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1 **Free movement: a key for welfare improvement in sport horses?**

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21        **ABSTRACT**

22        Horses, and in particular sport horses, remain housed predominantly in single stalls. One of the  
23        main reported reasons is the fear that they will become agitated and injure themselves and thereby  
24        impair their performance if released in paddocks. The hour spent daily at work is also assumed to be  
25        sufficient to satisfy the horses' needs for locomotion. Growing scientific evidence shows that single  
26        stall housing has negative consequences on horses' welfare and that time for free movement is  
27        necessary. Our aim was to assess the feasibility of allowing sport horses used to staying permanently  
28        in their stall (except for 1hour riding/day) daily free time in a paddock and to evaluate its potential  
29        impact on their welfare using two behavioural reliable indicators (stereotypic behaviours and ear  
30        position), as well as selected blood parameters (blood cell count, oxytocin and serotonin  
31        concentrations). Two experiments were conducted on the same site. The first experiment evaluated  
32        sport horses' habituation to the novel situation of daily sessions in a paddock, and recorded welfare  
33        indicators in their stall before and during the experiment, comparing horses that were taken outdoors  
34        (experimental) and those that stayed in their stall (control). The second experiment evaluated the  
35        impact of this daily free time in a paddock on horses' welfare and its durability, focusing on positive  
36        indicators.. Behavioural observations in paddocks showed that even horses that had never experienced  
37        free movement outside their stall habituated rapidly to this situation. The presence of hay in the  
38        paddock, may have speeded up habituation. Their restricted living conditions were associated with  
39        abnormalities in blood cell count that were not overcome during the time of daily paddock sessions but  
40        behavioural indicators showed that their welfare improved. In the second study, the experimental  
41        horses' welfare improved during the paddock release period, in particular their stereotypic behaviours  
42        decreased and oxytocin levels increased. No effects on serotonin concentrations could be evidenced.  
43        These effects were directly associated with being in paddock, as the indicators returned rapidly to their  
44        previous levels indicating compromised welfare when the paddock release sessions stopped. In  
45        conclusion, it can be recommended to release sport horses for free movement in paddocks as welfare  
46        is improved and subjective assessment by caretakers indicated minimal risks.

47        **Keywords:** horse, housing, free movement, horse welfare, stereotypic behaviour, oxytocin

## 48 I-INTRODUCTION

49 Under natural conditions, horses' home ranges vary in size according to resource availability (up to  
50 250km<sup>2</sup> in arid areas, Waring, 2003). This species has evolved to adapt to an almost permanent slow  
51 locomotion, mostly when feeding. In the horse industry, particularly for sport horses, constant single  
52 stall housing (>20h/day) is still predominant, despite its proven negative impact on horses' welfare.  
53 Beyond social constraints horses housed in individual stalls are deprived of free movement, *i.e.* slow  
54 walk observed quasi-permanently under natural conditions (*e.g.* Waring, 2003). Most domestic  
55 animals, including horses, show a rebound effect when released in larger spaces, with high locomotor  
56 patterns and excitement components (Albentosa and Cooper, 2004; Lesimple et al., 2011). Spatial  
57 confinement is associated with restriction of the expression of specific behaviours and thus impairing  
58 their welfare (Dawkins, 1988; Dixon et al., 2010). Captive laboratory animals, when kept under  
59 restricted spatial conditions, develop particular types of abnormal behaviours including stereotypic and  
60 abnormal repetitive behaviours (SB/ARB) that have been interpreted as attempts to flee (mice: Lewis  
61 and Hurst, 2004; Würbel et al., 1996; 1998; starlings: Feenders, 2012; Coulon et al., 2015; non-human  
62 primates: Draper and Bernstein, 1963). Similarly, single stall housing of horses has been shown  
63 experimentally to be a factor eliciting rapid emergence and the prevalence of abnormal repetitive  
64 behaviours (Visser et al., 2008) and identified by questionnaire-based (*e.g.* McGreevy et al., 1995) and  
65 observational (*e.g.* Lesimple et al., 2016) epidemiological studies. Reports suggest that weaving, a  
66 well-known locomotor stereotypic behaviour reflects the frustration of not being able to go out of the  
67 stall (Mills, 2005). SB and other indicators of compromised welfare are associated with changes in  
68 horses' learning abilities (*e.g.* Hausberger et al., 2007; Parker et al., 2009, see Hausberger et al., 2019  
69 for review) and attentional impairments (Rochais et al., 2016) potentially leading to a drop in  
70 performance that can become problematic for sport horses.

71 A daily training session is generally considered sufficient to satisfy horses' needs in terms of  
72 locomotion. Nevertheless, Christie et al. (2006) reported that the frequency of SB/ARB of stall-housed  
73 riding horses increased with time spent working (either ridden or harnessed), whereas other authors  
74 (*e.g.* Lesimple et al., 2016) showed that it decreased when horses benefited regularly from several  
75 hours for free movement. Moreover, when given the choice, animals prefer to go to a larger arena

76 (cattle: Bak Jensen et al., 1999) and are even ready to work actively to gain access to larger spaces  
77 (laboratory mice: Sherwin et al 2003, hens: Nicol, 1989; Lagadic and Faure, 1987). In choice tests,  
78 horses significantly preferred going to a paddock to staying in their stall, but also significantly  
79 preferred to go back to their stall to a forced 20-minute exercise on a treadmill (Lee et al., 2011).  
80 Similarly, when faced with an obstacle to reach food, leisure horses significantly preferred to go  
81 around the obstacle and the motivation of both leisure and sport horses to jump decreased with the  
82 obstacle's height (Gorecka-Bruzda et al., 2013). Thus, horses clearly appear motivated to gain free  
83 exercise but avoid, when possible, forced locomotion, maybe because it involves higher locomotion  
84 patterns (trotting, jumping). These preferences and observations of compromised welfare when horses  
85 are constantly in stalls outside work converge to indicate that free movement (at least part time) is a  
86 behavioural /physiological need. As mentioned by Popescu et al. (2014), many owners consider that a  
87 working horse does not need free exercise but rather needs rest to save and rebuild energy for work,  
88 although there are many arguments against this false belief. Due (2006) summarized guidelines  
89 proposed by different European associations for the protection of horses and veterinary associations,  
90 they state that "...the daily free running out ... has a positive influence on the general health,  
91 especially on the respiratory tract, the locomotor system as well as on the immune system and helps  
92 preserving health. Daily work, training machines (treadmill/horse walkers) are no adequate  
93 replacement for the free running out." At present, there is no legal frame concerning free exercise for  
94 horses. The European Union official text (Council Directive 98/58/CE, L221/26/7 of the directive)  
95 states that "the freedom of movement of an animal [...] must not be restricted in such a way as to  
96 cause it unnecessary suffering or injury" but also that continuous or regular confinement is accepted if  
97 "appropriate space is given to fulfil the physiological and ethological needs in accordance with  
98 established experience and scientific knowledge". Large discrepancies also arise between national  
99 countries' laws (Owers and Tabram, 2005), which all state that an animal has to be able to lie down  
100 and to stand up (French rural code 2018, Hungarian law on animal welfare and protection 1998, Polish  
101 Animal Protection Act 2010, Swiss Ordinance on animal protection 2018), but may even forbid  
102 outdoor housing in the absence of a shelter including (French rural code 2018) or not (Swiss

103 Ordinance on animal protection 2018) natural shelter (e.g. hedges). There is therefore a real need for  
104 further scientific evidence.

105 Beyond the ethical question of animal welfare, performance level of sport horses is important and  
106 time spent outside work may have an influence. Maintaining muscular activity after an intense  
107 exercise (also called active recovery) has proven efficacious to improve human athletes' recovery time  
108 (metabolic return to basal state, e.g. Greenwood et al., 2008; Kalen et al., 2017) and subsequent  
109 performance (Monedero and Donne, 2000). Indeed, keeping up mild aerobic and stretching activities  
110 was shown to fasten lactate removal and to increase its potential use as fuel for heart and working  
111 muscles (e.g. Gisolfi et al., 1966; Menzies et al., 2010). To our knowledge, the impact of recovery  
112 techniques on horses' recovery time and subsequent performance has not been investigated. However,  
113 as the metabolic consequences following intense exercise are similar for horses and humans (e.g.  
114 Assenza et al., 2014; Binda et al., 2016), active recovery is likely to have positive consequences in  
115 terms of recovery time and performance. Thus offering regularly sport horses time for free locomotion  
116 should improve their welfare, help them recover after an effort and maintain their physical capacities.  
117 The main reasons why most sport horses do not benefit from free locomotion times in paddocks are: 1-  
118 the fear potential injuries (e.g. wounds and lameness) could occur if they become more agitated after  
119 being released, and thus impair their subsequent performance, and 2- the belief that performing horses  
120 need rest more than free exercise (Popescu and Diugan 2017).

121 This study aimed: (1) to assess the feasibility (in terms of habituation and potential harm) of  
122 allowing adult sport horses that had always been housed in single stalls daily sessions in a paddock,  
123 and (2) to evaluate the consequences of these daily sessions on their welfare and the durability of the  
124 effects. Two experiments were carried out successively. The first experiment, including 24 horses,  
125 evaluated the feasibility of a daily paddock release. More precisely, potential habituation to this novel  
126 situation and the short-term impact on the horses' welfare (measured in stall) were assessed. The  
127 second experiment, including 20 horses, evaluated the impact of daily periods of free time in paddock  
128 on horses' welfare, focusing in particular on positive indicators. We assessed the durability (after  
129 cessation of the free release period) of the potential effects. Both studies compared pairs of horses: one  
130 experimental (*i.e.* released daily in paddock) to one control (*i.e.* remaining in stall). Observations were

131 performed before (study 1 and 2), during (study 1 and 2) and after (study 2) the period of regular  
132 paddock release. The reactions of the experimental horses during their daily release in a paddock were  
133 recorded (studies 1 and 2). Moreover, their welfare, as a chronic state (e.g. Broom, 1991), was  
134 assessed when horses were in their usual stall, using behavioural (*i.e.* stereotypic behaviours, postural  
135 indicators), as well as physiological parameters (*i.e.* complete blood cell count, oxytocin and serotonin  
136 plasma levels).

137 Humans' total blood cell counts have proven to be able to indicate depression (higher counts of  
138 white and red blood cells) and anxiety syndromes (e.g. Köhler Forsberg et al., 2017; Shafiee et al.,  
139 2017). Numbers of cows' red blood cells and lymphocytes increased under spatial restriction  
140 (Nakajima et al., 2018). Working horses' neutrophil/leucocyte ratios were negatively correlated with  
141 welfare (Popescu and Diugan, 2017), and many riding school horses living under restricted conditions  
142 were found to suffer from anaemia and abnormal neutrophil levels (Pawluski et al., 2017). The  
143 relationship between chronic stress and cortisol levels is less straightforward with, on one hand,  
144 rapidly changing individual cortisol levels (e.g. McBride and Cuddeford, 2001), and, on the other  
145 hand, heterogeneous results from one study to another. Some studies reported higher (e.g. McGreevy  
146 and Nicol, 1998; Bachmann et al., 2003) or lower levels (Pawluski et al., 2017) of cortisol when  
147 horses' welfare was compromised, but many other authors found no significant differences neither in  
148 salivary nor in plasma cortisol levels between horses' presenting stereotypic and non-stereotypic  
149 behaviour (Pell and McGreevy, 1999; Clegg et al., 2008; Hemman et al., 2012; Fureix et al., 2013).  
150 Other physiological markers may be of interest: humans' low plasma serotonin levels are associated  
151 with the prevalence of depression (Young, 2007) and aggressiveness (see Wallner and Machatschke,  
152 2009 for a review), and high plasma oxytocin levels were shown to enhance the processing of positive  
153 emotions (DiSimplicio et al., 2008) and increase generosity (Zak et al., 2007). Several studies have  
154 shown that aggressive dogs present lower blood serotonin levels (Cakiroglu et al., 2017; Jacobs et al.,  
155 2007; Leon et al., 2012; Rosado et al., 2010), while positive interactions with humans are associated  
156 with increase of serotonin (Alberghina et al., 2017) and oxytocin (Nagasawa et al., 2015) levels.  
157 Therefore, we focused on complete blood cell counts, but also on serotonin and oxytocin levels as  
158 potential physiological indicators of positive emotions.



159

## 160 **II-GENERAL METHODS**

### 161 **2.1-General procedure (Fig. 1)**

162 This experimentation involved two successive studies (Fig. 1). The first experiment (March 12<sup>th</sup> to  
163 April 6<sup>th</sup> 2018) assessed horses' behavioural reactions and habituation to paddock release, and the  
164 impact of these daily release on selected blood parameters (complete blood cell count, sedimentation  
165 rates as well as oxytocin and serotonin levels before and after the period of daily release) and  
166 behavioural welfare expressions (SB/ARB, ear positions). The second experiment (May 14<sup>th</sup> to June  
167 15<sup>th</sup> 2018) assessed the impact during and after (cessation) the daily release period. We included  
168 measures of oxytocin and serotonin levels as potential positive physiological indicators.

169 Two types of comparisons were used: (1) between the different phases, *i.e.* during the daily release  
170 periods *versus* outside daily release periods, and (2) between experimental (*i.e.* released daily in  
171 paddock) and control horses (*i.e.* stayed in stall).

172 We hoped to assess the impact of daily paddock free sessions on the horses' performance.  
173 Unfortunately, for sanitary reasons, all the competitions in the region were stopped for 2 months  
174 shortly after our experiments so we could not evaluate the horses' performance after the experiments.

### 175 **2.2-Animals**

176 These experiments took place at the "Ecole Nationale d'Equitation" (ENE) in Saumur (France). In  
177 all, 29 horses (16 geldings and 13 mares, 9 to 15 years old), mostly French Saddlebreds (N=26; Anglo  
178 Arabians: N=3) were observed. All horses were used in courses for riding instructors. They had all  
179 been in this same facility for at least one year and most had arrived when 4 or 5 years old.  
180 Management conditions were thus identical for all horses: single stall housing with one hour riding (or  
181 occasionally automated horse walker) per day, and none of them had had previous experience of free  
182 time in a paddock. They were housed in an indoor barn with no outdoor openings, but half side walls  
183 replaced with grids, enabling sight and nose to nose contact with their two neighbours. A grid above  
184 their door enabled them to see their neighbours across the corridor: they could therefore see more than  
185 5 neighbours (1 on each side and at least 3 in front). They were kept in straw bedded single stalls  
186 equipped with automatic drinkers. The stalls were cleaned every morning and fresh straw was added.

187 All horses were fed commercial pellets, using an automated feeding system that controlled the  
188 distribution of pellets in the individual feeders, three times a day (7.30 am, 11.30 am, and 5.30 pm).  
189 The amount given ( $X \pm SE = 3.0 \pm 0.0$  L; range = 2.0–4.0 L) was determined by the facility's  
190 veterinarian according to the size and mass of each individual. The amount of hay provided per day (9  
191 kg for all horses) was also determined by the veterinarian and distributed half in the morning (8am),  
192 and half in the afternoon (15pm). For both studies, horses were paired with their closest counterpart (in  
193 terms of sex, age, breed and caretaker). The two horses of each pair were then randomly allotted, one  
194 to the experimental (went to paddock) group and one to the control (stayed in stall) group. At ENE, the  
195 health routine procedure consists in a monthly examination by the facility's veterinarian. None of the  
196 horses involved in our study presented any declared pathological disorder at the time of the  
197 experiments.

198

### 199 **2.3-Paddock release procedure**

200 Two adjacent paddocks with sandy soil (190 and 220m<sup>2</sup>) separated by a 1m-wide path and located  
201 in the immediate surroundings of the horses' stables (to facilitate the caretakers' work) were used for  
202 this study. Each paddock was equipped with an automatic water-trough and a slow-feeder  
203 (Pacefeeder®, Bray, Ireland) full of hay in order to provide *ad libitum* access to water and fibre diet.  
204 The amount of hay distributed in the stall was lowered for the experimental horses as they had access  
205 to hay in paddocks, so as to insure that horses in both groups received the same quantity of roughage.

206 The experimental horses were released in paddock for 39-62 minutes ( $X \pm es = 46 \pm 1$  min, no  
207 significant differences between horses: Kruskal Wallis Anova,  $\chi = 10.2$ ,  $p = 0.5$ ) per day, 5 days a week  
208 (from Monday to Friday), between 8am and 4pm and in pairs with one horse in each paddock (same  
209 pair every day, same caretaker for horses of a same pair). They could see, hear and smell their  
210 neighbours, but physical contact was not possible. The horse's location of release (Right paddock, R  
211 or Left paddock, L), as well as the release time slot were shifted every day; thus, a horse that went out  
212 at 8am to R paddock on Monday went at 9am to L paddock on Tuesday, and so on. As for a given pair  
213 horses' arrival times differed a few minutes, only the periods when both horses had a neighbour were  
214 analysed here.

215 At the end of each study, caretakers were asked to give their subjective perceived feasibility (in  
216 terms of risks and handling).

#### 217 **2.4-Observations in the paddocks (experimental horses)**

218 The behaviours of the horses while in the paddocks were recorded using two camcorders (JVC Full  
219 HD EverioR 60x) placed in each paddock, at two opposite angles (close to the entrance and close to  
220 the slow-feeder) in order to have a full visibility of the horse, whatever its position in the paddock. The  
221 videos were analysed subsequently, using the Instantaneous Scan Sampling method (Altman, 1974)  
222 with a scan every 2 minutes. In addition, all occurrences of rare/short behaviours and postures were  
223 recorded (Altman, 1974). The activities and behaviours observed are described in Table 1.

#### 224 **2.5-Welfare evaluation in stalls (experimental and control horses): behavioural and postural** 225 **indicators**

226 All horses were observed in their own individual stall during the week prior to the beginning of the  
227 paddock releases and then during the three (Study 1) or two (Study 2) weeks of daily paddock release  
228 sessions.

229 The same observer (LRB, the experimenter) recorded all the observations in the two studies using  
230 classical ethological methods. Direct observations of behaviour using Instantaneous Scan Sampling  
231 were performed as this method reflects reliably individual time-budgets (*i.e.* time spent in different  
232 activities, Altman, 1974). The procedure used for the present study was similar to that used in other  
233 studies and proved successful in assessing welfare (Benhajali et al., 2010; Heleski and Murtazashvili,  
234 2010; Lesimple et al., 2019; Rochais et al., 2018): twice a day, once in the morning (between 6am and  
235 7.15am) and once in the evening (when horses had hay, between 3pm and 4.30pm), the experimenter  
236 walked slowly and silently along the stalls and noted the behaviour and posture (see below) of each  
237 horse, at the instantaneous time of observation, that is every 5 min (time between 2 circuits). Horses  
238 were used to see people walking in the corridor at the time these observations were made (riders,  
239 students, caretakers) and they did not show any change of activity when the experimenter walked in  
240 front of their stall. Moreover, both experimental and control horses lived in the same stables and thus  
241 were exposed to the same observational procedure. In order to ensure that time between two  
242 observations of the same horse was constant, they were always observed in the same order.

243 Occasionally, a horse had to leave its stall during the observation period (for care or work), leading to  
244 small discrepancies in the numbers of observations per horse.

245 The observations concentrated on two indicators of compromised welfare:

246 - **Presence of SB/ARB** related to chronic stress for captive and for domestic species (Mason, 1991)  
247 known to be associated with impaired fertility (Benhajali et al., 2014) and altered cognitive abilities  
248 (review in Hausberger et al., 2019) in horses. For an activity to be considered a SB/ARB, the sequence  
249 had to be repeated at least three times in succession and observed five times, independently of the  
250 period of observation (Lesimple et al., 2016).

251 - **ear position** (backwards, forwards, asymmetrical, see Fureix et al., 2010) **when foraging**, ears  
252 backwards in this context reflecting impairment of welfare (e.g. Hausberger et al., 2016; Henry et al.,  
253 2017; Fureix et al., 2010)

254

## 255 **2.6-Physiological parameters**

256 As a modification of their welfare state may impact the horses' physiology (e.g. Pawluski et al.,  
257 2017; Popescu and Diugan, 2017), we investigated the relationships between the horses' welfare  
258 states, paddock releases and some blood parameters (complete blood cell count, serotonin and  
259 oxytocin blood levels).

260 Blood sampling is part of the routine sanitary control of horses at the ENE, to monitor horses'  
261 rehydration, muscular condition and food intake adequacy with exercise. Therefore the Comité  
262 Rennais d'Ethique en matière d'Expérimentation Animale considered that no approval was required,  
263 since blood sampling was part of the usual examinations and not conducted especially for our  
264 experiment. It was undertaken with the approval of the facility's senior veterinary and conducted by  
265 the usual experienced technician (LB) in charge of blood sampling at the ENE. All horses were used to  
266 the blood sampling procedure and accepted it well during both studies. It is worth nothing that  
267 particular attention was given by the technician to minimize potential aversive effects of blood  
268 sampling, which was confirmed here by the absence of any reaction by the horses. The entire  
269 procedure, from halter fitting to the end of the sampling session lasted less than one minute. Besides,  
270 horses were systematically given a food reward (one sugar lump) at the end of each sampling session.

271 The following analyses were performed: complete blood cell counts (CBCC) and sedimentation rates  
272 (at 0.5h and 1h) were analysed by the veterinary laboratory LABEO Franck (Caen, France) for study 1  
273 and the CBCC results were compared to physiological norms for sport horses provided by LABEO. As  
274 the results of study 1 showed positive improvement of horses' welfare we chose to focus study 2 on  
275 potential indicators of positive emotions, *i.e.* plasma levels of oxytocin and serotonin (Alberghina et  
276 al, 2017; Nagasawa et al, 2015).

## 277 **2.7-Statistical analyses**

278 As data were not normally distributed (Shapiro-Wilk test,  $p < 0.05$ ), non-parametric statistical tests  
279 were used. The statistical tests were performed using Statistica® 13 (Statsoft, Tulsa, USA).

280 Friedman and subsequent Wilcoxon tests were used to evaluate the behavioural and physiological  
281 changes in relation to time, *e.g.* between the first days (D1 to D4) and the last days (D13 to D14) of  
282 the daily paddock release period. Wilcoxon tests were used to compare physiological data before and  
283 after the daily release period.

284 Mann-Whitney U tests (MW U test) were used to compare the behaviour and physiological  
285 parameters of experimental horses and control horses.

## 287 **III- STUDY 1: RELEASE OF SPORT HORSES IN A Paddock: FEASIBILITY AND** 288 **IMPACT ON WELFARE**

289 This study aimed to assess the potential habituation of horses to the novel situation of the possibility  
290 for free movement, as well as its short-term impact on their welfare during the period of daily release  
291 sessions. Twenty-four horses (8 mares and 16 geldings), 9-15 years old ( $X \pm es = 12.0 \pm 0.3$ ), mostly  
292 French Saddlebreds (N=21) (Anglo-Arabian, N=3) took part in this study. The experimental horses  
293 were released daily during three consecutive weeks (14 paddock releases per horse). Moreover,  
294 observations in stalls yielded 5618 scans ( $X \pm es = 207 \pm 9$  scans/horse), and 84 hours of video recording  
295 in paddocks were analysed (N= 1599 scans;  $135.9 \pm 5.3$  scans/horse). Based on the facility routine,  
296 blood samples were taken between 9 and 11.30am on March 8<sup>th</sup>, *i.e.* before the start of the experiment,  
297 and on April 10<sup>th</sup>, *i.e.* at the end of the experiment. In addition to routine sampling, five millilitres of  
298 blood were collected in heparinised EDTA K3 tubes (BD Vacutainer®, Becton, Dickinson &

299 Company, Franklin Lakes, USA), then placed in a refrigerator at 4°C before being sent to a veterinary  
300 laboratory for analysis (LABEO Franck Duncombe, Caen, France) within the day. They were used to  
301 assess the horses' complete blood cell count (CBC) and sedimentation rates that were not usually  
302 controlled.

### 303 **3.1. Results**

#### 304 *3.1.1 Behaviour in the paddock (experimental horses)*

305 On day 1 (D1) behaviours reflecting alarm (vigilance posture) and excitement (*e.g.* snores, blows,  
306 kicking, rearing, active locomotion) were frequent, but their frequency decreased quickly over the  
307 following days so that they were almost absent after D4 and remained rare until D14 (Friedman F,  
308  $p < 0.005$  in all cases; Fig. 2). In contrast, feeding activity was rare on days 1 to 4 (Friedman test,  
309  $p > 0.05$ ), but increased significantly on the last experimental days (Friedman test: D1-D14,  $p < 0.01$ ).  
310 Excitement/alert behaviours decreased significantly and time spent eating increased significantly  
311 between D1 and D14 (Wilcoxon tests: respectively  $p < 0.01$  and  $p < 0.005$ ), whereas no significant  
312 differences in behaviour between D13 and D14 could be evidenced (Wilcoxon tests,  $p > 0.05$  in all  
313 cases), suggesting stabilization of horses' behaviours.

314 Subjective reports by the caretakers indicated no particular difficulty in leading horses to the  
315 paddocks or back to the stalls, and no major injury, that would have requested special care or overt  
316 lameness.

#### 317 *3.1.2. Impact of free time in paddock on horses' welfare state (evaluated in stalls)*

318 In order to assess the impact of the daily paddock release sessions on horses' welfare, the  
319 behaviours of the experimental and control (remaining in stalls) horses were compared before and  
320 during the paddock release period.

##### 321 *3.1.2.1. Stereotypic / Abnormal repetitive behaviours (SB/ARB)*

322 The prevalence of SB/ARB did not differ between the two groups before the paddock release  
323 period ( $N_{\text{Experimental}}=12$ ,  $N_{\text{Control}}=12$ ,  $\bar{X}_{\text{Experimental}} \pm \text{se} = 12.88 \pm 1.9$ ,  $\bar{X}_{\text{Control}} \pm \text{se} = 13.13 \pm 2.6$ , MW U test,  
324  $U=85.5$ ,  $p=0.63$ ). At the end of the three weeks of daily paddock release, the experimental horses  
325 tended to produce significantly less SB/ARB than the control horses ( $\bar{X}_{\text{Experimental}} \pm \text{se} = 9.96 \pm 2.2$ ,  
326  $\bar{X}_{\text{Control}} \pm \text{se} = 17.96 \pm 3.2$ , MW U test,  $U=41$ ,  $p=0.07$ ).

### 327 3.1.2.2. Ear positions during feeding

328 The short-term positive impact of paddock release was confirmed by ear position records: times  
329 spent with ears backwards by the experimental horses while foraging decreased significantly during  
330 the first week of paddock release (Friedman and Wilcoxon tests,  $p < 0.05$  in all cases) whilst it did not  
331 vary for control horses (Friedman test,  $\chi = 1.17$ ,  $p = 0.5$ ) (Fig. 3). Thus, whilst times spent with ears  
332 backwards did not differ significantly between the two groups of horses before the paddock release  
333 period ( $\bar{X}_{\text{Experimental}} \pm \text{se} = 47.40 \pm 5.2$ ,  $\bar{X}_{\text{Control}} \pm \text{se} = 38.92 \pm 5.8$ , MW U test,  $U = 52$   $p = 0.26$ ), the experimental  
334 horses spent significantly less time than controls with ears backwards while foraging after three weeks  
335 of daily paddock sessions ( $\bar{X}_{\text{Experimental}} \pm \text{se} = 21.73 \pm 5.2$ ,  $\bar{X}_{\text{Control}} \pm \text{se} = 40.08 \pm 7.2$ , MW U test,  $U = 37$ ,  
336  $p = 0.04$ ) (Fig. 3).

### 337 3.1.2.3. Horses' welfare and complete blood cell count (CBCC) parameters

338 All horses involved in this study presented blood cell count abnormalities (*e.g.* abnormal count of  
339 red and white blood cells). For example, the mean corpuscular haemoglobin values were abnormally  
340 low for the whole population, suggesting anaemia, while 92% of horses ( $N = 22/24$ ) presented  
341 abnormally low concentrations of leucocytes (Table 2). No effects of free time in paddock could be  
342 evidenced: CBCC parameters did not differ significantly between before and after the paddock period  
343 (Mann Whitney U tests,  $p > 0.1$  in all cases).

344 It is worth noting that before the experiments, stereotypic horses presented higher sedimentation  
345 rates at 0.5h and 1h than non-stereotypic horses (MW U tests,  $N_{\text{SB}} = 20$ ,  $N_{\text{Non-SB}} = 4$ ,  $U_1 = 9$ ,  $U_2 = 11$ ,  
346  $p = 0.02$  and  $p = 0.03$  respectively).

## 347 3.2. Conclusion

348 Thus, these sport horses habituated rapidly to the novelty of daily release in a paddock, and despite  
349 excitement behaviours at first, did not come to any harm. Moreover, some improvement of  
350 experimental horses' welfare could be observed in the stalls, suggesting that daily free time in a  
351 paddock was beneficial in the short-term. The physiological data revealed that their restricted living  
352 conditions were in any case impacting the horses' health although the paddock release period did not  
353 have any impact on CBCC parameters. Maybe given the horses' condition before the experiment, the  
354 duration of the experiment of daily paddock release was not last long enough.

355

356 **IV- STUDY 2: IMPACT OF FREE TIME IN PADDOCK ON HORSES' WELFARE AND**  
357 **ITS DURABILITY**

358 The second experiment focused on the impact of time for free locomotion on horses' welfare and  
359 its impact over the two following weeks, after cessation of the daily release in paddock. We had the  
360 possibility to observe the behaviour of some horses that changed status (experimental/control) between  
361 the two experiments. Twenty horses (7 mares and 13 geldings), 9 to 15 years old ( $X \pm es = 12 \pm 0.4$ ),  
362 mostly French Saddlebreds (N=17; Anglo-Arabian: N=3) were used. Fourteen (7 pairs  
363 experimental/control) of these horses that had been involved in the first study were available, but we  
364 reversed their status (experimental or control): the 7 study 1 control horses now had daily access to  
365 paddock, whereas the 7 study 1 experimental horses became controls, *i.e.* remained in their stall.  
366 Although the distribution between the experimental and control groups had been random in study 1,  
367 the group of these 14 horses was automatically reversed for study 2. In order to have a larger sample  
368 and thus increase statistical power, 6 more horses (5 experimental and 1 control assigned randomly)  
369 were added for study 2. There were therefore twelve experimental and 8 control horses. The duration  
370 of the paddock release period was limited to two weeks (9 paddock releases) due to logistic constraints  
371 of the facility. Thus, horses were observed 1 week prior to the experiment, one week during the  
372 paddock release period and then for 2 weeks in their stalls after the cessation of paddock release. In  
373 all, we recorded a total of 6357 scans in the stalls (range: 288-347,  $X \pm es = 322 \pm 4.6$  scans/horse). Some  
374 horses had to leave their stall during the observations (care, work), which explains discrepancy in  
375 numbers of observations per horse.

376 Horses' oxytocin and serotonin plasma levels were assessed. As for study 1, according to the  
377 facility routine procedures, blood sampling was performed between 9 and 11 am on May 15<sup>th</sup> 2018,  
378 *i.e.* before the paddock release period, and on June 1<sup>st</sup> 2018 *i.e.* after the paddock release period and  
379 ten millilitres (2x5mL) of blood were collected additionally and put in two heparinised EDTA K3  
380 tubes (BD Vacutainer®, Becton, Dickinson & Compagny), then placed in crushed ice with salt. Tubes  
381 were centrifuged 15 min at 3000 g to extract the plasma (6-9mL/blood sample), which was then  
382 harvested and frozen at -20 °C pending completion of analysis. Analyses were performed within three



383 weeks following collection by the same technician specialized in hormonal analyses at the Veterinary  
384 School (ONIRIS) in Nantes (France). In order to check the effects of variations due to circadian  
385 rhythm of secretion, horses' blood samples were collected in the same order on both dates. Hormonal  
386 levels were assessed using commercially available Elisa kits specific for oxytocin and serotonin  
387 measurements.

## 388 **4.1. Results**

### 389 4.1.1. Welfare assessment in stall

390 Frequencies of SB/ARB decreased significantly (Friedman test S-1/S3,  $\chi=9$ ,  $p=0.03$ ; Wilcoxon  
391 tests: S-1/S1,  $Z=2.36$ ,  $p=0.02$ ; S1/S4,  $Z=1.7$ ,  $p=0.07$ , not significant in the other cases) (Fig. 4) and of  
392 the time spent with ears backwards in the experimental group (% time spent with ears backwards,  
393 Friedman tests,  $\chi=36.7$ ,  $p<0.001$ ) during the daily release period, while no changes could be evidenced  
394 in the control group (Fig. 6). These results were confirmed for the 7 pairs of horses from study 1,  
395 showing that the study 2 experimental horses (controls in study 1) tended to express less SB/ARB  
396 (Friedman test,  $\chi=6.9$ ,  $p=0.07$ ), and spent significantly less time with ears backwards (Friedman test,  
397  $\chi=16.1$ ,  $p=0.001$ ) during the paddock release period. This behavioural flexibility confirms clearly the  
398 impact of sessions of free exercise. In parallel, experimental horses spent more time with ears forwards  
399 during the daily release period (Friedman test,  $\chi=14.1$ ,  $p=0.003$ ).

400 However, this effect was only short-term as these two indicators increased during the first week  
401 after the daily paddock sessions had stopped, and still more so during the second week, reaching  
402 values above the basal level observed before the paddock release period (increased frustration?) (Fig.  
403 4, Fig. 5).

### 404 4.1.2 Hormonal measures

405 Serotonin levels before and after the paddock release period (Wilcoxon test,  $p>0.05$ ) were similar  
406 and no differences could be evidenced between the experimental and control groups (MW U test,  
407  $p>0.05$ ). However, oxytocin levels of experimental horses increased significantly after the period of  
408 daily paddock release (Wilcoxon test,  $t=1.9$ ,  $p=0.05$ ; Fig. 6).

## 409 **4.2. Conclusion**

410 The positive impact of daily periods of free movement in a paddock on horses' welfare is thus  
411 major and, associated with an increase of oxytocin levels, suggests a possible increase of positive  
412 emotions. However, their cessation was associated rapidly with an increase of the values of indicators  
413 of compromised welfare. Here again, subjective assessments by caretakers indicated no major risk or  
414 difficulty associated with this procedure.

#### 415 **V-DISCUSSION**

416 Our analyses of horses' behaviours showed (1) that daily release of inexperienced (with paddock)  
417 adult sport horses in a paddock is feasible, as they habituate rapidly to this situation and (2) that  
418 regular time for free movement has a clear positive impact on horses' welfare and emotional state.  
419 Thus, these times for free movement were associated with an immediate decrease of indicators of  
420 compromised welfare (SB/ARB and time spent with ears backwards), but also with an increase of the  
421 levels of plasma oxytocin in experimental horses. These results are remarkable, since the horses were  
422 released alone and the duration of the daily release sessions were very short (39-62 min.) and over  
423 limited periods of time (14 and 9 days). However, this was probably not enough to improve blood  
424 parameters which showed abnormalities before and still after the procedure. Further experiments  
425 should involve longer daily release sessions and longer periods of daily turnout. The presence of hay  
426 in the paddock might have been a major factor facilitating the horses' habituation to this novel  
427 situation.

428 During the first days of the release period, all the experimental horses expressed some excitement  
429 (trot, canter and passage) and alarm (vigilance posture), confirming the high emotional arousal of  
430 single-stall housed horses when released in a larger space (Lesimple et al., 2011). However, this  
431 emotional arousal disappeared quickly and frequencies of quieter behaviours such as feeding and slow  
432 locomotion (walk) increased. This positive behavioural transition, leading to a more naturalistic time-  
433 budget (Waring, 2003), was probably further enhanced by the access to hay in the slow-feeder. Thus,  
434 both experimental and control horses had a semi-continuous access to hay, thus fulfilling their  
435 foraging needs (*e.g.* Benhajali et al., 2009; Goodwin et al., 2002; Rochais et al., 2018; Waring, 2003).  
436 In addition, being able to forage probably helped redirect the horses' focus, speed up their habituation  
437 and decrease the potential novelty stressor effect of the paddock situation. Subjective assessment by

438 the familiar caretakers indicated no major problem associated with the procedure and the release in  
439 paddock never led to requirement of sanitary measures or vet examinations.

440 Constant single-stall housing is known to be detrimental for horses at any age (*e.g.* Heleski et al.,  
441 2002; Lesimple et al., 2016, Waters et al., 2002), while spending time in a paddock seems to reduce  
442 welfare impairment (McGreevy et al., 1995; Lesimple et al., 2016; Normando et al., 2002). Our  
443 observations show that even if it is only for short periods (1h/ day, 5 days a week, for 2 or 3 weeks)  
444 and without the presence of conspecifics daily access to a larger area enabling free movement led to a  
445 significant decrease of the values of indicators of compromised welfare (Mills, 2005; Wiedenmayer,  
446 1996), while no significant changes in control horses' behavioural patterns were observed. This is in  
447 accordance with other studies conducted on captive animals showing a decrease of SB/ARB when  
448 animals were given access to larger spaces (parrots: Polverino et al., 2015; cheetahs: Quircke et al.,  
449 2012). Our results were confirmed by the data for the behaviour of the horses that were in the study 2  
450 experimental group and had been in the study 1 control group: the positive impact of regular paddock  
451 release was not related to individual characteristics. Moreover, the values of compromised welfare  
452 indicators increased as soon as the period of daily paddock release was over, reaching and even, after  
453 two weeks, becoming above the pre-experiment basal level. Such rapid changes in the indicators of  
454 compromised welfare are in accordance with Lesimple et al.'s (2019) recent findings highlighting an  
455 immediate modification of the expression of SB/ARB in response to changes in stall architecture.  
456 Thus, in addition to the direct relationship found between welfare improvement and paddock release,  
457 our results highlight the importance of regular, uninterrupted access to periods for free movement.

458 The results of study 2 even suggest that daily paddock release may promote positive emotions.  
459 Thus, experimental horses' oxytocin levels increased after 5 days of paddock release while no such  
460 change was observed in the control horses. Authors generally consider that an increase of oxytocin  
461 blood levels is related to positive emotions, as reported for several species (*e.g.* humans: Feldman et  
462 al., 2010; Turner et al., 1999; dogs: Mitsui et al., 2011; Nagasawa et al., 2015; sheep: Laine et al.,  
463 2016). There is some controversy about the significance of oxytocin in terms of emotional valence as  
464 positive visual attention between humans and dogs is associated with an immediate increase of  
465 oxytocin levels (Nagasawa et al., 2015) whereas two recent studies suggest that humans' gentle

466 handling of horses and lambs induces a decrease of oxytocin levels (Lansade et al., 2018; Coulon et  
467 al., 2013). A first possible explanation is that this discrepancy could be related to differences in  
468 hormonal sampling and analyses (Neumann, 2008) or the situations involved (changes in housing  
469 versus human-animal interactions and the interpretation of its impact on animals). A second  
470 hypothesis could be that in our study, the duration of the paddock release period was too short to elicit  
471 a modification of the basal level of oxytocin. Thus, the increase of blood oxytocin level would be  
472 related to the occurrence of short term positive events, here paddock release (Mitsui et al., 2011;  
473 Nagasawa et al., 2015). Our results did not reveal however any impact of paddock release on serotonin  
474 blood levels. Serotonin levels vary throughout the day (together with the functioning of the  
475 corticosteroid system), and are higher during the activity phase of an individual (rodents: Chalet,  
476 2007). Exposure to stressors throughout the day impacts changes in serotonin levels (Chaouloff,  
477 2000). Possibly, rather than a single sampling before and after the paddock release period, evaluation  
478 of the speed serotonin levels decline throughout the day would be more efficient to detect potential  
479 positive impacts of such periods for free movement on horses' serotonergic system (see Daut and  
480 Fonken, 2019 for a review). On the other hand, despite the absence of any declared pathological  
481 disorders (monthly check-up by a dedicated senior veterinarian), as mentioned above, the horses in  
482 this study all presented important physiological disorders that remained after the paddock release  
483 period, as revealed by the CBCC analyses (e.g. 87% of the horses presenting low leucocyte levels).  
484 The short duration of the daily release period and the total duration of the release period seemed to be  
485 insufficient to improve the horses' internal state reflected by CBCC. The serotonergic system could  
486 possibly require a longer period of daily paddock release to show an increase. Interestingly, our results  
487 show unusual relationships between the horses' welfare state and physiological data. Recently,  
488 Popescu and Diugan (2017) reported an increase of inflammation indicators (neutrophils/leucocytes  
489 ratio) together with alteration of working horses' welfare state, and riding school horses under  
490 constraining working conditions presented abnormal neutrophil levels (Pawluski et al., 2017). Thus,  
491 horses with SB/ARB or that spent more time with ears backwards when foraging presented high rates  
492 of indicators of inflammation (e.g. sedimentation rate, white cells) showing a relationship between  
493 behavioural and postural indicators of compromised welfare and physiological disorders.

494 Given the major positive impact of experimental paddock release on horses' behaviours, it would  
495 be interesting to determine whether longer release durations could have an impact on the horses'  
496 internal state (CBCC and serotonin levels). Moreover, free movement is not part of any current  
497 international regulation. The results of the present study, which add to the already existing scientific  
498 literature on other types of ridden horses (e.g. Bachman et al., 2003; Lesimple et al., 2011, 2016)  
499 suggest that requirement for sessions for free movement should be added. Finally, as social contacts  
500 are a crucial need it would be interesting to repeat this experiment with horses released in pairs or in  
501 groups. Overall, this study highlights the fact that providing sport horses some time for daily free  
502 locomotion (ideally with roughage/grass resources) is possible and incurs no particular risks and that it  
503 is an essential requisite for promoting good welfare for horses.

504

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512

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## 752 VIII- TABLES

753 **Table 1.** Behaviours expressed and recorded during behavioural (Instantaneous Scan Sampling and  
754 all occurrence) observations in paddock. The behaviours are presented in relation to sampling method  
755 used and definitions or examples are given. \*Waring 2003, \*\*Kiley Worthington 1976, \*\*\*Mills  
756 2005, Lesimple et al 2016.

	Behaviour	Définition / Example
Instantaneous Scan Sampling	Feeding	Eat straw/hay
	Drink	Drink
	Rest*	The horse is still, eyes half-closed or closed, standing or lying (sternal or lateral)
	Observation	Monitoring the environment, still, eyes open, with head and ears mobile
	Look*	The horse looks at a precise object or direction of the environment, ears pointed towards the stimulus, neck at or above horizontal plane.
	Exploratory walk	Slow walk, neck below horizontal plane, loosened tail.
All occurrence	Active locomotion	Active walk, trot, canter, passage
	Excitation behaviour	Kicking, rearing, jumping

Vigilance**	Alarm posture: the horse stares at a particular point in the environment, with its neck and tail high
Stereotypic behaviours***	Including stereotypic (weaving, cribbing, wind-sucking, pacing, headshaking) and abnormal repetitive behaviours (head, tongue, lip movements, repetitive licking and biting, etc).
Whinnies	Whinnies
Blow*	Non-vocal sound produced by a forceful expulsion of air through the nostrils, emitted as an expression of alarm
Snore*	Non-vocal raspy inhalation sound often related to the exploration of a new object or environment.
Maintenance	Defecation, urination, rubbing, scratching, shaking, rolling

758

759 Table 2. Prevalence of complete blood cell count parameters abnormalities in our study population  
760 before the daily paddock release period. The numbers and percentages of horses presenting  
761 abnormally high or low levels are presented for each parameter as well as the total numbers and  
762 percentages of abnormalities. Veterinary norms (mean and range) are presented in the first two  
763 columns. Only the large lymphocyte concentration was normal for all horses, while MCV and MCHC  
764 were abnormal for the whole population.

765

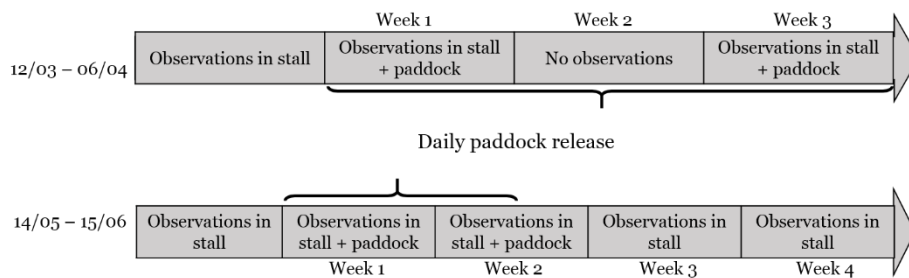
Blood cell count parameter	Norms		% of horses with abnormal values of BCC abnormalities		
	Mean values	Range values	Horses with values above norms	Horses with values below norms	Total Abnormalities
			Nb (%)	Nb (%)	Nb (%)
Red blood cells ( $10^6/\text{mm}^3$ )	7.9	7.1-8.8	0 (0%)	11 (45%)	11 (45%)
Leucocytes ( $10^6/\text{mm}^3$ )	8.4	7.1-10	0 (0%)	22 (91.6%)	22 (91.6%)
Haemoglobin (g/dL)	12.8	11.7-14	0 (0%)	5 (20.8%)	5 (20.8%)
Hematocrit (%)	34.3	31.2-37.3	1 (4.2%)	3 (12.5%)	4 (16.7%)
Neutrophils (%)	56.8	51.3-66.4	4 (16.7%)	4 (16.7%)	8 (33.4%)
Eosinophils (%)	2.3	1.3-3.9	5 (20.8%)	4 (16.7%)	9 (37.5%)
Basophils (%)	0.4	0.3-0.7	12 (50%)	0 (0%)	12 (50%)
Monocytes (%)	4.1	3.0-5.1	1 (4.2%)	2 (8.3%)	3 (12.5%)
Large lymphocytes (%)	0.6	0.3-0.9	0 (0%)	0 (0%)	0 (0%)
Small lymphocytes (%)	32.6	25.6-39.4	4 (16.7%)	4 (16.7%)	8 (33.4%)
Platelets ( $10^3/\text{mm}^3$ )	135	112-159	0 (0%)	20 (83.3%)	20 (83.3%)
Mean Globular Rate (pg)	15.7	15.2-16.6	20 (83.3%)	0 (0%)	20 (83.3%)
Mean Corpuscular Volume ( $\mu\text{m}^3$ )	41.1	38.4-42.7	24 (100%)	0 (0%)	24 (100%)
Mean Corpuscular Haemoglobin Concentration	37.9	36.8-39.7	0 (0%)	24 (100%)	24 (100%)

766

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768 **IX- FIGURES**

**Study1: testing habituation** and effects on welfare indicators in stalls during the release period



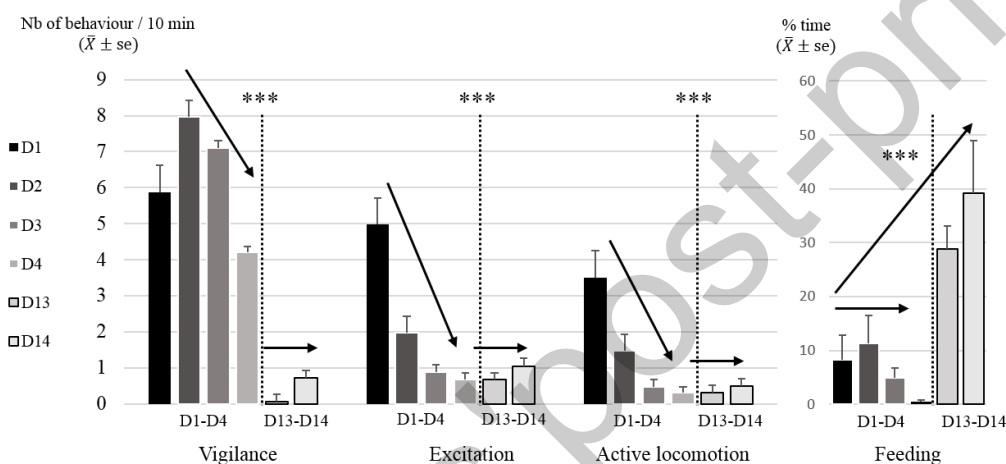
**Study2: testing habituation and effects on welfare indicators after the release interruption: sustainability of the positive effects**

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Fig. 1. Experimental procedure and calendar for the different phases.

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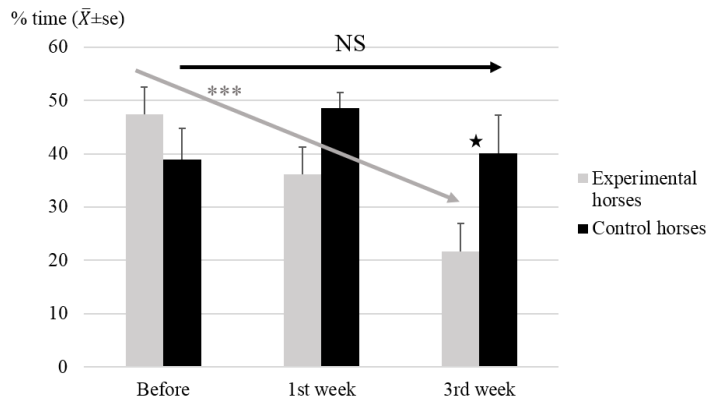
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Fig. 2 Study 1: Behavioural changes in relation to time (D1= day 1 etc. to Day14) of the 12 experimental horses while in paddock. Friedman tests, \*\*\*p<0.001. Vigilance, excitement and active locomotion are represented in numbers of occurrences expressed in 10 min (rare behaviours, *ad libitum* sampling). Feeding is represented in % of time (scan sampling). The prevalence of behaviours reflecting emotional arousal (vigilance, excitement and active locomotion) decreased from D1 to D4 and remained low until D14, while feeding increased.

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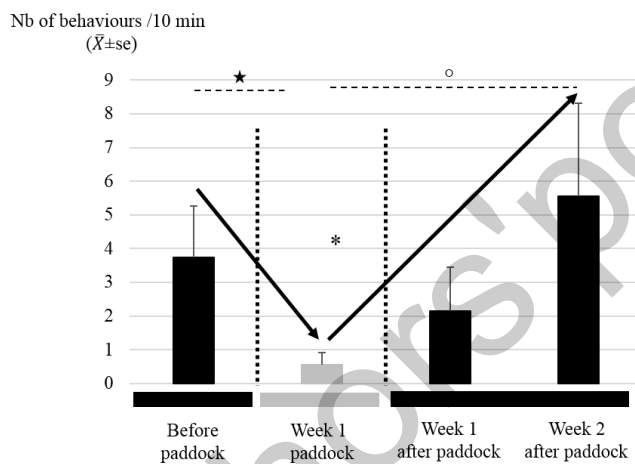
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Fig. 3. Study 1: time spent with ears backwards while foraging in stalls. Friedman (\*\* $p < 0.001$ ) and MW U ( $\star p < 0.05$ ) tests. Times spent with ears backwards did not differ between experimental and control groups before the experimentation. After 3 weeks, experimental horses spent significantly less time with ears backwards when foraging than control horses.



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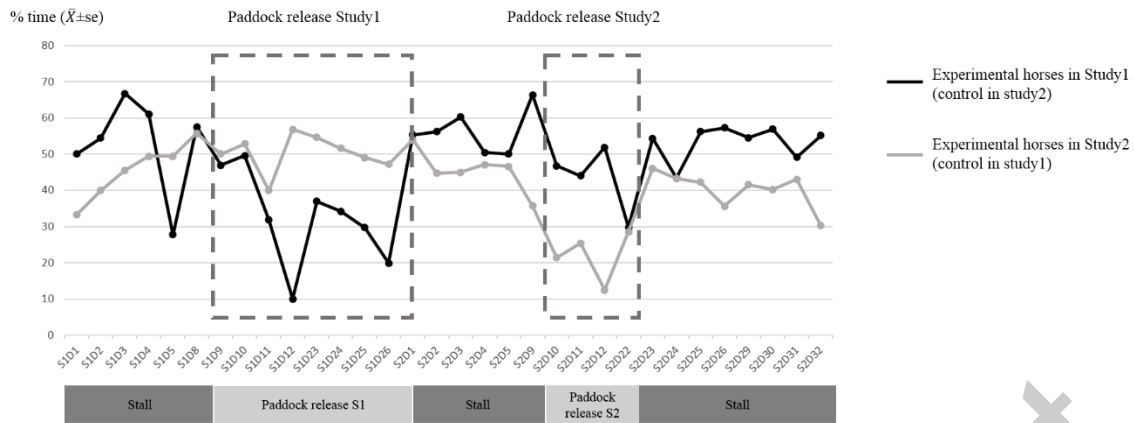
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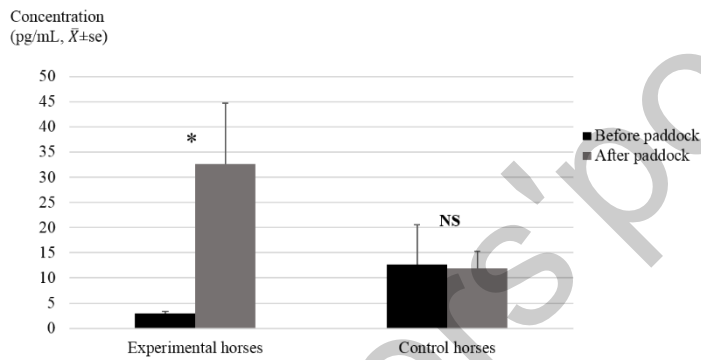
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Fig. 4. Experimental horses' stereotypic behaviours (frequencies) during Session 2, Friedman test,  $\star p < 0.05$ , Wilcoxon tests,  $\star p < 0.05$ ,  $^\circ p < 0.01$ . The frequency of SB/ARB decreased significantly during the period of paddock release, increased after the paddock release period had ended and reached the SB/ARB basal level and even reached levels above that.



791

792 Fig. 5. Time spent with ears backwards while foraging. Variations during the experiment of time spent by  
 793 horses that switched status between study 1 and study 2 (S1D1 = Study 1-Day 1; S2D1 = Study 2-Day 1). In  
 794 black: horses that were released in a paddock during the first study; in grey: horses that were released in a  
 795 paddock during the second study. In both case, the time spent with ears backwards decreased immediately at the  
 796 beginning of the paddock release period and increased instantaneously after its cessation.



797

798 Fig. 6. Oxytocin levels before and after the study 2 paddock release period, Wilcoxon test, \* $p < 0.05$ . After the  
 799 paddock release period, experimental horses (that went to paddock) presented higher oxytocin levels, while  
 800 control horses' levels did not change significantly.

801

802