that got progressively larger by moving a single lead fence daily (G1; n=2 ponies); and 3) a

field that was strip grazed by moving a lead and a back fence the same distance daily (G2; n=4 ponies). Larger numbers of ponies were assigned to the control and G2 paddocks compared to the G1 paddock as these were the two extremes of the strip grazing methods i.e., no strip grazing at all and true strip grazing in which the paddock area does not increase.

The first 10-15 m of every paddock had been mown to a height of 2.5-3 cm to act as a starting paddock for G1 and G2 animals. These starting paddocks were necessary to provide sufficient paddock area; had the ponies been started from day 1 on the longer (initially 15 cm high) herbage, paddock sizes would have been unacceptably small. The size of the starting paddocks was adjusted for each animal in G1 and G2 to provide 1.5% of individual BW as DM for days 1 and 2. Fences for the G1 and G2 study group were moved daily from day 3 $\frac{1}{26}$ th of the length of the total allocated paddock area. Thus, the area of the control paddocks remained constant and ranged from 340 to 750 m²; the G1 paddocks ranged from 70-100 m² on days one and two and gradually increased in size up to 200-290 m² on day 14; and the area of the G2 paddocks remained constant and ranged from 110-160 m². Due to the high DM yield of the herbage, the lead fences were moved only a short distance each day. In G2, the lead and back fences were moved the same amount as the lead fences each day, however, the length that the fences were moved did not mean that the entire available area was comprised of 'new' grass each day. The grass available each day was the new 'strip' and any residual herbage remaining after having been grazed over the previous day(s) on the part of the 'old' strip that still remained within the area.

The twelve individual paddocks were side by side in a rectangular field and the treatments were randomised. Ten ponies (in paddocks 2 to 11) could have direct eye contact with the two neighbours on either side of their own paddock. The ponies in paddocks 1 and 12 had direct eye contact with one neighbour. However, all ponies could see, even if not in direct eye contact with, ponies undergoing each of the three treatments.

Ponies were removed from their individual paddocks and turned out into a sand and rubber outdoor arena for approximately 50 min each day to allow the faeces to be collected from each paddock. Accelerometer data from this time period each day was discarded.

Behavioural monitoring was carried out on days 12-14 of this study. These days were chosen as the ponies were still wearing the physical activity monitors and were habituated to their conditions such that there should have been less of an impact of novelty or any initial stress on behaviour. Additionally, it was halfway through the larger study so that G1 paddocks were about average size. Observations were undertaken by a single observer and took place between approximately 09:00 and 18:30 on days 12 and 13, and 09:00 and 13:00 on day 14. The ethogram developed and used was based on 'The Equid Ethogram' [27] (Supplementary Table). Instantaneous scan sampling [28] was conducted at 3-minute intervals. The day was broken into observation sessions that lasted approximately 45-90 minutes, with groups of between two and six ponies being observed during each session depending on the observer's location in the field but outside the study paddocks. Observations of these groups of ponies each day was rotated, allowing ponies from different grazing conditions to be observed at similar times in relation to the moving of the fences and also across a variety of times of the day. Ponies were not observed whilst they were in the outdoor arena or during the time when the fence was actually being moved. In total, ponies were observed for approximately five hours (mean \pm SD 5.2 \pm 0.6 hours) over the three days. Observed behaviours were categorised as grazing (new grass, under fence, elsewhere in paddock), standing (alert, at rest [includes lying at rest]), locomoting (walking, trotting/cantering) and 'other' behaviours including agonistic behaviour (ears back, lunge, retreat), auto-grooming, frustration related behaviour (pace, paw, tail swish), greeting behaviour, repetitive head movements (shake, nod), repetitive oral movements, spooking/startling and vocalising (squeal, whicker, whinny).

Each day, the fences for G1 and G2 were moved in succession, always starting with the same pony, and ending with the same pony each day. On the behavioural observation days, fences began to be moved between 09:00 and 10:30 and it took approximately one and a half hours to move all fences.

2.3 Statistical analysis

For all data analyses, data were assessed for normality using Shapiro-Wilk normality test and significance was accepted at $P < .05$.

2.3.1. Actigraph data

Actigraph data were downloaded using the accompanying ActiLife software package (Actigraph LLC) and exported into Microsoft Excel. In all analyses, the vector magnitude (the square root of the sum of the squares of all three axes) was used as the measurement for physical activity intensity. Each 10 second epoch was assigned to one of three categories, namely standing, grazing and locomoting, using previously determined vector magnitude cut-offs [20]. The time spent in each category was calculated for each 24-hour period and for the total study period for each study.

For study one, statistical analysis was performed using GraphPad Prism version 8.0 (La Jolla, California, USA) and the effect of field type and day on the time spent undertaking each activity determined using a two-way ANOVA with Tukey's multiple comparisons *post hoc* tests. For study two, statistical analysis was performed using SPSS version 26 Statistics software (IBM, New York, USA) and the effect of field treatment, day, and individual animal on the time spent in each physical activity category was determined using a univariate general linear model with Bonferroni *post hoc* tests. For both studies, the strip grazing method x day interaction was investigated.

2.3.2. Behavioural data

Behavioural data were analysed using SPSS version 26 Statistics software. For time-of-day comparisons, the data were categorised into three time periods according to when the fences were moved for each individual paddock. Before strip grazing fences had been moved each day was classed as period one, when fences had been moved less than or equal to four hours ago this classed as period two, and period three was when fences had been moved more than four hours ago. The choice of a four hour time period was based on observation of foraging behaviour periods used in previous publications [29]. The effect of grazing method on the proportion of scans ponies were observed performing the different behaviours, both overall and for each of the three time periods, was assessed using Kruskal-Wallis tests. *Post hoc* Dunn's tests with a Bonferroni adjustment were then used to make pairwise comparisons between conditions where a significant result was found.

A Wilcoxon's test was used to compare the percentage of scans ponies in the G1 and G2 study groups (individually and combined) were observed grazing new grass or grazing elsewhere in their paddocks. New grass refers to the fresh grass G1 and G2 ponies could access once their electric fences had been moved each day. Therefore, control ponies were not included in this analysis. Only periods two and three were included as in period one the previously uncovered grass (period one) was no longer considered to be new grass.

3. Results

3.1 Actigraph Data

The mean \pm standard deviation amount of time spent in each of the three activity categories for both studies is presented in Table 1.

3.1.1. Study one

There was no significant difference in the amount of time spent in each activity category when comparing the ponies in paddocks A and B over the entire study (Table 1). In both paddocks, the majority (70 and 74%) of the time was spent grazing and only a very small proportion of time (1 and 3%) was spent locomoting. The ponies in paddock B spent significantly more time locomoting on day 4 compared to days 1 ($P = 0.003$), 2 ($P = 0.003$), 5 ($P = 0.007$) and 6 ($P = 0.01$; Fig. 1). There was no interaction between strip grazing method and day.

3.1.2. Study two

There was no significant difference in the amount of time spent in each activity category over the 14-day study period when the three grazing methods were compared (Table 1). There was also no significant effect of day (days 2-13 only) on the amount of time spent undertaking all three activities (Fig. 2); days 1 and 14 were not included in this analysis as they were not complete 24-hour periods. There was no interaction between strip grazing method and day.

3.2. Behavioural data

There was no significant difference in the percentage of scans ponies were observed in each activity category (grazing, standing, walking) or performing 'other' observed behaviours over the entire behavioural monitoring period when the three grazing methods (control, G1 and G2) were compared (Table 2). In addition, no significant differences were found when the type of standing (standing alert or standing at rest) and type of locomoting (walking or trotting/cantering) were examined separately, nor when grazing under the fence was tested (Table 2).

When the behaviours that made up the 'other' behaviour category were tested individually, the only behaviour that was significantly different between grazing methods was repetitive head movements

(Table 2). G1 ponies (median 2.7%) were observed displaying repetitive head movements (head shake, head nod) in a significantly ($P = 0.04$) greater percentage of scans than G2 ponies (0.0%). However, no significant differences were found between control (median 1.5%) and G2 or G1 ponies.

When time periods one, two and three were examined separately, it was found that grazing method had a significant impact on the percentage of scans ponies were observed grazing during period three (when fences had been moved >4 hours ago), but not during periods one or two (Table 3). Control ponies spent a significantly ($P = 0.02$) greater proportion of scans grazing in period three than G2 ponies. However, there were no significant differences between G1 and control or G2 ponies. Grazing method also had a significant impact on the percentage of scans ponies were observed standing during period three (Table 3). G2 ponies spent significantly ($P = 0.02$) more scans standing than control ponies, the opposite finding to grazing behaviour. Again, there were no significant differences between G1 and control or G2 ponies. No significant differences between time periods were found for any of the other observed behaviours (Table 3).

There was no significant difference in the percentage of scans G1 or G2 ponies were observed grazing new grass compared to grazing elsewhere in their paddocks (Fig. 3). However, when the data from G1 and G2 was combined, ponies spent a significantly ($P = 0.03$) greater proportion of scans grazing new grass (median 53.2%) than grazing elsewhere (median 3.6%) in their paddocks during periods two and three.

4. Discussion

In the present study, the reduction of space associated with strip grazing using either just a lead fence or a lead and a back fence combined was not associated with a significant change in physical activity determined using physical activity monitors in ponies kept individually or in groups.

The effect of paddock size on the physical activity assessed using physical activity monitors in horses has been investigated once previously in horses grazed individually 24 hours a day for 10 days in smaller (60 x 10 m) and larger (60 x 40 m) paddocks [20]. Horses spent a significantly larger percentage of their time standing and smaller percentage of time locomoting when kept in the smaller paddocks, but there was no significant difference in the time spent grazing between the two sized paddocks [20]. In comparison, in the present studies, ponies spent similar proportions of their time undertaking the three activities (standing, grazing and locomoting) compared to the previously reported values for horses grazed in the larger paddock [20], regardless of the strip grazing method. Whilst it is possible that even the most area constrained paddocks were not small enough to induce a significant effect on the time spent undertaking each activity, this is unlikely as these paddocks were only one sixth of the area of those previously reported to have such an effect [20].

There was a significant effect of day in study one with ponies in the strip grazed paddock spending a significantly greater amount of time locomoting on day 4 (mean 67 minutes) compared to three of the other five days (mean 27, 30 and 32 minutes). However, this equates to a difference of between 35 and 40 minutes, which is within the previously reported between day difference range for locomoting (6.8 - 40.2 minutes) in horses at pasture [20]. Additionally, there were no obvious external influences such as inclement weather or management activities that would explain this difference

It has been previously demonstrated that accelerometer cut off points can be used to distinguish between rest, walking, trotting and cantering [30]. Using the validated cut off points determined by Maisonpierre et al. (2019) in the present study allowed for a grazing category and so was more appropriate. However, future studies could try to include distinguishing between different behaviours exhibited in the paddock, such as grooming or rolling, and the various different gaits and investigating the effect of strip grazing methods on these. This would require use of accelerometers

attached to both the poll and the trunk or a limb to help differentiation between activities that involve only the head moving and those that involve the whole horse moving.

Results from the behavioural monitoring during study two supported the results obtained using the physical activity monitors, with there being no significant differences in the ponies' physical activity between the grazing methods. In a previous study, physical activity assessed using direct observation was compared in horses turned out individually for two hours once daily for seven days into small (150 m²), medium (300 m²) and large (450 m²) bare paddocks [8]. Horses spent a significantly larger percentage of their time standing in the small paddock (62%), with standing time decreasing as paddock size increased (50% in medium and 34% in large paddock). In comparison, in the present studies, ponies spent a similar percentage of their time standing as these previously reported values for horses in the large paddock, spending between a quarter and a third of their time standing in paddocks that ranged in size from being smaller to being much larger (70 m²-5500 m²). Horses were also previously reported to spend less time grazing in the small paddocks (22.5%) compared to the medium (31%) and large paddocks (44%) [8]. However, it should be noted that the paddocks in the previous study were bare and grazing was only possible along the fence line at the edge of the paddock. In comparison, in the present studies in which the ponies were fully kept at pasture, ponies unsurprisingly spent a much greater proportion of their time grazing, spending between half and three quarters of their time grazing, regardless of paddock size.

Whilst grazing method did not impact the overall amount of time spent grazing, behavioural observation revealed that it did have a significant impact on the time of day and where ponies concentrated their grazing efforts. Ponies in study two spent the greatest proportion of their time grazing in period two (within four hours of fences being moved) regardless of grazing method. This is in agreement with a study on lactating mares grazing with front and back strips moved every two days [31] which reported a lower grazing time on the smaller area (and smaller forage supply) on the second day on a strip. This is interesting as even the control ponies who had no fences moved

spent more time grazing during this time period. One could speculate that this was due to social facilitation resulting in all ponies developing behavioural synchronicity, as is observed in free-ranging horses [32]. In period three (>4 hours since fences had been moved), control ponies spent a significantly greater proportion of time grazing than G2 ponies. This suggests that ponies strip grazed with a lead and back fence (G2) concentrated their grazing efforts during period two when fresh grass had become available, whilst control ponies spent a similar proportion of time grazing in periods two and three, possibly due to having constant grass availability. After a period of restriction, horses may engage in compensatory eating and have been recorded to consume 49% of their daily dry matter intake in just three hours at pasture after a period of being stabled [33]. Though the ponies in this study had constant access to grazing, the fact that strip grazed ponies spent the greatest proportion of time grazing in period two just after the fence(s) had been moved suggests they may have engaged in compensatory eating, after having less grass available in quantity and quality to eat overnight. Similarly, G1 and G2 ponies grazed the newly revealed strip of grass for most of their total time observed grazing, spending significantly more time grazing this area than anywhere else in the paddock. This was to be expected as this strip contained newly available grass, while the rest of their paddocks had previously been grazed down and affected by trampling. Future research should investigate the potential physiological impacts of this preferential grazing of new grass and hence inconsistent intake of pasture on the ponies. It may be of relevance for example to animals at increased risk of endocrinopathic laminitis, as changes in grass availability and grazing management were found to be a risk factor for laminitis in some [34, 35], but not all studies [36].

It should be acknowledged that horses can compensate for poorer quality grass by increasing the amount of grass intake through increasing the frequency and/or mass of bites as well as by increasing the time spent grazing [37]. The effect of strip grazing on the act of grazing itself was not determined in this study. Additionally, it should be remembered that paddock area and forage supply are not always associated. Whilst a reduction in paddock area suggests that there is a decrease in forage supply, this is not always the case. However, the larger 28-day study did

demonstrate that the strip grazing methods used in study two reduced estimated daily pasture dry matter intake [23].

Analysis of the individual behaviours revealed that G1 ponies were observed displaying repetitive head movements in significantly more scans than G2 ponies. However, there was a low performance of this behaviour in all conditions and actual differences between conditions were small. Therefore, it is unlikely that the differences seen were due to the impact of grazing method, and more likely to be due to other factors. For example, these behaviours are a common response to harassment by flies and the amount of harassment experienced by ponies is likely to be impacted by the location of their paddock in the field and the weather conditions at the time of observation [38, 39]. Individual differences in ponies and the fact that this behaviour often occurred in short bouts that could have been missed between scanning intervals also likely had an effect.

Grazing method did not affect performance of any other behaviours, including those related to frustration or distress, and there was overall low performance of these behaviours. This suggests that under our study conditions ponies did not find strip grazing aversive compared to the control treatment where ponies were given access to their full grazing ration in one go. However, it should be acknowledged that physiological parameters were not measured. Whilst Squibb et al. (2018) found no correlation between behaviour and physiological signs of stress [40], publications investigating correlations between behaviour and physiological signs of stress on Equidae are rare in comparison with other domestic animal species, therefore future studies into strip grazing should also aim to record physiological measures of stress. Additionally, it was necessary for the aims of the larger study [23] for ponies in study two to be kept in individual paddocks. However, horses are a social species and show increased physiological signs of distress when prevented from making physical contact [41]. Therefore, when implementing strip grazing, horse owners should consider keeping horses in groups or allowing horses socialisation time outside of being strip grazed.

Small subject size was a limitation of this study, particularly for study two. Whilst four subjects were allocated control and G2 paddocks, only two subjects were allocated G1 paddocks. This was due to the fact that only 10 accelerometers were available for use. However, this limitation was at least in part negated by the behavioural data supporting the results of the physical activity monitors. Future studies should involve larger numbers of subjects, evenly divided between the groups. It should also be acknowledged that the intention in study two was that all horses had the same degree of dietary restriction over a 28-day period [23]. As the present study was performed over only days 2-15, the control ponies are likely to have consumed >50% of their 28-day ration over this time period as they had access to the entire field, whereas those in G1 or G2 would have had access to only 50% of the ration. Ideally, the accelerometers should have been left in place for the entire 28 period of the larger study and/or behavioural observations should have been carried out intermittently through this longer period. The battery life of the Actigraphs prevented continuous monitoring, but they could have been recharged and replaced after a 12-hour delay.

It should also be acknowledged that the behavioural observations were only undertaken between 9am and 6.30pm and so behaviour in the early morning, evening and overnight was not recorded. It has been reported that horses at pasture have a peak of activity in the morning and evening [4] and that time of day affects grazing bouts [42] with longer feeding bouts being recorded at dawn and in the later afternoon [43] or at night [44]. Future studies should ensure that behavioural observations are undertaken throughout the day and night.

5. Conclusion

In conclusion, strip grazing assessed using physical activity monitors and behavioural observation did not significantly alter physical activity in ponies regardless of the method employed. However, whilst there was also no significant effect on the overall time spent grazing, strip grazed ponies concentrated their grazing efforts during the period when the fences had been moved most recently and preferentially grazed on the additional grass that had become available. The potentially

deleterious effect of this selective grazing and/or inconsistent pasture intake on the physiological health of the pony requires further investigation.

6. Acknowledgements

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7. Funding Sources

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8. Data statement

A copy of the study data can be obtained from the corresponding author upon reasonable request.

Ethical statement

The research described in this manuscript followed international, national, and institutional guidelines for humane animal treatment and complied with relevant legislation in the country in which the study was conducted. The studies were approved by the Royal Veterinary College Clinical research ethical review board (study one) and the Royal College of Veterinary Surgeons Ethical Review Panel (study two).

Conflict of interest statement

Professor Harris is employed by the study funder.

Table 1: Mean (\pm standard deviation) total time (hours) spent in each of the three activity categories (standing, grazing locomoting) in study one (group A and B; n=5 per group) over the 7-day study period and study two (Control [total area available; n=4], G1 [lead fence only; n=2], G2 [lead and back fence; n=4]) over the 14-day study period. The mean (\pm standard deviation) time (hours) spent in each activity over each 24-hour period is also presented.

Activity Category	Study one	Study one	Study two	Study two

	A		B		Control		G1	
	7 day	24-hour	7-day	24-hour	7-day	24 hour	7-day	24
	Total	Total	Total	Total	Total	Total	Total	Total
Standing	47.9 ± 6.3	6.8 ± 0.9	41.0 ± 4.5	5.9 ± 0.6	96.1 ± 16.3	7.5 ± 0.9	89.7 ± 25.1	
Grazing	116.4 ± 6.8	16.6 ± 1.0	121.6 ± 5.3	17.4 ± 0.8	150.8 ± 16.0	11.6 ± 3.7	157.9 ± 10.4	
Locomoting	2.7 ± 0.8	0.4 ± 0.1	4.4 ± 1.9	0.6 ± 0.3	47.9 ± 15.4	0.9 ± 1.5	29.3 ± 17.4	

Table 2. The median percentage of scans ponies in each grazing method were observed performing each behaviour, and significance (P) obtained from Kruskal-Wallis tests.

Behaviour	Control Median % Scans	G1 Median % Scans	G2 Median % Scans	P
Grazing (Total)	64.7	59.0	65.3	0.8
Grazing Under Fence	0.9	4.1	1.8	0.8
Standing (Total)	24.5	31.5	30.0	0.7
Standing Alert	13.7	19.4	19.2	0.7
Standing at Rest	11.4	12.2	10.8	0.9
Locomoting (Total)	3.0	1.8	3.3	0.7
Walking	2.0	1.8	3.3	0.7
Trotting/Cantering	0.5	0.0	0.0	0.4
All Other Behaviours Grouped	5.5	7.7	2.3	0.3
Agonistic	0.0	0.9	0.0	0.6
Auto-grooming	1.4	0.9	0.0	0.1
Frustration Related	0.0	0.5	0.0	0.8
Greeting	0.0	0.0	0.4	0.5

Repetitive Head Movements	1.5 ^{a,b}	2.7 ^a	0.0 ^b	0.04 [*]
Repetitive Oral Movements	0.0	0.5	0.0	0.4
Spooking/Startling	0.5	0.9	0.0	0.3
Vocalising	0.0	0.5	0.4	0.9
Other Non-categorised	0.0	0.9	0.5	0.6

Values with the different letter superscripts were significantly different from each other.

*indicates a significant result (P<0.05)

Table 3. The median percentage of scans ponies in each grazing method and during each time period were observed performing each behaviour, and significance (P) obtained from Kruskal-Wallis tests.

Behaviour	Period 1				P	Period 2			
	Control % Scans	G1 % Scans	G2 % Scans	P		Control % Scans	G1 % Scans	G2 % Scans	P
Grazing (Total)	28.6	7.6	35.3	0.05	70.3	86.1	94.1	0.2	
Grazing Under Fence	0.0	0.0	14.7	0.04 [*]	0.0	0.0	0.0	0.4	
Standing (Total)	57.2	66.7	40.0	0.1	17.1	10.6	3.4	0.1	
Standing Alert	42.9	45.5	32.4	0.7	10.9	9.6	3.4	0.3	
Standing at Rest	14.3	21.2	0.0	0.7	4.7	1.1	0.0	0.2	
Locomoting (Total)	0.0	0.0	0.0	0.7	0.0	1.1	4.7	0.2	
Walking	0.0	0.0	0.0	0.7	0.0	1.1	4.7	0.2	
Trotting/Cantering	0.0	0.0	0.0	1.000	0.0	0.0	0.0	0.5	
All Other Behaviours	9.1	25.8	2.9	0.5	7.5	2.2	0.0	0.08	

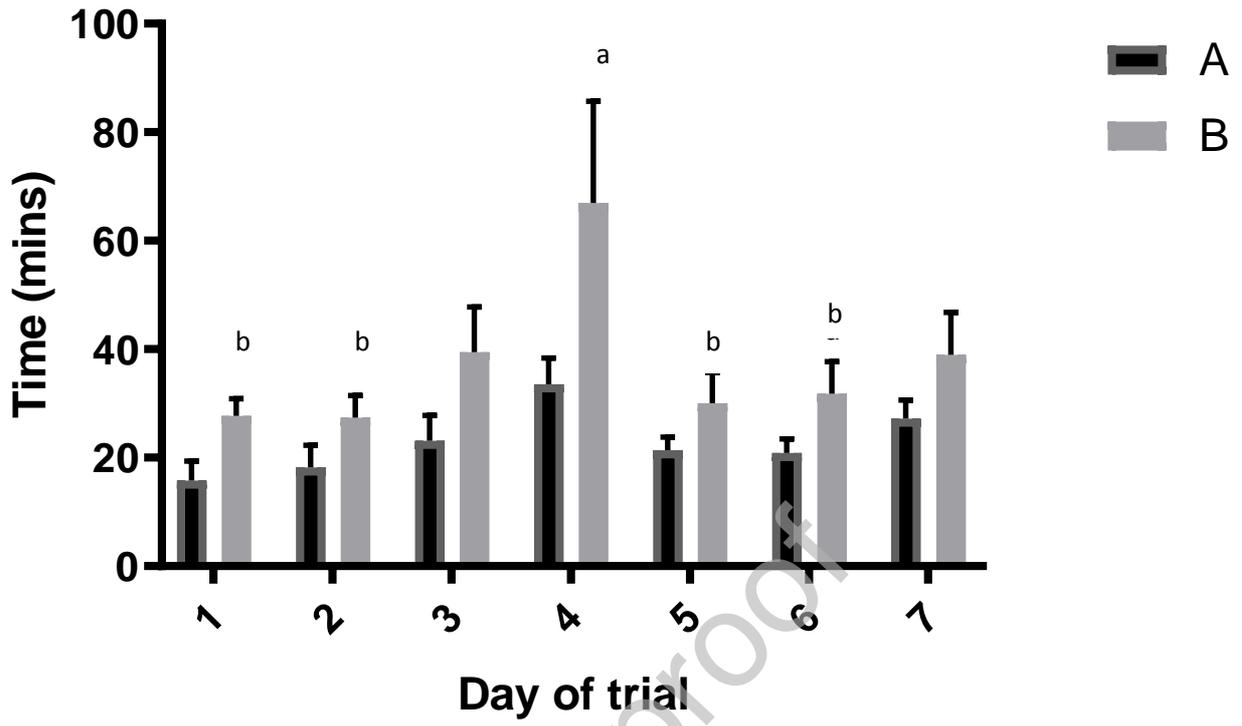
Grouped									
Agonistic	0.0	1.5	0.0	0.4	0.0	1.1	0.0	0.4	0
Auto-grooming	3.0	1.5	0.0	0.3	0.0	0.0	0.0	0.5	0
Frustration Related	0.0	0.0	0.0	0.7	0.0	0.0	0.0	1.0	0
Greeting	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0
Repetitive Head Movements	0.0	12.9	0.0	0.06	1.1	0.0	0.0	0.2	0
Repetitive Oral Movements	0.0	1.5	0.0	0.2	0.0	0.0	0.0	1.0	0
Spooking/Startling	0.0	0.0	0.0	1.0	1.1	1.2	0.0	0.3	0
Vocalising	0.0	8.3	0.0	0.2	0.0	0.0	0.0	0.7	0
Other Non-categorised	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.5	0

Results of all *post hoc* Dunn's pairwise comparison tests were non-significant

* indicates a significant result

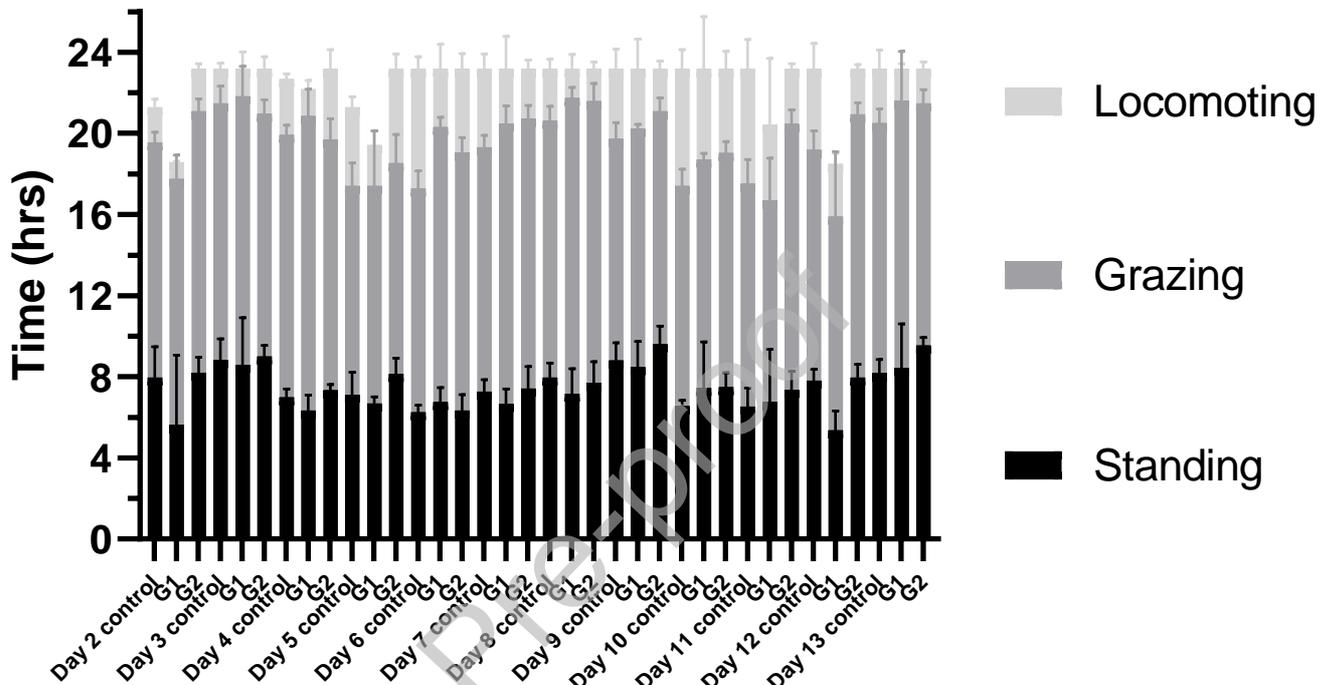
Figures

Figure 1: The mean \pm SD time (minutes) spent locomoting each day by groups of ponies (n=5 per group) in a large paddock (A) and a strip grazed paddock with only a lead fence (B) on days 2-7 of the study period. Bars with different letter superscripts were significantly ($P < 0.05$) different from each other.



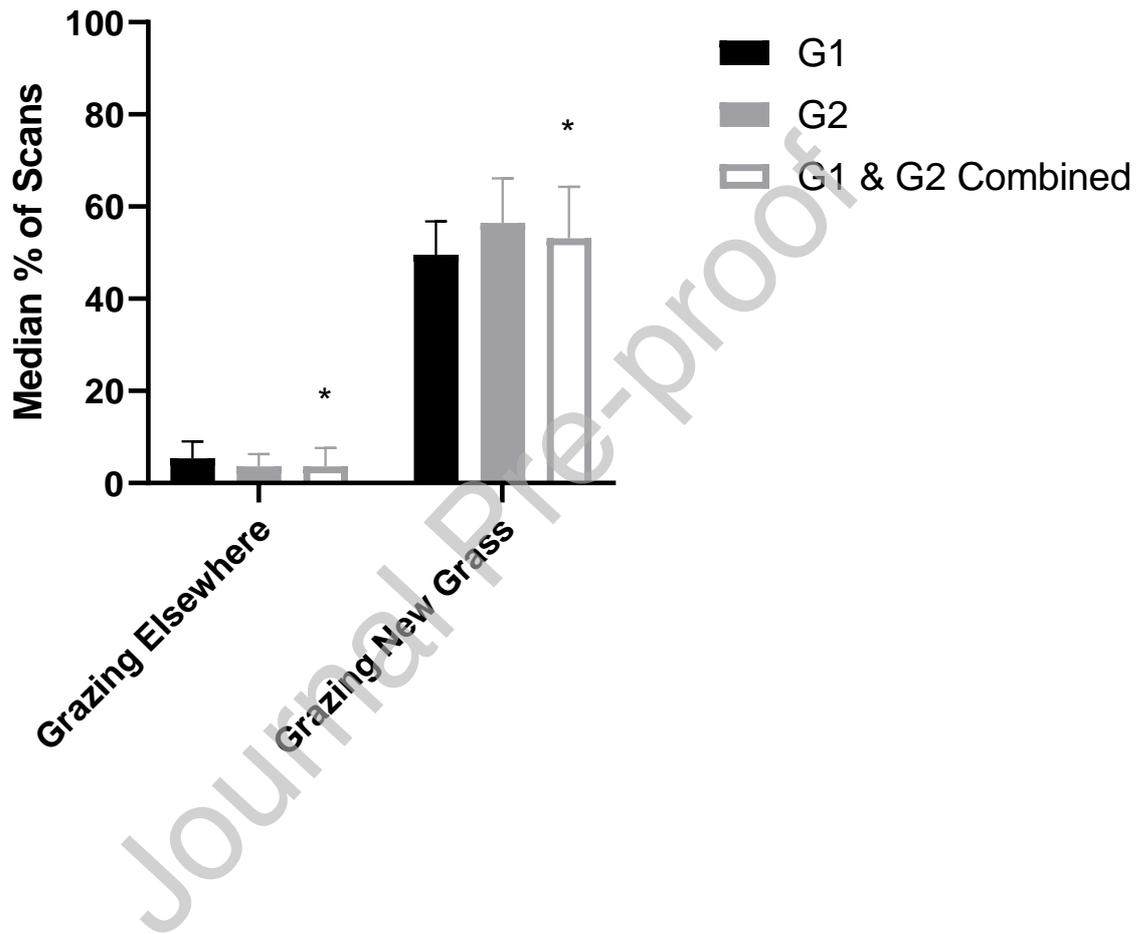
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Figure 2: The mean \pm SD total time (hours) spent standing, grazing and locomoting on each day by ponies kept for 14 days individually in either a large paddock (control) or a strip grazed paddock with only a lead fence (G1) or a lead and a back fence (G2).



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Figure 3: The median (IQR) percentage of scans ponies kept individually in a strip grazed paddock with only a lead fence (G1) or a lead and a back fence (G2) were observed grazing new grass and grazing elsewhere in the paddock during periods two and three across days 12-14. Data is shown for G1 and G2 alone and combined. * indicates a significant ($P < 0.05$) difference between grazing sites within the paddock.



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Behavioural Classification	Behaviour	Description
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Supplementary Table: Ethogram of observed behaviours.

Agonistic	Ears back (at another pony)	Ears facing backwards, pinned flat down against head
	Lunge (at another pony)	Swift thrust of body from close range towards another pony, usually with ears back
	Retreat	Moving away from another pony in response to the other pony's behaviour
Auto-grooming	Auto-grooming	Nibbling and biting, or using the hoof to scratch own body
Frustration Related	Pace	Walks either a circle following the perimeter of the paddock or back and forth along one fence line
	Paw	A foreleg is lifted off the ground slightly, extended quickly forward and the toe is then dragged backwards across the ground towards the body, this is often done repetitively
	Tail swish	Tail is swiftly moved in a flicking motion
Grazing	Grazing new grass (condition 1 & 2 ponies only, periods 2 & 3 only)	Grazing only the fresh grass the pony had been provided access to that day, once the strip grazing fence had been moved
	Grazing under fence	Reaching under the paddock fence with the muzzle to graze grass on the other side of the fence
	Grazing elsewhere in paddock	Ingesting grassy vegetation in any area of the paddock other than the new grass strip or under the fence
Greeting Behaviour	Greet	Ponies approach nose to nose with deliberate sniffing of one another's exhaled breath, ponies are separated by an electric fence and do not make physical contact with one another
Locomoting	Walking	Movement forward in a four-beat gait
	Trot/Canter	Movement forward in a two-beat gait/running in a three-beat gait
Repetitive Head Movements	Head nod	Repeated, rhythmic up and down movement of the head in a 'yes' motion
	Head shake	Repeated, rhythmic side to side movement of the head
Repetitive Oral Movements	Lick and chew	Side to side grinding of the upper and lower jaw whilst sticking the tongue in and out
Spooking/Startling	Spook	Startle response where the pony either suddenly jumps or runs away from the stimulus that caused it
	Startle	Pony flinches or jumps slightly but without travelling, usually turns to look at the stimulus that caused it

Standing	Stand alert	Standing still, either relaxed, whilst seemingly paying attention to surroundings, likely to be turning head to look at different stimuli
	Stand at rest/Lie down	Standing inactive or asleep in a relaxed position with eyes partly or nearly closed, or closed, head lowered, relaxed lips, ears rotated laterally and often bearing weight on three legs/Lying recumbently on the ground either sternally or laterally
Vocalising	Squeal	High pitched, short vocalisation, mouth is typically closed
	Whicker	Low pitched, gutturally pulsated vocalisation
	Whinny	Loud, prolonged call, typically beginning high pitched and ending lower pitched
Other	Blow nose	Forceful, extended exhalation clearing the nostrils
	Drinking	Ingesting water
	Investigating pony droppings	Looking at and smelling faeces
	Urinating	Expelling of urine through the urethra