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

Alice Ruet, Cécile Arnould, Justine Levray, Julie Lemarchand, Núria Mach, Marie-Pierre Moisan, Aline Foury, Christine Briant, Léa Lansade

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

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

[1] *INRAe, UMR 85 PRC, CNRS, UMR 7247, IFCE, University of Tours, 37380 Nouzilly, France.*
[2] *INRAe, UMR 1313 GABI, AgroParisTech, University of Paris-Saclay, 78352 Jouy-en-Josas, France.*
[3] *Univ. Bordeaux, INRAe, Bordeaux INP, NutriNeuro, UMR 1286, 33076 Bordeaux, France.*

* Corresponding author: alice.ruet@sfr.fr

Abstract

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behavioural indicators reflecting a compromised welfare state: stereotypies; aggressive behaviours towards humans; the "withdrawn posture" reflecting unresponsiveness to the environment; and the "alert posture" indicating hypervigilance.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 However, the expression of the "withdrawn posture" increased during the first five days on pasture (Friedman: $P 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

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

Keywords

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

1. Introduction

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

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

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

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

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

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

2. Materials and methods

2.1. Animals

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

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

2.2. Housing and management conditions

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

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

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 ± 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).

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

2.4. Statistical analyses

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

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

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

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

2.5. Ethics statement

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

3. Results

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

Stereotypies. Intra-group comparisons for the "Pasture" group showed that the proportion of horses expressing stereotypies significantly differed between periods ($Q\ (4) = 28.3$, $P < 0.001$). The proportion was higher in the "Post-pasture (0 to 5 days)" period than in the three preceding periods ("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 (χ^2 (1) = 3.9, P = 0.05). No
218 significant differences were observed in the "Pre-pasture" (P = 1) and "3 months after pasture" (χ^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 (χ^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 (χ^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 (χ^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.

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

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

Stereotypies were no longer observed when the horses were on pasture. This result is consistent with numerous studies in horses and cattle, which observing a decrease in or a total absence of stereotypies in animals that had access to pasture (Bachmann et al., 2003; Christie et al., 2006; Hockenhull and Creighton, 2014; Redbo, 1992, 1990; Topczewska, 2018). In the current study, this decrease was not related to the fact that horses were prevented from expressing stereotypies due to the environment. For example, repetitive licking could have been performed on the metal barriers, the fence posts or the shelters present on pasture. The occurrence of the "alert posture" also decreased, although the difference with the pre-pasture level was not significant. This result suggests that horses experienced less hypervigilance on pasture than in individual boxes, despite the unfamiliar environmental and social stimuli. The increase in locomotion, exploration, resting and social behaviours indicate a beneficial effect of the pasture environment on the expression of natural behaviours, which probably contributed to the decrease in the occurrence of stereotypies and hypervigilance. In addition, the quality of the human-animal relationship seemed to improve after 20 days on pasture, as no horses showed aggressiveness towards humans during the approach test. This result was observed despite the slightly different way of assessing this behavioural indicator between boxes (scans) and pasture (test). As aggressiveness may be related to a long-lasting negative affective state (Henry et al., 2017) and could reflect motivation to escape from an aversive situation (i.e., human approach; Ödberg, 1987), our result would be consistent with the study of Löckener et al., 2016, which demonstrated that horses showed an affective improvement after 10 days on pasture following a period of confinement. Being housed with congeners also positively influenced the aggressiveness of young horses during training, as they were less likely to bite the trainer (Søndergaard and Ladewig, 2004). Altogether, the decrease 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")
321 immediately upon return to individual boxes. A drastic marked increase in the occurrence of
322 stereotypies has previously been observed in cattle moving from pasture to confinement (Redbo, 1992,
323 1990). In these studies, the stereotypies recorded were similar to those in our study which increased
324 sharply after the period on pasture (i.e., repetitive tongue movements and licking). The author
325 suggested that these could be symptoms of feeding frustration related to the physiological and
326 behavioural need for prolonged fibre ingestion. Indeed, we observed that the "Pasture" horses ate less
327 than the "Control" horses for the first five days following their returned to individual boxes. It is also
328 likely that the rise of stereotypies reflected a high level of stress related to the novel situation (i.e.,
329 isolation, confinement) and indicate an attempt to cope with this situation (Broom, 2019; Cooper and
330 Albentosa, 2005; Redbo, 1992). The increased occurrence of hypervigilance measured in the present
331 study is similar to that observed in foals stabled for the first time (Visser et al., 2008). In addition, the
332 expression of locomotion, exploration, resting and social behaviours immediately returned to the "Pre-
333 pasture" level. Overall, the sharp rise in these three indicators combined with the decrease in
334 expression of natural behaviours suggest that the return to individual boxes had a deleterious effect on
335 the welfare state of horses. In addition, no benefits of pasture were observed on horses regarding the
336 quality of the human-animal relationship after their return to individual boxes. Indeed, the occurrence
337 of aggressive behaviours did not differ from the pre-pasture level. It is possible that animals associated
338 the box environment with past human-related and negatively perceived experiences. Indeed, horses
339 have several-month memory capacities of their relationship with humans (Lansade et al., 2018;
340 Sankey et al., 2010). It is likely that the constrained environment of the boxes, which prevents the
341 animals from escaping from humans, exacerbates the expression of this indicator. Three months after

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

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

Declarations of interest

The authors have no conflicts of interest to report.

Acknowledgments

This project was funded by the IFCE and the "Fonds Eperon". This funding source had no role in the study design, data collection and analysis or in the preparation and submission of the manuscript. The authors are very grateful to the staff of the French Horse and Riding Institute (IFCE) and INRA*e* (Nouzilly, France) and in particular to Patrick Galloux, Xavier Goupil, Isabelle Burgaud, Sophie Biau, Colonel Patrick Teisserenc and all the animal keepers for their collaboration during the experiment. We would like to thank the Springer Nature Author Services team and Sue Edrich from Interconnect for correcting the English manuscript.

References

Altmann, J., 1974. Observational Study of Behavior : Sampling. Behaviour 49, 227–267. https://doi.org/10.1080/14794802.2011.585831.

AWIN, 2015. AWIN welfare assessment protocol for horses. https://doi.org/10.13130/AWIN_HORSES_2015.

Bachmann, I., Bernasconi, P., Herrmann, R., Weishaupt, M.A., Stauffacher, M., 2003. Behavioural and physiological responses to an acute stressor in crib-biting and control horses. Appl. Anim. Behav. Sci. 82, 297–311. https://doi.org/10.1016/S0168-1591(03)00086-8.

Bøe, K.E., Færevik, G., 2003. Grouping and social preferences in calves, heifers and cows. Appl. Anim. Behav. Sci. 80, 175–190. https://doi.org/10.1016/S0168-1591(02)00217-4.

392 Broom, D.M., 2019. Abnormal behavior and the self-regulation of motivational state. J. Vet. Behav.
393 29, 1–3. https://doi.org/10.1016/j.jveb.2018.09.001.

394 Broom, D.M., Leaver, J.D., 1978. Effects of group-rearing or partial isolation on later social behaviour
395 of calves. Anim. Behav. 26, 1255–1263.

396 Carenzi, C., Verga, M., 2016. Animal welfare: review of the scientific concept and definition. Ital. J.
397 Anim. Sci. 8, 21–30. https://doi.org/10.4081/ijas.2009.s1.21.

398 Christensen, J.W., Ladewig, J., Søndergaard, E., Malmkvist, J., 2002a. Effects of individual versus
399 group stabling on social behaviour in domestic stallions. Appl. Anim. Behav. Sci. 75, 233–248.
400 https://doi.org/10.1016/S0168-1591(01)00196-4.

401 Christensen, J.W., Zharkikh, T., Ladewig, J., Yasinetskaya, N., 2002b. Social behaviour in stallion
402 groups (Equus przewalskii and Equus caballus) kept under natural and domestic conditions.
403 Appl. Anim. Behav. Sci. 76, 11–20. https://doi.org/10.1016/S0168-1591(01)00208-8.

404 Christie, J.L., Hewson, C.J., Riley, C.B., Mcniven, M.A., Dohoo, I.R., Bate, L.A., 2006. Management
405 factors affecting stereotypies and body condition score in nonracing horses in Prince Edward
406 Island. Can Vet J 47, 136–143.

407 Cooper, J.J., Albentosa, M.J., 2005. Behavioural adaptation in the domestic horse: potential role of
408 apparently abnormal responses including stereotypic behaviour. Livest. Prod. Sci. 92, 177–182.
409 https://doi.org/10.1016/j.livprodsci.2004.11.017.

410 Degabriele, R., Fell, L.R., 2001. Changes in behaviour, cortisol and lymphocyte types during isolation
411 and group confinement of sheep. Immunol. Cell Biol. 79, 583–589.
412 https://doi.org/10.1046/j.1440-1711.2001.01040.x.

413 Dellmeier, G.R., 1989. Motivation in relation to the welfare of enclosed livestock. Appl. Anim. Behav.
414 Sci. 22, 129–138. https://doi.org/10.1016/0168-1591(89)90049-X.

415 Enriquez-Hidalgo, D., Teixeira, D.L., Lewis, E., Buckley, F., Boyle, L., O'Driscoll, K., 2018.
416 Behavioural responses of pasture based dairy cows to short term management in tie-stalls. Appl.

417 Anim. Behav. Sci. 198, 19–26. https://doi.org/10.1016/j.applanim.2017.09.012.

418 Flauger, B., Krueger, K., 2013. Aggression level and enclosure size in horses (Equus caballus).
419 Pferdeheilkunde 29, 495–504. https://doi.org/10.21836/pem20130404.

420 Fraser, D., Duncan, I.J.H., 1998. “Pleasures”, “pains” and animal welfare: Toward a natural history of
421 affect. Anim. Welf. 7, 383–396.

422 Fureix, C., Beaulieu, C., Argaud, S., Rochais, C., Quinton, M., Henry, S., Hausberger, M., Mason, G.,
423 2015. Investigating anhedonia in a non-conventional species: Do some riding horses Equus
424 caballus display symptoms of depression? Appl. Anim. Behav. Sci. 162, 26–36.
425 https://doi.org/10.1016/j.applanim.2014.11.007.

426 Fureix, C., Jego, P., Henry, S., Lansade, L., Hausberger, M., 2012. Towards an ethological animal
427 model of depression? A study on horses. PLoS One 7, 1–9.
428 https://doi.org/10.1371/journal.pone.0039280.

429 Fureix, C., Menguy, H., Hausberger, M., 2010. Partners with bad temper: Reject or cure? a study of
430 chronic pain and aggression in horses. PLoS One 5(8).
431 https://doi.org/10.1371/journal.pone.0012434.

432 Hallam, S., Campbell, E.P., Qazamel, M., Owen, H., Ellis, A.D., 2012. Effects of traditional versus
433 novel feeding management on 24 hour time budget of stabled horses, in: Forages and Grazing in
434 Horse Nutrition. Wageningen Academic Publishers, Wageningen, pp. 319–321.
435 https://doi.org/10.3920/978-90-8686-755-4_39.

436 Hao, Y., Ge, H., Sun, M., Gao, Y., 2019. Selecting an appropriate animal model of depression. Int. J.
437 Mol. Sci. 20, 1–16. https://doi.org/10.3390/ijms20194827.

438 Hartmann, E., Søndergaard, E., Keeling, L.J., 2012. Keeping horses in groups: A review. Appl. Anim.
439 Behav. Sci. 136, 77–87. https://doi.org/10.1016/j.applanim.2011.10.004.

440 Henry, S., Fureix, C., Rowberry, R., Bateson, M., Hausberger, M., 2017. Do horses with poor welfare
441 show ‘pessimistic’ cognitive biases? Sci. Nat. 104, 8. https://doi.org/10.1007/s00114-016-1429-

442 1.

443 Higashiyama, Y., Nashiki, M., Narita, H., Kawasaki, M., 2007. A brief report on effects of transfer
444 from outdoor grazing to indoor tethering and back on urinary cortisol and behaviour in dairy
445 cattle. Appl. Anim. Behav. Sci. 102, 119–123. https://doi.org/10.1016/j.applanim.2006.03.007.

446 Hockenhull, J., Creighton, E., 2015. The day-to-day management of UK leisure horses and the
447 prevalence of owner-reported stable-related and handling behaviour problems. Anim. Welf. 24,
448 29–36. https://doi.org/10.7120/09627286.24.1.029.

449 Hockenhull, J., Creighton, E., 2014. Pre-feeding behaviour in UK leisure horses and associated
450 feeding routine risk factors. Anim. Welf. 23, 297–308.
451 https://doi.org/10.7120/09627286.23.3.297.

452 Houpt, K., Houpt, T.R., Johnson, J.L., Erb, H.N., Yeon, S.C., 2001. The effect of exercise deprivation
453 on the behaviour and physiology of straight stall confined pregnant mares. Anim. Welf. 10, 257–
454 267.

455 King, S.S., Jones, K.L., Schwarm, M., Oberhaus, E.L., 2013. Daily horse behavior patterns depend on
456 management. J. Equine Vet. Sci. 33, 365–366. https://doi.org/10.1016/j.jevs.2013.03.105.

457 Lansade, L., Nowak, R., Lainé, A.-L., Leterrier, C., Bonneau, C., Parias, C., Bertin, A., 2018. Facial
458 expression and oxytocin as possible markers of positive emotions in horses. Sci. Rep. 8, 14680.
459 https://doi.org/10.1038/s41598-018-32993-z.

460 Lansade, L., Valenchon, M., Foury, A., Neveux, C., Cole, S.W., Layé, S., Cardinaud, B., Lévy, F.,
461 Marie, M., Moisan, 2014. Behavioral and transcriptomic fingerprints of an enriched environment
462 in horses (Equus caballus). PLoS One 9, 1–19. https://doi.org/10.1371/journal.pone.0114384.

463 Leme, D.P., Parsekian, A.B.H., Kanaan, V., Hötzel, M.J., 2014. Management, health, and abnormal
464 behaviors of horses: a survey in small equestrian centers in Brazil. J. Vet. Behav. Clin. Appl.
465 Res. 9, 114–118. https://doi.org/10.1016/j.jveb.2014.01.004.

466 Löckener, S., Reese, S., Erhard, M., Wöhr, A.C., 2016. Pasturing in herds after housing in horseboxes

induces a positive cognitive bias in horses. J. Vet. Behav. Clin. Appl. Res. 11, 50–55. https://doi.org/10.1016/j.jveb.2015.11.005.

Mason, G.J., 1991. Stereotypies: a critical review. Anim. Behav. 41, 1015–1037. https://doi.org/10.1016/S0003-3472(05)80640-2.

McHugh, M.L., 2012. Interrater reliability : the kappa statistic. Biochem. Medica 22, 276–282.

Mellor, D.J., 2017. Operational details of the five domains model and its key applications to the assessment and management of animal welfare. Animals 7. https://doi.org/10.3390/ani7080060.

Nakajima, N., Doi, K., Tamiya, S., Yayota, M., 2018. Physiological, immunological, and behavioral responses in cows housed under confinement conditions after grazing. Livest. Sci. 218, 44–49. https://doi.org/10.1016/j.livsci.2018.10.011.

Nuñez, C.M.V., Adelman, J.S., Smith, J., Gesquiere, L.R., Rubenstein, D.I., 2014. Linking social environment and stress physiology in feral mares (Equus caballus): Group transfers elevate fecal cortisol levels. Gen. Comp. Endocrinol. 196, 26–33. https://doi.org/10.1016/j.ygcen.2013.11.012.

Ödberg, F.O., 1987. Chronic stress in riding horses. Equine Vet. J. 19, 268–269. https://doi.org/10.1111/j.2042-3306.1987.tb01402.x.

Panksepp, J., 2011. The basic emotional circuits of mammalian brains: Do animals have affective lives? Neurosci. Biobehav. Rev. 35, 1791–1804. https://doi.org/10.1016/j.neubiorev.2011.08.003.

Ransom, J.I., Cade, B.S., 2009. Quantifying equid behavior — A research ethogram for free-roaming feral horses, Geological Survey Techniques and Methods. Cambridge University Press, Cambridge. https://doi.org/10.1017/CBO9781107415324.004.

Redbo, I., 1992. The influence of restraint on the occurrence of oral stereotypies in dairy cows. Appl. Anim. Behav. Sci. 35, 115–123. https://doi.org/10.1016/0168-1591(92)90002-S.

Redbo, I., 1990. Changes in duration and frequency of stereotypies and their adjoining behaviours in heifers, before, during and after the grazing period. Appl. Anim. Behav. Sci. 26, 57–67.

492 https://doi.org/10.1016/0168-1591(90)90087-T.

493 Rochais, C., Henry, S., Hausberger, M., 2018. “Hay-bags” and “Slow feeders”: Testing their impact
494 on horse behaviour and welfare. Appl. Anim. Behav. Sci. 198, 52–59.
495 https://doi.org/10.1016/j.applanim.2017.09.019.

496 Ruet, A., Lemarchand, J., Parias, C., Mach, N., Moisan, M., Foury, A., Briant, C., Lansade, L., 2019.
497 Housing Horses in Individual Boxes Is a Challenge with Regard to Welfare. Animals 9, 621.
498 https://doi.org/10.3390/ani9090621.

499 Salomons, A.R., Arndt, S.S., Ohl, F., 2009. Anxiety in relation to animal environment and welfare.
500 Scand. J. Lab. Anim. Sci. 36, 37–45.

501 Sankey, C., Richard-Yris, M.A., Henry, S., Fureix, C., Nassur, F., Hausberger, M., 2010.
502 Reinforcement as a mediator of the perception of humans by horses (Equus caballus). Anim.
503 Cogn. 13, 753–764. https://doi.org/10.1007/s10071-010-0326-9.

504 Sarrafchi, A., Blokhuis, H.J., 2013. Equine stereotypic behaviors: Causation, occurrence, and
505 prevention. J. Vet. Behav. Clin. Appl. Res. 8, 386–394.
506 https://doi.org/10.1016/j.jveb.2013.04.068.

507 Søndergaard, E., Ladewig, J., 2004. Group housing exerts a positive effect on the behaviour of young
508 horses during training. Appl. Anim. Behav. Sci. 87, 105–118.
509 https://doi.org/10.1016/j.applanim.2003.12.010.

510 Souris, A.C., Kaczensky, P., Julliard, R., Walzer, C., 2007. Time budget-, behavioral synchrony- and
511 body score development of a newly released Przewalski’s horse group Equus ferus przewalskii,
512 in the Great Gobi B strictly protected area in SW Mongolia. Appl. Anim. Behav. Sci. 107, 307–
513 321. https://doi.org/10.1016/j.applanim.2006.09.023.

514 Sylvers, P., Lilienfeld, S.O., LaPrairie, J.L., 2011. Differences between trait fear and trait anxiety:
515 Implications for psychopathology. Clin. Psychol. Rev. 31, 122–137.
516 https://doi.org/10.1016/j.cpr.2010.08.004.

517 Topczewska, J., 2018. An attempt to assess the welfare of horses maintained in herd systems. Arch.
518 Anim. Breed. 57, 1–9. https://doi.org/10.7482/0003-9438-57-024.

519 Van Dierendonck, M.C., Bandi, N., Batdorj, D., Dügerlham, S., Munkhtsog, B., 1996. Behavioural
520 observations of reintroduced Takhi or Przewalski horses (Equus ferus przewalskii) in Mongolia.
521 Appl. Anim. Behav. Sci. 50, 95–114. https://doi.org/10.1016/0168-1591(96)01089-1.

522 Visser, E.K., Ellis, A.D., Van Reenen, C.G., 2008. The effect of two different housing conditions on
523 the welfare of young horses stabled for the first time. Appl. Anim. Behav. Sci. 114, 521–533.
524 https://doi.org/10.1016/j.applanim.2008.03.003.

525 Yngvesson, J., Rey Torres, J., Lindholm, J., Pättiniemi, A., Andersson, P., Sassner, H., 2019. Health
526 and Body Conditions of Riding School Horses Housed in Groups or Kept in Conventional Tie-
527 Stall/Box Housing. Animals 9, 73. https://doi.org/10.3390/ani9030073.

528

Figures

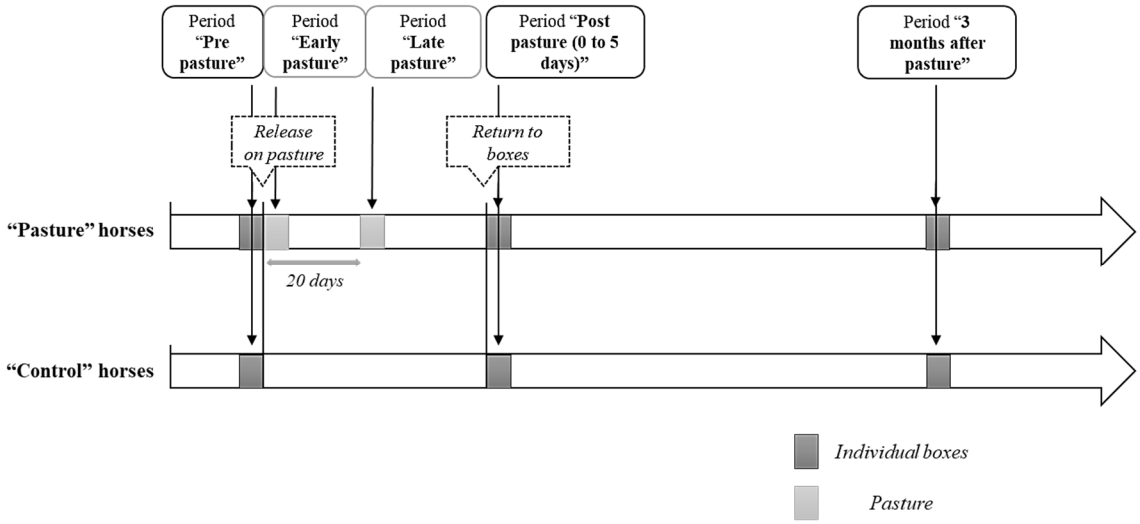

Figure 1 Time schedule. "Pre pasture": five days before pasture; "Early pasture": the first five days including the day of release after waiting for 2 hours; "Late pasture": five days after 20 days on pasture; "Post pasture (0 to 5 days)": the first five days immediately on the return in individual boxes 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 ± SD).

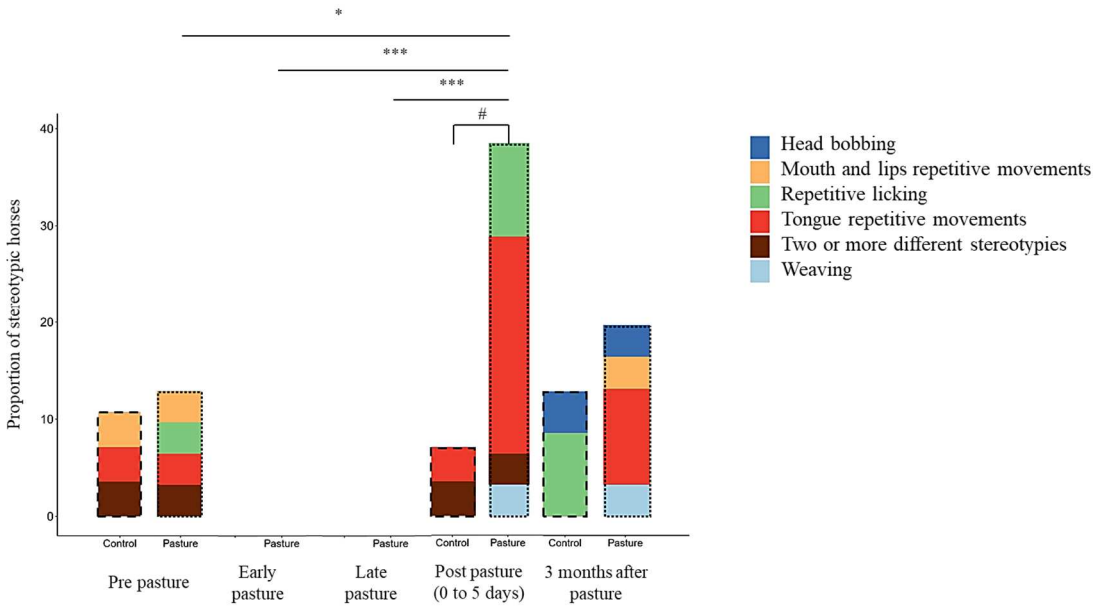

*Figure 2 Proportion of stereotypic horses depending on the type of stereotypy performed and according to the five periods of observation for the "Control" (discontinuous lines) and the "Pasture" (dots) groups. Number of "Control" horses = 29. Number of "Pasture" horses = 31. No observations are available 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: Chi² tests of homogeneity. # $P \leq 0.05$.*

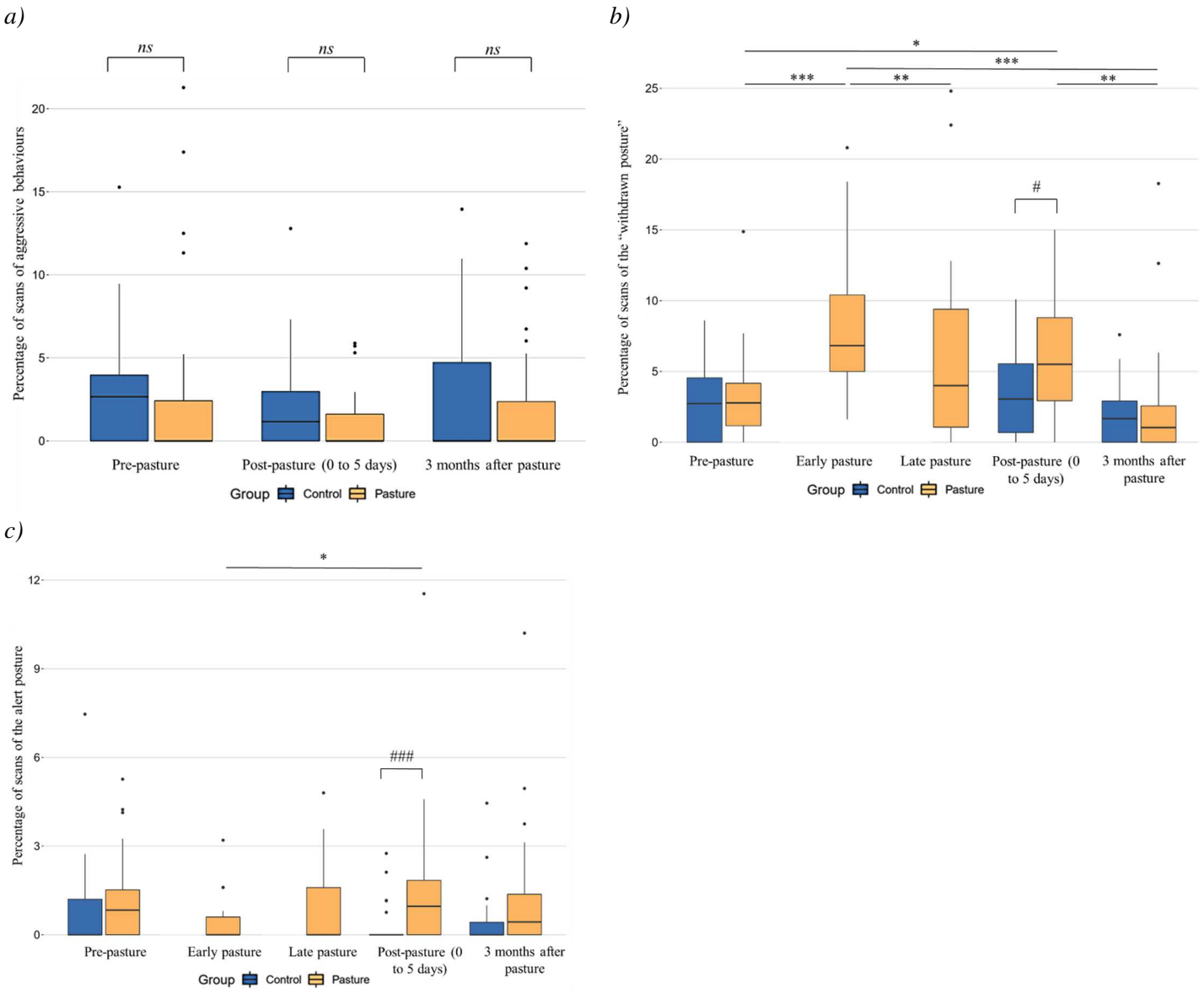

*Figure 3 Percentage of scans of aggressive behaviours towards humans (a), the "withdrawn posture" (b) and the alert posture (c) according to the periods of observation in the "Control" group (blue) and the "Pasture" group (yellow). Number of "Control" horses = 29. Number of "Pasture" horses = 31. The 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 ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001. Inter-group comparisons: Wilcoxon rank-sum tests with continuity correction. # P ≤ 0.05; ### P ≤ 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).

Behavioural indicator	***Description***
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