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1 **Effects of a temporary period on pasture on the welfare state of horses** 2 **housed in individual boxes**

3 Alice Ruet ^{1,*}, Cécile Arnould ¹, Justine Levray ¹, Julie Lemarchand ¹, Núria Mach ², Marie-Pierre
4 Moisan ³, Aline Foury ³, Christine Briant ¹, and Léa Lansade ¹

5 ¹ INRAe, UMR 85 PRC, CNRS, UMR 7247, IFCE, University of Tours, 37380 Nouzilly, France.

6 ² INRAe, UMR 1313 GABI, AgroParisTech, University of Paris-Saclay, 78352 Jouy-en-Josas,
7 France.

8 ³ Univ. Bordeaux, INRAe, Bordeaux INP, NutriNeuro, UMR 1286, 33076 Bordeaux, France.

9
10 * Corresponding author: alice.ruet@sfr.fr

11

12 **Abstract**

13 Domesticated horses mainly live in individual boxes, a housing system reported as compromising
14 animal welfare. A common practice in riding schools involves offering a temporary period on pasture
15 with conspecifics to alleviate the impact of long-term deprivation triggered by boxes. The aim of this
16 study was to assess the influence of this practice using four behavioural indicators reflecting a
17 compromised welfare state: stereotypies; aggressive behaviours towards humans; the “withdrawn
18 posture” reflecting unresponsiveness to the environment; and the “alert posture” indicating
19 hypervigilance. A group of 31 horses was monitored before, during and after a period of 1.5 months
20 on pasture (intra-group comparisons) and their behaviours were compared to those of 29 horses kept in
21 individual boxes during the study (inter-group comparisons). On pasture, no stereotypies and
22 aggressive behaviours towards humans were observed, and the occurrence of the “alert posture”
23 decreased, although the results were not significant. An increase in the expression of natural
24 behaviours such as locomotion, exploration and social behaviours was observed. However, the
25 expression of the “withdrawn posture” increased during the first five days on pasture (Friedman: $P <$
26 0.001 ; Wilcoxon signed-rank: $P < 0.001$) before a decrease was observed after 20 days, returning to
27 the level previously observed when horses were in boxes (Wilcoxon signed-rank: $P < 0.01$). These
28 results suggest that going out to pasture can positively influence the welfare state of horses, but also
29 that several days of adaptation are needed, probably due to the novelty of the environmental and social

30 conditions. The most noticeable result occurred when horses returned to individual boxes. A sharp
31 increase in the occurrence of stereotypies (Cochran test: $P < 0.001$; Chi² of homogeneity: $P = 0.05$), of
32 the “withdrawn” (Friedman: $P < 0.001$; Wilcoxon rank-sum: $P < 0.05$) and the “alert” postures
33 (Friedman: $P < 0.01$; Wilcoxon rank-sum: $P < 0.001$) was observed during the first five days of
34 returning to confinement. The expression of the majority of natural behaviours immediately returned
35 to the level observed during the pre-pasture period. After three months, the expression of the four
36 welfare indicators was not different from that in the pre-pasture period. These results demonstrate that
37 the beneficial effects likely to be induced by the pasture do not last when horses return to individual
38 boxes and that the environmental change causes deleterious short-term effects on the animals’ welfare
39 state. It would thus be recommended to keep domestic horses permanently on pasture when possible.

40

41 **Keywords**

42 Aggressiveness, Behaviour, Human-animal relationship, Management, Stereotypies,
43 Unresponsiveness

44

45 **1. Introduction**

46 The welfare of animals living in human-organised environments is now recognized as a critical issue.
47 This concept considers both physical and psychological components interacting with each other
48 (Carenzi and Verga, 2016). Among domesticated species, horses may be subject to welfare alterations,
49 as up to 90 % mainly live in individual boxes (Leme et al., 2014). This housing system was reported to
50 prevent animals from performing natural behaviours due to environmental deprivations. For example,
51 feeding can only accounts for 16.5 % of the time budget in individual boxes (Hallam et al., 2012)
52 while it represents 46.0 to 66.8 % under free-roaming conditions (Souris et al., 2007; Van
53 Dierendonck et al., 1996). Similarly, moving and social interactions are sometimes totally prevented
54 (Christensen et al., 2002a; Houpt et al., 2001). The failure to perform natural behaviours can lead
55 animals to experience chronic negative internal experiences (Mellor, 2017), motivating the expression

56 of potentially adaptive behaviours such as stereotypies (Dellmeier, 1989; Fraser and Duncan, 1998). It
57 could therefore be postulated that the impact of these long-term deprivations may be alleviated by
58 allowing horses to benefit from access to a more natural environment, such as pasture with
59 conspecifics. To this aim, a common practice in riding schools consists in offering usually-stabled
60 horses a temporary period on pasture during the summer, when customers are absent.

61 It has been reported that horses continuously living on pasture with conspecifics present a more
62 natural expression of the species' behaviours such as feeding and social interactions (Christensen et
63 al., 2002b; King et al., 2013) and less health impairments (Yngvesson et al., 2019), suggesting a better
64 welfare state than horses kept in individual boxes (Hartmann et al., 2012). The more horses spend time
65 on pasture, the less likely they are to express stereotypies (Christie et al., 2006). However, it is not
66 known whether a temporary period on pasture for horses usually housed individually has the same
67 beneficial effects. Indeed, sudden marked environmental changes between individual boxes and
68 pasture with conspecifics, and vice versa, could affect the welfare of horses, as reported in other
69 species. Grouping of individuals could be perceived as an aversive event in cattle (Bøe and Færevik,
70 2003). Major effects have also been specifically reported the first few days after cattle and sheep are
71 confined, following a grazing period. This change of environment has been observed to induce
72 behavioural and physiological stress responses (Nakajima et al., 2018), oxidative stress and a higher
73 susceptibility to infections (Degabriele and Fell, 2001), as well as a decrease in the expression of
74 natural behaviours (e.g., lying, rumination; Enriquez-Hidalgo et al., 2018; Higashiyama et al., 2007). It
75 would thus be interesting to know whether a temporary period on pasture effectively improves horse
76 welfare or induces excessive environmental changes leading to deleterious effects.

77 To this aim, we focused on four behavioural indicators reflecting a compromised mental welfare state
78 (Ruet et al., 2019). The first indicator concerns stereotypies, defined as “repetitive unvarying and
79 functionless behaviours” (Mason, 1991) that are presumably induced by boredom, chronic stress and
80 frustration (Sarrafchi and Blokhuis, 2013). The second indicator is related to aggressiveness towards
81 humans, which has been correlated to a pessimistic judgement bias reflecting a long-lasting negative
82 affective state (Henry et al., 2017) and chronic health impairments (Fureix et al., 2010). This

83 behavioural indicator, reinforced by the affective system of rage/anger, allows animals to defend
84 themselves against constraints (Panksepp, 2011). The third indicator is the “withdrawn posture”,
85 reflecting unresponsiveness to the environment (Fureix et al., 2012). The behavioural and
86 physiological profile (anhedonia; Fureix et al., 2015) of unresponsive horses shows strong similarities
87 with depressive states of animal models and humans (Hao et al., 2019), that could indicate a decrease
88 in the arousal of the reward system (Panksepp, 2011). The fourth indicator is a high occurrence of the
89 “alert posture” (Ransom and Cade, 2009), indicating hypervigilance and potentially revealing an
90 internal state of anxiety which allows the animals to avoid danger (Panksepp, 2011; Sylvers et al.,
91 2011). Although anxiety has an adaptive value, it constitutes a welfare concern when it compromises
92 the mental and physical functions of individuals and prevents them from adapting to external factors
93 (Salomons et al., 2009).

94 A group of horses was observed before, during and after a period of 1.5 months on pasture (“Pasture”
95 group). We hypothesised that the animals would express the four behavioural indicators mentioned
96 above less frequently while on pasture. We also monitored the possible increase in the expression of
97 these indicators in the first five days after the environmental changes, especially when horses returned
98 to their boxes (intra-group comparisons). To control for seasonal effects, the behaviours of the
99 “Pasture” horses following their return to individual boxes were also compared to those of “Control”
100 horses that had not been released to pasture (inter-group comparisons). We hypothesised that the
101 occurrence of the behavioural indicators would be higher in the “Pasture” group than the “Control”
102 group during the days following their return to individual boxes.

103

104 **2. Materials and methods**

105 *2.1. Animals*

106 This study was carried out at a riding school (France) and included 60 Warmblood horses aged 11.03
107 \pm 2.9 years [6 – 21 years] (mean \pm SD; [min – max]) that had lived in individual boxes since they were
108 three years old. Prior to this, these horses had lived outside on pasture in groups on their breeding

109 farms. The “Pasture” group consisted of 31 horses (20 geldings and 11 mares) and the “Control” group
110 consisted of 29 horses (17 geldings and 12 mares) kept in individual boxes. The two groups were
111 balanced regarding age, and the absence of mean difference in the expression of the four behavioural
112 indicators was statistically tested between the two groups, before release to pasture.

113 *2.2. Housing and management conditions*

114 *Individual boxes.* All horses in both groups lived in individual 9 m² boxes, that were cleaned six
115 mornings out of seven. They were fed with hay (9 ± 1.5 (mean \pm SD) kg divided into two meals per
116 day) and concentrated feed of varying quantities according to body condition and physical activity.
117 Water was provided *ad libitum* by automatic drinkers with pressure valves. All horses had visual
118 contact with conspecifics and reduced tactile contacts through a grilled window on the wall between
119 two boxes. They were trained for sport purposes six days out of seven. The horses were occasionally
120 (less than four hours per week) released for free exercise into individual sand paddocks of
121 approximately 200 m², equipped with a drinking trough and a slow feeder containing hay, but no
122 shelter (Rochais et al., 2018).

123 *Pasture.* Horses of the “Pasture” group spent 41.7 ± 16.8 days on pasture between August and
124 September. The pastures were located 5 ± 1.5 kilometres from the riding school, and the “Pasture”
125 horses were transported together by truck. All the animals were familiar with transportation. They
126 were released onto unfamiliar pastures in randomly constituted groups consisting of a minimum of six
127 and a maximum of eight individuals. The groups were mixed (both geldings and mares), except one
128 that consisted only of geldings. The average surface area of the pastures was 5.02 ± 0.4 hectares,
129 which was much larger than the minimum recommended surface area ensuring a low level of
130 aggression among horses (0.03 hectares per horse; Flauger and Krueger, 2013). All pastures were
131 equipped with one or two human-made shelters. Water, hay and concentrated feed (the same as in
132 individual boxes) were provided on a daily basis by a caretaker who monitored the animals for early
133 detection of injuries or health impairments.

134 *2.3. Behavioural assessment*

135 The “Pasture” group was studied during five different periods and the “Control” group was studied
136 during three of these periods. For each period (“Pre-pasture”, “Early pasture”, “Late pasture”, “Post-
137 pasture (0 to 5 days)” and “3 months after pasture”), the horses were observed over five consecutive
138 days using the scan sampling method (Altmann, 1974; Figure 1). Per period, behavioural observations
139 were carried out during 10 sessions of 90 minutes each (two sessions between 09:00 and 10:30, 10:30
140 and 12:00, 12:00 and 13:30, 13:30 and 15:00, 15:00 and 16:30). Thirteen scans per horse were
141 recorded per session. The average number of scans per horse and per period of observation was 105.3
142 ± 13.02 (mean \pm SD); variations resulted from the absence of the horse in the box or the presence of
143 the caretaker in the box or pasture at the time of the observation. The details of the total number of
144 scans recorded per period and per group are presented in the supplementary materials (Table S1).

145 When horses were in individual boxes, the observers regularly walked in front of the box doors, at a
146 distance of at least 1.5 m, making as little noise as possible. On pasture, the observers familiarised the
147 horses to their presence before starting the observations; to do this, they slowly and quietly approached
148 the horses, stopped at a distance of at least 30 metres and stood motionless for 10 minutes. If the
149 horses approached during the familiarization process, the experimenter moved to a different place and
150 remained motionless until the horses lost interest. Observations were performed in the same way both
151 in the boxes and on pasture: the observers looked at the horse for 5 seconds and then recorded whether
152 the animal expressed the behavioural indicators studied or not. The descriptions of the four
153 behavioural indicators recorded are presented in Table 1.

154 Natural behaviours of the time budget of the horses were also recorded to provide a more complete
155 picture of the horses’ activities in the boxes and on pasture. These natural behaviours are defined in
156 the supplementary materials (feeding, locomotion, exploration, resting, observation, body movements
157 due to insect harassment, positive and negative social interactions; Table S2). Two observers
158 experienced in equine ethology carried out the observations, sharing the sessions equally between the
159 two groups. The interrater reliability was estimated on 100 scans prior to the study. Agreement
160 between observers was considered very high (percentage of agreement = 91 %; Cohen’s kappa = 0.9;
161 Mchugh, 2012).

162 As aggressive behaviours towards humans could not be observed through scan sampling for horses on
163 pasture in the same way as in boxes because the observer stood at least 30 meters from the animals,
164 this indicator was assessed using a human-animal relationship test. This test was performed only once
165 in the period “Late pasture” and consisted of slowly approaching the horse’s head with the arm raised
166 at a 45 ° angle from the chest (1 step per second), and then trying to touch the horse from the neck to
167 the back on the left side (see the AWIN protocol adapted to horses housed in groups; AWIN, 2015).
168 The expression of aggressiveness was recorded in a binary manor during this test as previously
169 described (presence or absence, behaviours described in Table 1).

170 *2.4. Statistical analyses*

171 The percentage of scans recorded for each behavioural indicator per period was calculated from the
172 total number of observations per horse. The percentage of scans of aggressive behaviours towards
173 humans when horses were in individual boxes, and that of the “withdrawn” and “alert” postures were
174 analysed as continuous variables. However, stereotypies were processed as the proportion of horses
175 expressing these indicators because of the high number of null values among the sample, as well as
176 aggressive behaviours towards humans on pasture. Nonparametric tests were used for the four
177 behavioural indicators because the data were not normally distributed.

178 Intra-group comparisons (i.e., differences in the expression of the four behavioural indicators over the
179 five periods for the “Pasture” horses) were investigated first. The proportion of horses expressing
180 stereotypies was compared between the periods using Cochran’s *Q* test (*cochrans.q* function in the
181 *nonpar* R library) followed by Dunn’s test with Bonferroni’s correction for pairwise comparisons
182 (*dunn.test* function in the *dunn.test* R library). Nonparametric ANOVA (Friedman’s; *friedman.test*
183 function in the *stats* R library) was performed on the percentages of scans of aggressive behaviours
184 and “withdrawn” and “alert” postures followed by Wilcoxon signed-rank tests with Bonferroni’s
185 correction for pairwise comparisons (*pairwise.wilcox.test* function in the *stats* R library). Since
186 aggressive behaviours towards humans were binary assessed during the test on pasture, the result was
187 not included in the analysis of the evolution in the percentage of scans of this behavioural indicator
188 over the periods.

189 Inter-group comparisons were then performed between the “Pasture” and “Control” groups. These
190 comparisons only concerned the periods when the horses were in individual boxes (“Pre-pasture”,
191 “Post-pasture (0 to 5 days)” and “3 months after pasture”). The proportion of stereotypic horses was
192 compared between the two groups using Chi² tests of homogeneity (*chisq.test* function in the stats R
193 library). Wilcoxon rank-sum tests with continuity correction (*wilcox.test* function in the stats R
194 library) were used to compare the percentages of scans of aggressive behaviours and the “withdrawn”
195 and “alert” postures.

196 The percentages of scans of the horses’ natural behaviours in the time budget (feeding, locomotion,
197 exploration, resting, observation, body movements due to insect harassment, positive and negative
198 social interactions) were calculated from the total number of observations per horse. More details of
199 the statistical analyses are provided in the supplementary materials. All statistical analyses were
200 performed using R software (version 3.6.0, R Development Core Team, 2019) with a significance
201 level of $P \leq 0.05$.

202 2.5. Ethics statement

203 This study was conducted in compliance with the ethical policy of the International Society for
204 Applied Ethology and approved by the ethics committee of Val de Loire (2019012211274697.V4 –
205 18939).

206

207 3. Results

208 3.1. Evolution of the four behavioural indicators over the five periods

209 *Stereotypies.* Intra-group comparisons for the “Pasture” group showed that the proportion of horses
210 expressing stereotypies significantly differed between periods ($Q(4) = 28.3, P < 0.001$). The
211 proportion was higher in the “Post-pasture (0 to 5 days)” period than in the three preceding periods
212 (“Pre-pasture”: $P < 0.05$; “Early pasture”: $P < 0.001$; “Late pasture”: $P < 0.001$; Figure 2).

213 The increased expression of stereotypies in the “Post-pasture (0 to 5 days)” period in the “Pasture”
214 group mainly concerned repetitive tongue movements and to a lesser extent repetitive licking (Figure
215 2; Table 2).

216 Inter-group comparisons showed that the proportion of stereotypic horses was higher in the “Pasture”
217 group than the “Control” group in the “Post-pasture (0 to 5 days)” period ($\chi^2 (1) = 3.9, P = 0.05$). No
218 significant differences were observed in the “Pre-pasture” ($P = 1$) and “3 months after pasture” ($\chi^2 (1)$
219 $= 0.1, P = 0.76$) periods (Figure 2).

220 *Aggressive behaviours.* Intra-group comparisons for the “Pasture” group showed that the percentage of
221 scans of aggressive behaviours towards humans did not significantly differ between the three periods
222 in which horses were in individual boxes ($\chi^2 (2) = 4.1, P = 0.14$). In addition, no horses on pasture
223 showed aggressive behaviours towards the observer during the test performed in the “Late pasture”
224 period.

225 Inter-group comparisons showed that there were no significant differences between the “Pasture” and
226 “Control” groups in any of the periods (“Pre-pasture”: $W (1) = 507, P = 0.24$; “Post-pasture (0 to 5
227 days)”: $W (1) = 526.5, P = 0.12$; “3 months after pasture ”: $W (1) = 383, P = 0.44$; Figure 3.a).

228 “*Withdrawn posture*”. Intra-group comparisons showed that the percentage of scans of the
229 “withdrawn posture” was significantly different between periods ($\chi^2 (4) = 40.7, P < 0.001$). This
230 percentage was higher in the “Early pasture” period than the “Pre-pasture” ($P < 0.001$), “Late pasture”
231 ($P < 0.01$) and “3 months after pasture” ($P < 0.001$) periods. The percentage of scans of the
232 “withdrawn posture” was also higher in the “Post-pasture (0 to 5 days)” than the “Pre-pasture” ($P <$
233 0.05) and “3 months after pasture” ($P < 0.01$) periods.

234 Inter-group comparisons showed that the percentage of scans of the “withdrawn posture” was higher
235 in the “Pasture” group than the “Control” group in the “Post-pasture (0 to 5 days)” period ($W (1) =$
236 $267, P < 0.05$). There were no significant differences in the “Pre-pasture” ($W (1) = 423.5, P = 0.88$)
237 and “3 months after pasture” ($W (1) = 336.5, P = 0.78$) periods (Figure 3.b).

238 “Alert posture”. Intra-group comparisons showed that the percentage of scans of “alert posture” was
239 significantly different between periods ($\chi^2 (4) = 16.6, P < 0.01$). This percentage was higher in the
240 “Post-pasture (0 to 5 days)” period than the “Early pasture” ($P < 0.05$) period.

241 Inter-group comparisons highlighted that “Pasture” horses expressed or tended to express more “alert
242 postures” in the “Post-pasture (0 to 5 days)” ($W (1) = 232.5, P < 0.001$) and “3 months after pasture ”
243 ($W (1) = 256, P = 0.07$) periods, respectively, than the “Control” group. There were no significant
244 differences between the “Pasture” and “Control” groups in the “Pre-pasture” period ($W (1) = 372.5, P$
245 $= 0.32$; Figure 3.c).

246 3.2. Evolution of the natural behaviours of the time budget over the five periods

247 Intra-group comparisons showed that the percentage of scans for all the natural behaviours of the time
248 budget were significantly different between periods ($13.2 < \chi^2 (4) < 105.3, P < 0.05$ in all cases).
249 Details of the results of the statistical analyses for intra-group and inter-group comparisons for each of
250 these behaviours are presented in the supplementary materials (Table S3).

251

252 4. Discussion

253 When horses were on pasture, no stereotypies were observed and the occurrence of the “alert posture”,
254 indicating hypervigilance, decreased, although the results were not significant. The combined increase
255 in locomotion, exploration, resting and social behaviours confirms a beneficial effect of the pasture
256 environment on the expression of natural behaviours. However, the occurrence of the “withdrawn
257 posture” strongly increased during the five days following their release on pasture, indicating an
258 increase in unresponsiveness to the environment immediately after the environmental change and
259 suggesting that horses had some difficulties coping with their new living conditions. When returning
260 to individual boxes, a high occurrence of stereotypies and of the “withdrawn” and the “alert” postures
261 was observed. The expression of the natural behaviours immediately returned to the “Pre-pasture”
262 level, except for feeding behaviours that were lower during the first five days back in individual boxes.

263 All these results are supported both by intra-group (longitudinal study of the “Pasture” horses) and
264 inter-group comparisons (“Pasture” versus “Control” horses).

265

266 *Pasture improves the welfare state of horses despite a period of adaptation.*

267 Stereotypies were no longer observed when the horses were on pasture. This result is consistent with
268 numerous studies in horses and cattle, which observing a decrease in or a total absence of stereotypies
269 in animals that had access to pasture (Bachmann et al., 2003; Christie et al., 2006; Hockenull and
270 Creighton, 2014; Redbo, 1992, 1990; Topczewska, 2018). In the current study, this decrease was not
271 related to the fact that horses were prevented from expressing stereotypies due to the environment. For
272 example, repetitive licking could have been performed on the metal barriers, the fence posts or the
273 shelters present on pasture. The occurrence of the “alert posture” also decreased, although the
274 difference with the pre-pasture level was not significant. This result suggests that horses experienced
275 less hypervigilance on pasture than in individual boxes, despite the unfamiliar environmental and
276 social stimuli. The increase in locomotion, exploration, resting and social behaviours indicate a
277 beneficial effect of the pasture environment on the expression of natural behaviours, which probably
278 contributed to the decrease in the occurrence of stereotypies and hypervigilance. In addition, the
279 quality of the human-animal relationship seemed to improve after 20 days on pasture, as no horses
280 showed aggressiveness towards humans during the approach test. This result was observed despite the
281 slightly different way of assessing this behavioural indicator between boxes (scans) and pasture (test).
282 As aggressiveness may be related to a long-lasting negative affective state (Henry et al., 2017) and
283 could reflect motivation to escape from an aversive situation (i.e., human approach; Ödberg, 1987),
284 our result would be consistent with the study of Löckener et al., 2016, which demonstrated that horses
285 showed an affective improvement after 10 days on pasture following a period of confinement. Being
286 housed with congeners also positively influenced the aggressiveness of young horses during training,
287 as they were less likely to bite the trainer (Søndergaard and Ladewig, 2004). Altogether, the decrease
288 in the occurrence of the three indicators and the rise in several natural behaviours (locomotion,

289 exploration, resting and social interactions) suggest an improvement in the welfare state of horses on
290 pasture.

291 This suggestion should be qualified, as a strong increase in the occurrence of the “withdrawn posture”
292 during the first five days on pasture was also observed. However, the expression of this indicator
293 returned to the pre-pasture level after 20 days, indicating an improvement over time. It could be
294 assumed that this decrease would continue the longer the horses spent on pasture. The burst of
295 unresponsiveness to the environment in the first five days may indicate that the environmental change
296 from individual boxes to pasture with conspecifics was extreme and that certain animals needed time
297 to adapt. On pasture, the new environmental and social stimuli could overwhelm individuals’ coping
298 abilities, as suggested by Cooper and Albentosa (2005). In feral horses, grouping with unknown horses
299 is a stressful event, highlighted by an increase in faecal cortisol levels in individuals who voluntarily
300 join a new group (Nuñez et al., 2014). Furthermore, horses isolated for a long period may also present
301 inadequate social skills, as has been demonstrated in calves (Broom and Leaver, 1978). Horses which
302 were usually housed with conspecifics expressed approximately an equal proportion of positive and
303 negative social interactions when suddenly grouped with unfamiliar conspecifics (Christensen et al.,
304 2002a). However, in the current study, horses showed three to four times more negative than positive
305 interactions in a similar situation, suggesting altered social skills.

306 This study suggests that temporary release on pasture positively influences the welfare of horses which
307 usually live in individual boxes and enhances the expression of natural behaviours, but also that few
308 days of adaptation would be required. For this practice to be beneficial for the welfare of horses, the
309 minimum duration necessary should be 20 days, but further studies are needed to determine the
310 optimum period. One study has shown that the effect of individual housing on social behaviours of
311 horses could be observed up to six weeks following the release of animals to pasture (Christensen et
312 al., 2002b). It is thus possible that only very long durations on pasture could optimize the welfare state
313 of horses while countering the negative effects of the period of adaptation. Moreover, feeding and
314 lying behaviours have been found to decrease during the periods on pasture. This is probably linked to

315 insect harassment during the day, as increased insect-related behaviours have also been observed.
316 Protection against insects would reduce this discomfort when horses are on pasture in the summer.

317

318 *Deleterious short-term effects when horses returned to individual boxes.*

319 One of the noteworthy results of this study was the strong increase in the occurrence of stereotypies,
320 hypervigilance (“alert posture”) and unresponsiveness to the environment (“withdrawn posture”) immediately upon return to individual boxes. A drastic marked increase in the occurrence of
321 stereotypies has previously been observed in cattle moving from pasture to confinement (Redbo, 1992,
322 1990). In these studies, the stereotypies recorded were similar to those in our study which increased sharply after the period on pasture (i.e., repetitive tongue movements and licking). The author
323 suggested that these could be symptoms of feeding frustration related to the physiological and
324 behavioural need for prolonged fibre ingestion. Indeed, we observed that the “Pasture” horses ate less
325 than the “Control” horses for the first five days following their returned to individual boxes. It is also
326 likely that the rise of stereotypies reflected a high level of stress related to the novel situation (i.e.,
327 isolation, confinement) and indicate an attempt to cope with this situation (Broom, 2019; Cooper and
328 Albentosa, 2005; Redbo, 1992). The increased occurrence of hypervigilance measured in the present
329 study is similar to that observed in foals stabled for the first time (Visser et al., 2008). In addition, the
330 expression of locomotion, exploration, resting and social behaviours immediately returned to the “Pre-
331 pasture” level. Overall, the sharp rise in these three indicators combined with the decrease in
332 expression of natural behaviours suggest that the return to individual boxes had a deleterious effect on
333 the welfare state of horses. In addition, no benefits of pasture were observed on horses regarding the
334 quality of the human-animal relationship after their return to individual boxes. Indeed, the occurrence
335 of aggressive behaviours did not differ from the pre-pasture level. It is possible that animals associated
336 the box environment with past human-related and negatively perceived experiences. Indeed, horses
337 have several-month memory capacities of their relationship with humans (Lansade et al., 2018;
338 Sankey et al., 2010). It is likely that the constrained environment of the boxes, which prevents the
339 animals from escaping from humans, exacerbates the expression of this indicator. Three months after
340
341

342 pasture, the expression levels of the four behavioural indicators, and also the natural behaviours
343 mentioned above, including feeding behaviours, did not differ from those seen in the pre-pasture
344 period or from those of horses kept in individual boxes throughout the study. Thus, the effect of the
345 change in environment is transient.

346 All these results support the fact that a temporary period on pasture would not alleviate long-term
347 behavioural deprivation when horses return to individual boxes, and that the sudden environmental
348 change could even have harmful effects in the short term. As confinement has been implicated in
349 susceptibility to infections and the development of pre-pathological conditions in sheep and cattle
350 (Degabriele and Fell, 2001; Nakajima et al., 2018), it would be interesting to investigate physiological
351 and health measures to assess more effectively the cost of adaptive responses of usually-stabled horses
352 to such marked environmental challenges.

353 Regarding these results, it would therefore be recommended to maximise the time horses are kept on
354 pasture with conspecifics to avoid abrupt environmental changes. Individual boxes should only be
355 used occasionally, for example to isolate a sick animal. In cases where continuous housing on pasture
356 is not possible, one alternative could be to leave the horses outside during the night and kept them in
357 individual boxes during the day for care and availability for riders. This pattern was demonstrated to
358 impact positively welfare indicators expressed in boxes, such as stereotypies and “alert postures”
359 (Lansade et al., 2014) and the overall affective state of horses (Löckener et al., 2016). However,
360 further researches are needed to assess how horses cope with these regular environmental changes.

361

362 **5. Conclusion**

363 This study suggests that a temporary period on pasture improves the welfare state of usually-stabled
364 horses following a period of adaptation. However, sudden marked environmental changes can have
365 deleterious short-term effects, both when horses are released on pasture but more particularly when
366 they return to their individual boxes. In addition, the positive effects likely to be induced by the period

367 on pasture do not last when horses return to individual boxes. Considering these results, it is thus
368 recommended to maximise the time horses spend at pasture with conspecifics.

369

370 *Declarations of interest*

371 The authors have no conflicts of interest to report.

372

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381

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528

Figures

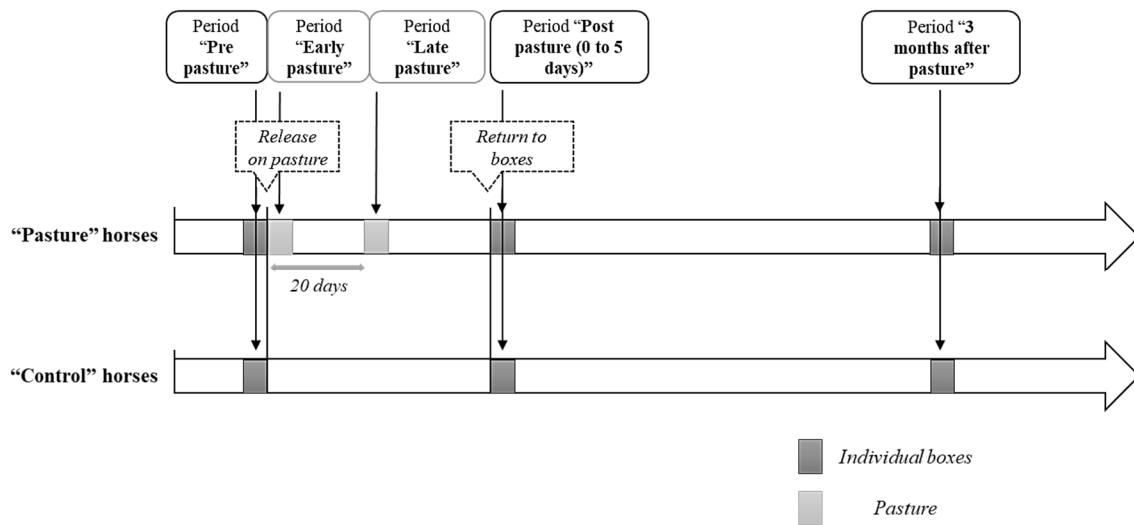


Figure 1 Time schedule. "Pre pasture": five days before pasture; "Early pasture": the first five days including the day of release after waiting for 2 hours; "Late pasture": five days after 20 days on pasture; "Post pasture (0 to 5 days)": the first five days immediately on the return in individual boxes after waiting for 2 hours; "3 months after pasture": five days after 3 months in individual boxes. The time spent on pasture was 41.7 ± 16.8 days (mean \pm SD).

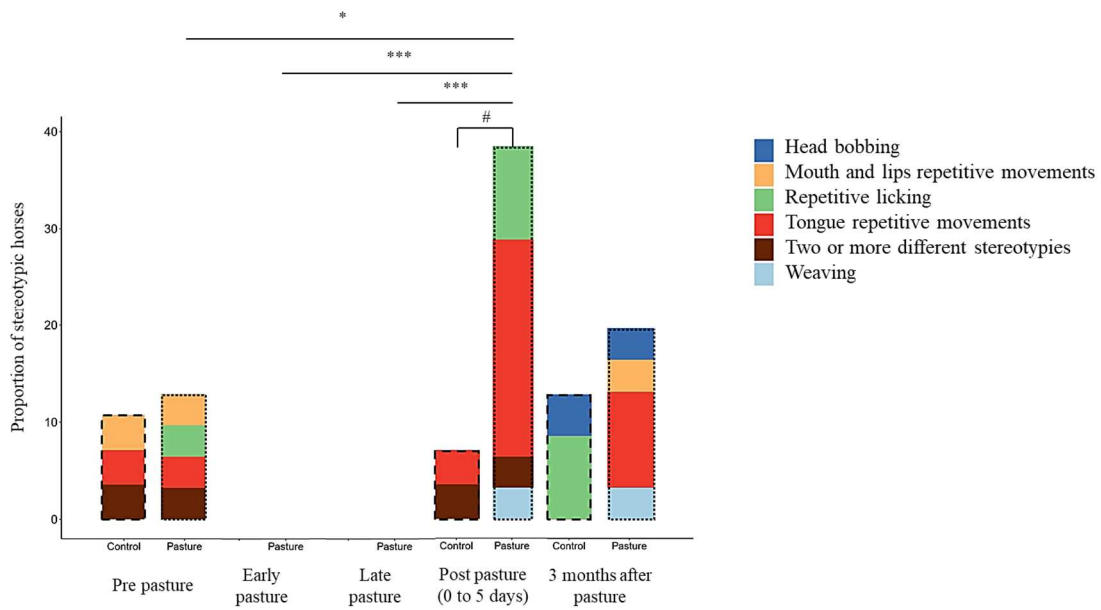


Figure 2 Proportion of stereotypic horses depending on the type of stereotypy performed and according to the five periods of observation for the “Control” (discontinuous lines) and the “Pasture” (dots) groups. Number of “Control” horses = 29. Number of “Pasture” horses = 31. No observations are available for “Control” horses in “Early pasture” and “Late pasture” periods as they were kept in individual boxes. Intra-group comparisons: Cochran’s *Q* test followed by Dunn tests with Bonferroni’s correction for pairwise comparisons. * $P \leq 0.05$; *** $P \leq 0.001$. Inter-group comparisons: χ^2 tests of homogeneity. # $P \leq 0.05$.

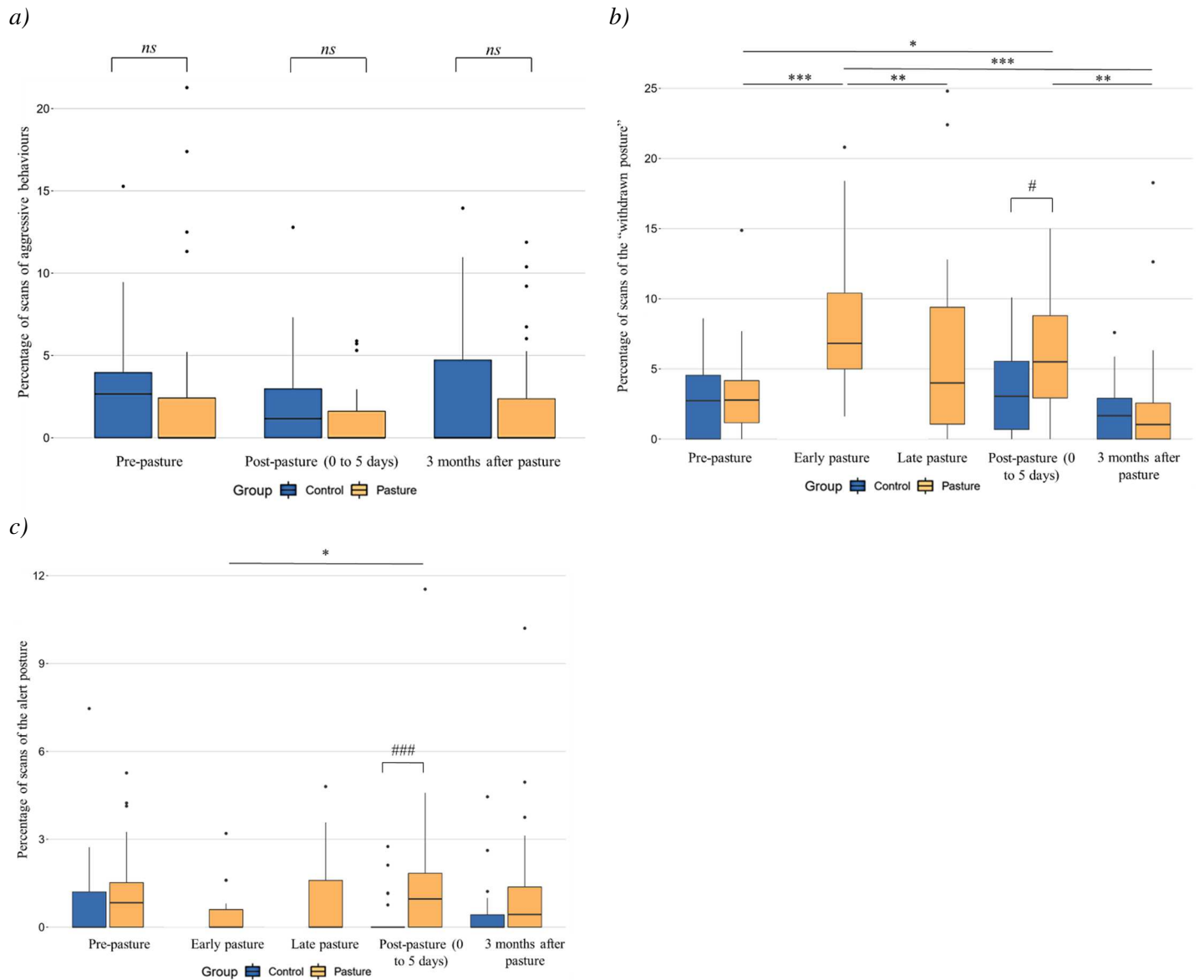


Figure 3 Percentage of scans of aggressive behaviours towards humans (a), the "withdrawn posture" (b) and the alert posture (c) according to the periods of observation in the "Control" group (blue) and the "Pasture" group (yellow). Number of "Control" horses = 29. Number of "Pasture" horses = 31. The boxplots represent the median (black line, located at 0 when not visible), 25 – 75 % quartiles and 95 % confidence intervals. No observations are available for "Control" horses in "Early pasture" and "Late pasture" periods as animals were kept in individual boxes. Intra-group comparisons: Friedman ANOVAs followed by Wilcoxon signed-rank test with Bonferroni's correction for pairwise comparisons. * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$. Inter-group comparisons: Wilcoxon rank-sum tests with continuity correction. # $P \leq 0.05$; ### $P \leq 0.001$; ns non-significant.

Tables

Table 1. Descriptions of the behavioural indicators reflecting a compromised mental welfare state in the sample observed through scan sampling or experimental tests (i.e., measures of aggressiveness towards humans on pasture).

<i>Behavioural indicator</i>	<i>Description</i>
Stereotypies	Head bobbing, weaving, repetitive licking, repetitive mouth and lip movements (e.g., clapping of lips), repetitive tongue movements
Aggressive behaviours	Simple threat (looking with ears pinned backward) Sustained threat (approaching with ears pinned backward and mouth open or turning hind quarters, sometimes raising a leg) Physical attack (bite or kick)
“Withdrawn posture” (Fureix et al., 2012)	Neck horizontal at same level as back, fixed stare, ears (mainly oriented backward) and head static, reflecting unresponsiveness to the environment
“Alert posture” (Young et al., 2012)	Elevated neck and ears pricked forward, looking intensely at the environment, reflecting hypervigilance

Table 2. Stereotypies performed among the “Pasture” group in “Pre pasture”, “Post pasture (0 to 5 days)” and “3 months after pasture” periods.

Horse	Pre pasture	Post pasture (0 to 5 days)	3 months after pasture
1	Tongue movements	Tongue movements	Tongue movements
2	Licking	-	-
3	Mouth and lips movements	-	Mouth and lips movements
4	Head bobbing + Tongue movements	Licking + Tongue movements	Tongue movements
5	-	Tongue movements	Tongue movements
6	-	Licking	-
7	-	Tongue movements	-
8	-	Licking	-
9	-	Tongue movements	-
10	-	Tongue movements	-
11	-	Tongue movements	-
12	-	Licking	-
13	-	Weaving	Weaving
14	-	Tongue movements	-
15	-	-	Head bobbing