MS for APPLIED ANIMAL BEHAVIOUR SCIENCE

No increased stress response in horses on small and electrically fenced paddocks

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Abstract

Regular free movement is an important factor for the wellbeing of horses, reducing the risk of behavioural disorders as well as maintaining bodily functions healthy. Currently animal welfare legislations in several countries require minimal stay times in outdoor enclosures. However, clear legal recommendations for the fence type enclosing or surrounding this area do not exist. With regard to electric fencing systems, some concerns exist that the latter could affect the behaviour of horses, cause chronic stress and reduce the accessible space. The aim of this study was to quantify stress responses in horses kept in small and big paddocks with wooden fencing compared to paddocks with electric fencing, focusing on possible differences concerning the utilisation of the paddock area between the two types of fencing and size. Twenty horses aged between 6 and 18 years were tested comparing two paddock sizes (12.25 m^2 / 36 m^2) either with wooden or electric fencing in order to evaluate physical and behavioural parameters. Each horse was tested in a randomized sequence in all four paddock types during 90 min per type. During the experiments the horses were continuously recorded on video for later analyses of behavioural patterns and the utilisation of the available paddock area. Additionally, periodic measurements of heart rate (HR), heart rate variability (HRV) as well as standardized salivary samplings for later cortisol determination were carried out. Horses used the available area significantly less both in electrically fenced and small paddocks ($p < 0.001$). The border area (50 cm) was used less both in electrically fenced and small paddocks ($p < 0.001$). The total amount of stress-indicating behaviour did not differ between the two fence types. Horses moved less in small ($p < 0.001$) and electrically fenced ($p < 0.005$) paddocks than in big and wooden fenced ones. Horses rolled less in small paddocks ($p < 0.001$). Fence contact was significantly less with electric fencing ($p < 0.001$). Stress-indicating behaviour tended to be more prominent in small paddocks. Salivary cortisol and HRV parameters did not differ between the paddocks but a slight tendency of alteration of the low frequency/ high frequency ratio indicating stress reactions was observed with electric fencing. Based on the measured physiological parameters there is no indication for stress in electrically fenced paddocks. However horses use less of the available paddock area both in electrically fenced paddocks and small paddocks, especially by avoiding the area near the fence.

Key words: horse, saliva, stress, cortisol, electric fence, heart rate variability

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1. Introduction

Current welfare laws regulate companion and farm animal husbandry including equine management. In many European countries, horses are required by law to be granted regular access to an exercise area or paddock. In Switzerland for example, turnout for riding horses is required at least two times per week for two hours on a defined minimum area dependent on the horse's size. The soil substrate and enclosure type are not clearly specified (TSchV, 2014). Consequently, different fencing systems can be encountered within horse farms. Because of the lack of legal prescriptions, factors such as economic considerations and rapid relocatability motivate about 50 percent of horse keepers in Switzerland to use electric fencing. Furthermore, electric fences mostly prevent tactile contacts between neighbouring horses, which is sometimes requested from anxious horse owners. However, the construction of fences surrounding any exercise areas, paddocks or pastures must not present an injury risk to the horse, must not cause unnecessary stress for the horse and must prevent the horse from escaping the enclosure ((BMELV, 2009; FAWAC Dublin, 2005; TSchV, 2014).

In this context, the use of electric fencing is discussed as a potential source of undue stress including fear of electrical shocks, particularly when the exercise area is small. Constant stress may lead to a compromised immune function and hence increased susceptibility to infections or prolonged recovery times (Kelley, 1980; Robson et al., 2003). Several studies have investigated various husbandry and management systems (Bachmann and Stauffacher, 2002; Knubben et al., 2008a, Mellor et al. 2001) as well as the influence of husbandry systems on the horse (Bachmann et al., 2003; Hoffmann et al., 2009; Knubben et al., 2008b; Leme et al., 2014; Niederhöfer, 2009), however the direct influence of electric fencing on a horse's wellbeing has not been examined in detail. A recent study compared the behaviour of horses in box stalls with enclosed paddocks of different sizes with or without electric fences (Moors et al., 2010). Yet, no systematic stress assessment was carried out. Previous studies examining the stress reaction of horses in certain situations have shown that horses' wellbeing cannot be expressed with a single measurable parameter, but that it involves a combination of various ethological and physiological parameters (Hoffmann, 2008; Rietmann et al., 2004; Schmidt et al., 2010a, 2010b, 2010c, 2010d). Thus, to compare the assumed stress situations, in this experiment not only ethological but also physiological parameters of the two main stress axes were measured: (i) heart rate (HR) and heart rate variability (HRV), sensitive criteria for the assessment of sympatho-vagal balance, and (ii) the concentration of plasma cortisol, a widely recognised parameter of the hypothalamo-pituitary-adrenocortical axis (HPA).. Previous studies have successfully used HR and HRV measurements to quantify stress in horses (Rietmann et al., 2004; Schmidt et al., 2010a, 2010b, 2010c). Others have used cortisol as a parameter to assess stress in a variety of situations including transportation, training, competition or stereotypies (Peeters et al., 2011). Due to the good correlation between salivary and plasma cortisol levels, the analysis of salivary cortisol concentrations represents a non-invasive, stress free method for measuring free, biologically active cortisol, and is therefore a suitable parameter for assessing stress in horses (Hughes et al., 2010; Hughes and Creighton, 2007; Schmidt et al., 2010a, 2010 b). Finally, activity and specific arousal-indicating behaviours, the extent to which the horses made use of the entire paddock area and the contact time with the fence were recorded with the aim to directly compare the effect of electric and non-electric fencing.

The aim of this study was to directly compare the effect of electric and non-electric fencing in small and large paddocks on stress levels in horses by assessing various physiological (HR, HRV, salivary cortisol) and ethological parameters. Additionally, we studied the extent to which the horses made use of the entire paddock area. We hypothesized that a short-term stay in experimental paddocks would lead to an increased HPA activity, a reduced utilisation of space and more stress indicating behaviours in the context of electric fencing.

2. Materials and methods

Animals, housing conditions and experimental paddocks

The experiment was carried out using 20 healthy horses, 17 mares and 3 geldings (11 Warmblood, 4 Standardbred, 2 Franches-Montagnes horses and 1 Pony) aged on average 10.1 ± 3.19 years at the Swiss National Stud (Avenches, Switzerland). The horses were kept in group or individual housing systems and all of them had previously encountered electric fencing, either on pasture or on the paddock of the group housing system. Standardized feed rations comprised 1.5 kg concentrates and 2.5 kg hay twice daily. During the experimental days the horses were stabled in individual boxes (3.5 x 3.5 m and bedded on straw) and provided with hay ad libitum.

For the experiment, 4 paddock types were used that resulted from a 2 x 2 factorial design with two paddock sizes (6 x 6 m and 3.5 x 3.5 m) and two fencing materials (wooden rails made from spruce and 40 mm electrical tape) (see Fig. 1). The fences were 1.5 m high with 3 rails or tapes 0.5 m apart. The electrical power was supplied by a 220 - 230 Volt power supply (Agraro N50, Landi, Switzerland). The output voltage between fence and ground was 7.16 - 10.3 kV at a current of 0.2 - 0.83 A. The four different experimental paddocks were arranged two-by-two on a field with 150 m^2 ground. Another four paddocks of the four different types were arranged in the same field forming a point reflection of the first four paddocks (mirrored configurations on the right and left hand side of the field). Halfway through the study, all electric and wooden fences were interchanged. This allowed to partially control for effects of the specific location.

Experimental design

Each horse underwent a 90 min experimental phase in one of each paddock type on four consecutive days at the same time of day (either in the morning or the afternoon). The 20 horses were randomly allocated to one of five groups of four horses. This constellation remained constant throughout the experiment. The groups' allocation to the left or right hand paddocks was alternated as well as randomised. All four horses of the same group were tested simultaneously always at the same time (12.00pm or 02.30pm, respectively) in the four paddocks available on the side assigned to each group.

During an adaptation period of 4 consecutive days for all horses before the experiments, the horses of each group were stabled in an allocated individual box for 1 h before being turned out for two hours to the experimental paddocks. The horses were rotated three times between the paddocks every 30 min to accustom them to each type of paddock. To make sure that each horse experienced each type of fencing, they were encouraged to approach the fences using a titbit (carrot). As a result, the horses frequently made contact with the wooden rails, but no horse actually touched the electric fencing. In addition, during the period of adaptation, the horses wore the girth that during the subsequent experimental period would be required to measure HR and HRV. Using a simulated procedure, the horses were also accustomed to saliva sampling in each paddock and were subsequently rewarded with a titbit (carrot).

In the morning of each experimental day, the horses were subjected to a short clinical examination with particular emphasis on heart rate, body temperature, general condition and gait. After the clinical examination, the horses were stabled in one of the experimental boxes throughout the experimental day from 10.00 am until 4.30 pm. Each horse remained stabled in its allocated box throughout the experiment.

Data acquisition

a) Surface analysis

To evaluate the horses' use of the available surface area using automated video-analysis, a red foam marker (20 x 10 cm) was attached to the Polar® girth at the horses' withers. The video-analysis was carried out with a previously specifically developed and visually validated software which divided the paddock area being analysed into 10 x 10 cm quadrants and noted the presence of the red marker in these quadrants, recorded the movement of the marker over time and calculated and displayed the utilised surface area as well as the length of time spent in any one location. The border area of the available surface was defined as the area within 50 cm of the fence. Any activity in this area was verified visually by consulting the video. The maximally utilised paddock area (in m^2 and %) and time spent in the border area (in s), was evaluated. In two groups, failure of video recording or Polar® data recording necessitated the repetition of an experimental day.

b) Ethogram

The entire experimental phases of 90 min each were recorded on camera using WLAN outdoor surveillance cameras (Vario-AI-CUT, Gmyrek Electronik GmbH, Braunsbedra, Germany) mounted on 6 m high wooden posts. Each camera recorded activity in one paddock using long-term surveillance software. The resulting films were analysed using an ethogram that involved recording nine mutually exclusive basic activities every s. These basic activities were chosen after literature study (Glatthaar, 2009; McDonnell and Haviland, 1995) and included: inactive stance, active stance, walking with head (held) low, walking with head high, trotting with head low, trotting with head high, cantering with head low, cantering with head high and rolling. In addition, the frequency and duration of specific stress–indicating behaviour (pawing, stamping, striking out with one leg, aggressive behaviour against other horses), loosely based on Glatthaar, (2009), Young et al. (2012), and McDonnell and Haviland (1995), was recorded. For all these behavioural analysis, each phase was separated into two observation periods, the first 30 min (adaptation time) and the subsequent 60 min. In addition the contact time with the fence was recorded (definition: any body part in contact with the fence).

In one horse the activity "walking with head high" recorded in the large electrically fenced paddock was excluded from statistical evaluation as this behaviour was shown excessively. This was also the case in another horse for the behaviour "head-throwing" which occurred in the large wooden paddock.

c) Salivary cortisol

Four saliva samples were collected from each horse per experimental day. The first sample was collected 20 min before the start of the experimental phase when the horse was in its box and served as a base-line value (time point = TP1). Two further samples were collected 25 min after the horse had been turned out in the paddock (TP2) and at the end of the experimental phase (TP3). The fourth sample was collected 20 min after the end of the experimental phase when the horse had been returned to its box (TP4). Samples were collected using a Salivette® sampling kit (Sarstedt AG & Co, Nümbrecht, Germany); the synthetic swabs held with a surgical clamp were inserted into the horse's buccal pouch and lightly rotated for a period of 30 s. Immediately after sampling, the swabs were placed into the Salivette® sampling tubes and centrifuged (3000 g for 15 min). The dispensed saliva was frozen at -80°C until analysis. Analysis of the frozen-thawed saliva samples was carried out using a commercial ELISA kit (Salimetrics Saliva Cortisol ELISA, Carlsbad, CA 92008, USA).

d) Heart rate and heart rate variability

The HR and HRV was recorded during the 90 min experimental phase, using the cardiometer system Polar® with the Polar Pro Trainer 5 Equine Edition software (Version 5.35.161) according to manufacturer's instructions. The Polar® watch recording the data was inserted into a protective pouch made of transparent film and attached to the horse's headcollar underneath the lower jaw. For data analysis, the video recordings of the horse during the experimental phase were used to select four two min sequences in which the horse was as calm as possible and engaged in inactive stance behaviour. HRV can be analysed using time domain or frequency domain methods as well as being subjected to a geometric transformation (Task force of the European Society of Cardiology and the North American Society of Pacing Electrophysiology, 1996). During the experimental phase, the following parameters were determined: i) HR: number of heart beats, average heart rate, and ii) HRV: average R-R interval, SD1, SD2, RMSSD, pNN50, LF (low frequency, 0.040-0.150Hz), HF (high frequency, 0.150-

0.400Hz), LF/HF ratio. Of the 20 horses, one had to be excluded from the analyses due to the presence of non-pathological second-degree atrio-ventricular blocks. Moreover, the data recorded by the electrode girths was liable to disturbances, and of the 304 remaining records only 284 could be evaluated.

Statistical analysis

The data were analysed using a linear mixed-effects model (R V2.14.1, R Development Core Team 2011; package nlme, Pinheiro et al., 2011; Pinheiro and Bates, 2000) with each measurement described above used as an outcome variable. The model was specified with material (two-level factor: wood, electric), size (two-level factor: small, large) and their interaction as fixed effects. Animal identity was specified as a random effect to accommodate repeated measures of single individuals. Main effects and their first-order interactions were included if $p \leq 0.1$. Otherwise, the interaction was dropped and only the F- and p- values of the two main effects are reported. If there was some indication of an interaction ($p \leq 0.1$), an additional model was calculated using only one fixed effect, a four-level factor reflecting all four combinations of material and size. In the latter, pair-wise post-hoc comparisons evaluated differences between the experimental treatments (package multcomp, Hothorn et al., 2008) and these comparisons are reported. For heart rate and cortisol measurements taken at several time points throughout each trial, trial was additionally nested in horse identity in the random effect. The cortisol measurement model also included the time-point effect (four-level factor). We started with the model with all potential interactions and reduced the model step-wise until the model including main effects only was reached (none of the interactions reached a p-value ≤ 0.1). Model assumptions were checked using a graphical analysis of residuals and normality of errors and random effects were assessed based on qq-plots. Additionally, homoscedasticity was evaluated using plots of the residuals against the fitted values and against the explanatory variables. Some right-skewed and proportion variables were transformed using a log and a logit transformation, respectively, to satisfy statistical assumptions. Some of these variables included 0s (log and logit) and/or 1s (logit) that did not allow a direct use of these transformations. These zeros/ones may not have been real in the sense that they reflected a very low/high motivation to perform a specific behaviour but this motivation was nevertheless not exactly nil/full. These values were smaller/larger than the detection level defined by the observed values. The zeros/ ones were therefore replaced by a value smaller/ larger than the detection level before transformation. If at all, this procedure made the data somewhat less extreme and, at most, a slightly conservative bias was introduced. Rare behaviours were dichotomised and analysed using a generalised linear mixed-effects model for which test-statistics and p-values are reported for the corresponding likelihood-ratio tests (package lme4, Bates et al., 2011).

3. Results

Surface area utilisation

The maximally utilised portion of available paddock surface area was lower in paddocks with electric vs. wooden fences ($p < 0.001$) and in small vs. large paddocks ($p < 0.001$; Fig. 2).) Horses spent the most time in the border area in the large wood-fenced paddocks compared to the other paddock types ($p < 0.001$). In addition, a significant difference for both fence material and area at disposal ($p < 0.001$) was shown for the average time spent in the border area between wood-fenced paddocks and electrically fenced paddocks and between large or small (see Fig. 3).

Behaviour

Outcome variables from the behavioural observations, estimated effects for the experimental conditions, test statistics, and p-values are shown in Tab. 1.

Salivary cortisol

Salivary samples were easily obtained and prompted no negative reaction from the horses. Over the entire experiment, salivary cortisol levels of the first three sampling times (TP1, 2 and 3) showed a significant decrease ($p < 0.001$; Fig. 4). However, no differences could be related to fencing material or size of paddock. The first (TP1) and last (TP4) sample, both taken when the horse was in a box, showed significantly higher levels of salivary cortisol than those taken in between on the paddock (TP2 and TP3).

Heart rate and heart rate variability

No significant differences between paddock size or fence type were shown in the time domain and geometrical analysis. However, in the first 30 min of the experiment, LF/HF ratio was lower in electrically fenced ($p < 0.01$) and small paddocks ($p = 0.015$) than in the wood-fenced and large paddocks, respectively.

4. Discussion

Our study clearly shows that the utilisation of available paddock space was lower in small paddocks and in paddocks with electric fences, but no physiological stress response could be demonstrated during the 90-minute experiments.

A study by Moors et al. (2010) showed similar findings in that the type of fencing in paddocks that were permanently accessible from stables had a major influence on the position of the horse in relation to the fence: in paddocks with electric fences, only 11.9% of the time spent in the paddock was spent in close proximity to the fence, whereas in paddocks without electric fences, horses spent more than 50% of their time in fence proximity. They also demonstrated that electric fencing restricted the relative frequency a horse spent engaged in social contact with its neighbours (0.6% compared to 19.1% in non-electrically fenced paddocks). Much the same effect was seen in our study, with the amount of time spent in the border area being much lower in paddocks with electric fencing. Based on the much higher frequency of fence contact in wood fenced paddocks, one can conclude, that horses in paddocks with electric fences actively avoid fence contact by keeping away from the border area.

According to McKillop and Silby (1988), the effect of electric fencing is an adapted behavioural response to receiving an electrical shock. Repeated fence contact associated with repeated electrical shocks result in an aversion of the horse towards the fence (Moors et al., 2010). As a result, electric fences directly influence horses' behaviour. In the present study, the aim was to document stress-indicating behaviour. Our results showed that the sum of the observed behaviours gave no indication of a significant difference in horses' behaviour with regards to paddock size or fence type. However, paddock size was a major limiting factor for horses' movement patterns, with horses rolling and walking less frequently in small paddocks. Jørgensen und Bøe (2007) also found that in large paddocks, horses spent significantly more time moving around and less time passively standing than in small paddocks. However, compared to the daily 16 hours of movement by a free-living horse whilst searching for food (Hoffmann, 2008), horses in the present study spent a greater proportion of their time in the paddocks standing around, even in the large paddocks where they could conceivably have covered a certain distance.

The behaviours pawing, stamping, kicking out with a hindlimb were observed with a slightly greater frequency in the small paddocks. This was also the case with aggressive behaviour towards neighbouring horses. Pawing has been classed as an indicator of medium stress in the scale of behavioural indicators of stress of Young et al. (2012). A horse may paw the ground simply to assess the type of footing it is standing on, but it can also be an expression of aggression or frustration (McDonnell and Haviland, 1995). Stamping and kicking out with a hindlimb can be interpreted as posturing, warning or distancing behaviour. The increased occurrence of these behaviours in small paddocks can be interpreted as a sign of boredom or frustration with the lack of space, or as an aggressive response due to the horse's inability to show defensive behaviour such as avoidance, flight or turning away in the restricted space available.

Fence contact was almost entirely restricted to the wood fenced paddocks: in the large paddocks, in the first 30 min and during the rest of the experimental time, 53% and 73%, respectively, of horses came into contact with the fence, which increased to 68% and 88%, respectively, in the small paddocks. Fence contact was not merely a passive occurrence – most horses actively sought fence contact, either to chew wood or to rub themselves, others stood with their heads over the fence towards the neighbouring paddocks. Predictably, fence contact was much lower in electrically fenced paddocks, with only 4% making fence contact in large paddocks in the first 30 min, and 7% in the following 60 min, respectively, and 7% and 17% in small paddocks. In effect, the purpose of an electric fencing system is to deter animals from crossing a boundary and therefore encourage them to keep away According to the video analysis, all contact with electric fencing was accidental, for instance with a leg when rolling, or with the hindquarters when turning. Immediately after contact, horses showed a flight response, which subsided after a few s.

Behavioural analysis allowed us to conclude that the presence of electric fencing was not connected to an increase in stress-indicating behaviour. Horses in small paddocks however, did show a tendency towards more stress-indicating behaviour. Furthermore, a wooden fence may provide a source of occupation as well as permitting more social interaction with a neighbouring horse than an electric fence.

Contrary to the behavioural observations, the classical physiological stress parameters showed no clear differences between the paddock types in our study. No significant differences in salivary cortisol levels with respect to paddock size or type of fencing were found. The relatively low and progressively declining levels of cortisol measured at TP2 and TP3 indicate that the period spent on the paddock did not result in an activation of the HPA axis indicative of a stress response. However, increased cortisol levels after a long-term strain due to restricted space or fear of electrical shock cannot be excluded, because of the limited exposure time of 90 minutes in our experiments.

In studies documenting increased cortisol levels in response to a road transport situation, cortisol values rose to five times the basal values (Schmidt et al., 2010a, 2010c) whereas basal values were similar to the concentrations found in our study. Cortisol values in our study were highest during the horses' confinement in the boxes. Two factors are likely to have contributed to this finding: handling of the horses, and physical confinement in the boxes. The majority of the horses in the study were normally kept in a group stabling system, that is, the removal of the animal from its herd and the subsequent individual confinement could have led to a slight activation of the HPA axis, despite the initial adaptation period.

Our results show also that neither the type of fencing nor the size of the paddock had any influence on HR or HRV reflecting the sympatho-vagal balance (Borell et al., 2007; Hoffmann, 2008) and serving as a marker of autonomic neural activity (Rietmann et al., 2004). The geometrical analysis showed neither a short (SD1) nor long term (SD2) change in HRV. The analysis in the frequency domain showed some significant differences in the LF/HF ratio with regards to area and type of fencing. HF is believed to reflect parasympathetic activity whereas LF is thought to be responsive to both sympathetic and parasympathetic tone. Therefore the LF/HF ratio allows an evaluation of the relative influence of sympathetic and parasympathetic nervous activity (Hoffmann, 2008). The significantly lower LF/HF ratio values in our study at the start of the experimental phase in the electric ($p < 0.01$) and small paddocks may have resulted from increased sympathetic activity and the corresponding increase in HF which can be brought about by a stress-inducing situation (Hoffmann, 2008). In previous studies HR and HRV were found to be a sensitive form of stress evaluation in horses, whereby changes were always observed in several HR and HRV parameters (Schmidt et al., 2010a, 2010b, 2010c, 2010d). As the changes observed in the present study were limited to the LF/HF ratio, while all other HRV parameters showed no significant differences with regards to paddock fencing or size – the significance of our findings should not be overrated. Moreover, the recording of HR and HRV is prone to disturbances, a fact also observed by Borell et al. (2007) who found various problems with the recordings, predominantly in the form of movement artefacts and low resting frequencies.

5. Summary and Conclusion

Electric fencing systems for horses are cheaper than conventional fences, well-respected by the animals and very flexible. Therefore, they are often used to corral outdoor areas to enable horses to stay outside and move freely. On the other hand, horses could be chronically frightened of electrical shocks and may therefore suffer from stress, especially in small enclosures. Our results indicate that electric fencing over 90 minute periods does not appear to lead to any changes in physiologically quantifiable stress variables in horses. The use of electric fencing does however limit the horse's utilisation of the available surface area by leading to avoidance of the border area. This behavioural adaptation appears to allow the horse a measurably stress-free stay in paddocks with electric fencing. Small paddocks and electrically fenced paddocks limit the amount by which a horse moves around. In small paddocks of either fence type, horses will likely show stress-indicating behaviour more frequently as the restricted space prevents them from showing defensive or avoidance behaviour.

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Fig. 1: Arrangement of experimental paddocks

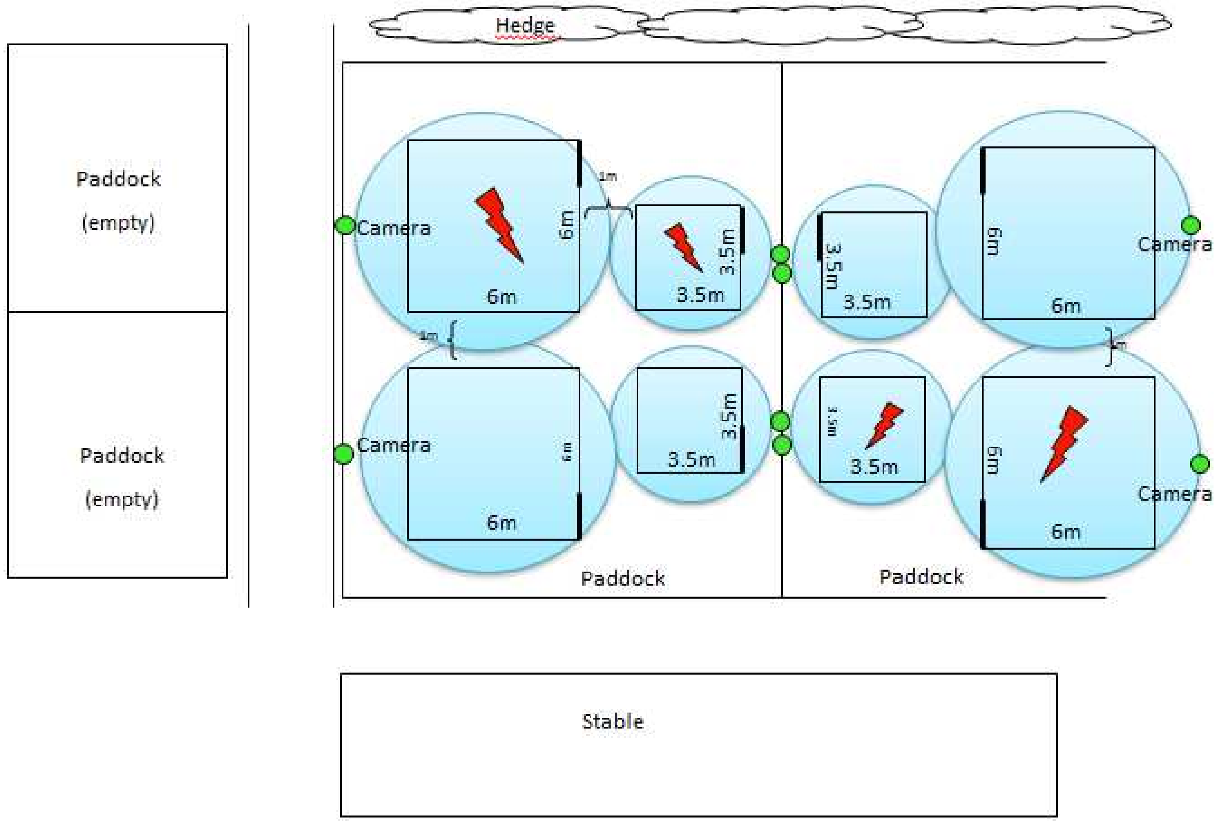
Fig. 2: Maximally utilised surface area (%) in small and large paddocks with wooden (W) or electric (E) fencing

Fig. 3: Amount of time (s) spent in border area (0 – 50 cm from fence) in small and large paddocks with wooden (W) or electric (E) fencing

Fig. 4: Salivary cortisol (nmol/l) at the sampling times TP1 (basal value), TP2: after 25 min on paddock, TP3: after 90 min on paddock, TP4: 20 min after return to stable in large (L) and small (S) paddocks surrounded by wooden (WF) or electric (EF) fencing.

Tab. 1: Behavioural outcome variables (%, s) in the two observation periods 0-30 min and 31-90 min including 95% confidence intervals and p-values for differences regarding the type of fencing (wood vs. electric) and the size (large vs. small)

Fig. 1:



Hedge
Paddock
(empty)
Paddock
(empty)
Camera
Camera
Camera
Camera
6m
6m
6m
6m
6m
6m
6m
6m
3.5m
3.5m
3.5m
3.5m
3.5m
3.5m
3.5m
3.5m
1m
1m
Paddock
Paddock
Stable

Fig 2:

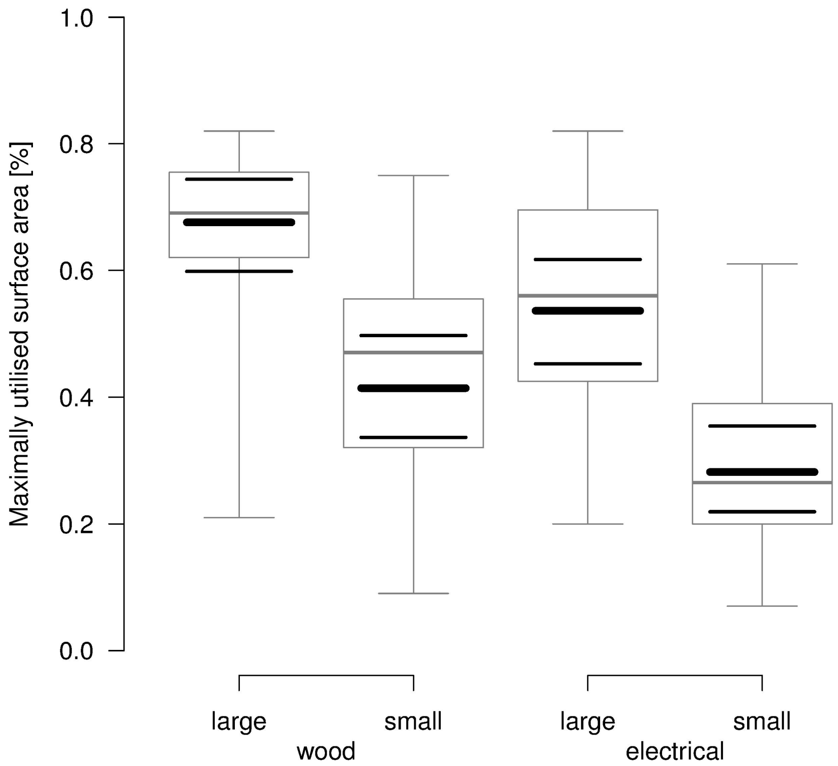
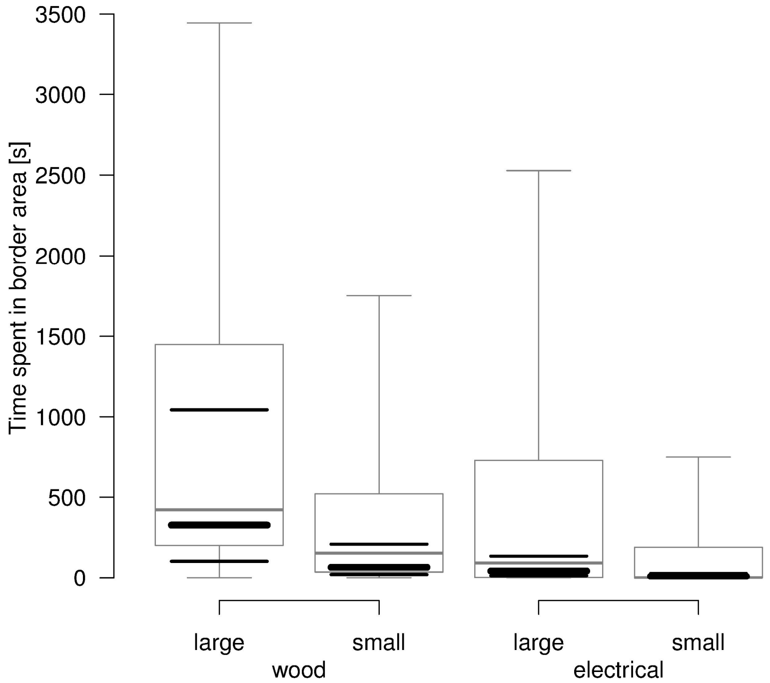
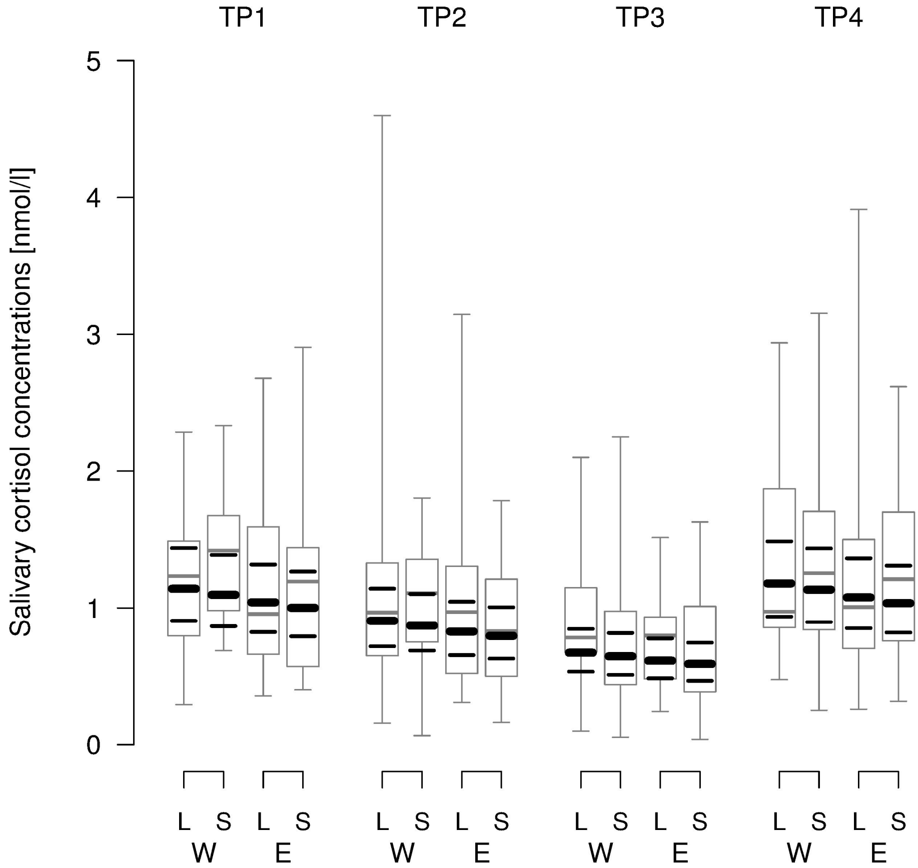

1.0
0.8
0.6
0.4
0.2
0.0
Maximally utilised surface area [%]
large
small
wood
large
small
electrical

Fig. 3:



Time spent in border area [s]
3500
3000
2500
2000
1500
1000
500
0
large
small
wood
large
small
electrical

Fig. 4:


TP1
TP2
TP3
TP4
Salivary cortisol concentrations [nmol/l]
0
1
2
3
4
5
L
S
L
S
W
E

Tab. 1:

		Wood		Electric		p-value	
		large	**small**	**large**	**small**	**Material**	**Space**
Rolling (%)	**0-30 min**	56 (29-80)	6 (1-21)	51 (25-77)	5 (1-19)	0.751	<0.001
	31-90 min	0	0	0	0	1.000	1.000
Walk, head low (s)	**0-30 min**	71 (43-118)	16 (10-26)	42 (25-69)	9 (6-15)	0.011	<0.001
	31-90 min	116 (62-217)	35 (19-66)	76 (41-143)	23 (12-43)	0.043	<0.001
Pawing (n)	**0-30 min**	6 (3-11)	8 (4-16)	7 (4-16)	10 (5-19)	0.548	0.210
	31-90 min	2 (1-4)	4 (2-8)	3 (2-5)	5 (3-10)	0.404	0.010
Stamping (n)	**0-30 min**	1 (1-2)	2 (1-3)	2 (1-2	3 (2-4)	0.085	0.018
	31-90 min	3 (2-5)	4 (3-7)	4 (3-7)	7 (4-12)	0.044	0.047
Striking out (1 leg) (%)	**0-30 min**	32 (15-57)	42 (21-66)	19 (7-41)	26 (11-50)	0.184	0.422
	31-90 min	22 (9-43)	56 (33-76)	38 (19-61)	73 (51-88)	0.127	0.003
Aggressive behaviour (%)	**0-30 min**	10 (2-34)	44 (21-70)	25 (89-52)	15 (4-40)	0.110	0.600
	31-90 min	13 (4-33)	38 (17-64)	14 (5-36)	42 (20-68)	0.774	0.013
Fence contact (%)	**0-30 min**	53 (28-77)	68 (40-87)	4 (1-17)	5 (2-25)	<0.001	0.346
	31-90 min	73 (50-88)	88 (68-96)	7 (1-34)	17 (6-39)	<0.001	0.130
Duration of all observed behaviour (s)	**0-30 min**	6 (3-11)	4 (2-8)	5 (2-9)	3 (2-6)	0.503	0.392
	31-90 min	6 (3-12)	10 (5-21)	5 (3-12)	9 (4-20)	0.922	0.094