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Events around weaning in semi-feral and stable-reared Konik polski foals: Evaluation of short-term physiological and behavioural responses

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ABSTRACT

The annual weaning, stabling and subsequent sale of semi-feral Konik polski foals born in forest reserves ensure a stable population of free-roaming Konik polski horses. However, welfare concerns regarding the maternal and herd separation of young horses, manipulation by humans, transportation and stabling of forest-reared Koniks have arisen. The aim of the present study was to examine the physiological and behavioural responses of weaned, 7-to-9-month-old forest-reared Konik polski foals (FR, $N = 26$) relative to those of stable-reared peers (SR, $N = 27$). Fifty-three weanlings (24 colts and 29 fillies) were studied. FR foals from five distinct herds were captured and transported and stabled together in loose-housing stables (FR weaning scheme). Three weeks later, SR foals were weaned by separating them from their dams and joining them with FR foals (SR weaning scheme). Faecal cortisol metabolites (FCM), which reflect adrenocortical activity on the day preceding weaning (WD − 1) and on the day of weaning (WD), were analysed to evaluate the effect of weaning on FCM concentrations. The time budget over the 6 h following weaning on the day of weaning (WD) and one day post-weaning (WD + 1) was recorded.

Weaning caused a significant increase ($P < 0.0001$) of adrenocortical activity in all horses. Sex ($P = 0.0064$), but not rearing condition ($P = 0.1542$) had an effect on FCM concentrations. Differences between FR and SR foals in terms of their behavioural responses to the two weaning schemes were observed, FR foals exhibiting less outward arousal (lower social and locomotor activity, less vocalisation) compared to SR foals. On WD + 1, both groups tended to adapt to the post-weaning scheme. However, FR foals still stood for longer periods than their SR peers and presented less alert behaviour and lower locomotor and social activity. Increased FCM levels on weaning day were correlated with longer periods of standing ($r_s = 0.32$, $P = 0.0149$) and shorter feeding bouts ($r_s = -0.42$, $P = 0.0017$).

It seems that forest foals were more disturbed by all events around weaning. They presented higher apathy and more forest horses were among those showing higher FCM concentrations. However, as the FCM did not differed significantly between groups from

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different rearing conditions, the claim of a higher "stress" response to the weaning scheme in forest foals cannot be unequivocally supported based on behavioural and physiological measures in our study. For both groups, feeding behaviour appears to be a good indicator of post-weaning stress.

1. Introduction

Over the course of human history, horses, primarily considered an important source of food (Levine, 2005), were gradually domesticated to provide power for work and enable quick transport over long distances. It is believed that the direct ancestors of present domestic horses are extinct, most likely due to human predation (Goodwin, 2002). In addition to horses that are bred and widely used for various activities, local free-roaming populations of horses have formed. These horses are generally very well adapted to the environments they inhabit, which are often restricted areas of islands. In the face of progressive decreases in the number of natural predators with the spread of civilisation or by the inadvertent introduction of animals into environments to which they are not naturally adapted, as in the case of Australian brumbies (Csurhes et al., 2009; Dawson and Hone, 2012), the uncontrolled reproduction of free-roaming horses has become a serious problem. Indeed, the overpopulation of feral horses may gravely jeopardise the biological balance of different environments (Ostermann-Kelm et al., 2009). Moreover, welfare problems may emerge due to periodical starvation or prolonged thirst in feral equines.

The Konik polski is a Polish breed that was back-selected from primitive horses to restore the wild-type conformation, coat and behaviour of wild Tarpan horses, crossed in XVII century with local equine populations (Jezierski and Jaworski, 2008). A number of Koniks are maintained under semi-feral conditions, both in Poland and in several wildlife sanctuaries in Europe (e.g. Konik-crosses: Dülmen, Germany; Oostvaarderplaasen, the Netherlands). In contrast to the free-roaming population in the Netherlands, where the progeny of 20 Koniks imported from Poland in 1985 amounted to approximately 1000 individuals (Jezierski, personal communication), the Polish population of free-living Konik horses is maintained at a stable level by the annual weaning of foals. The stabling, taming, broking and subsequent sale of forest-reared weanlings ensure a sustainable surplus forest horses. Only one foal every few years is left in the forest to replace its dam or sire, which prevents population overgrowth and ensures the biodiversity and environmental sustainability of the lands used by the horses.

The abrupt weaning of semi-feral Konik foals from their dams, handling by humans during capture and transportation and environmental changes (stable vs. prior forest reserve conditions) represent challenging situations for young horses and may compromise their welfare. In the horse-breeding industry, weaning is a routine procedure used to manage youngstock, and a number of studies have investigated the stressful effects of this procedure on stabled horses (for review see Waran et al., 2008). In our previous study (Jezierski et al., 1999), we observed that stable-born horses scored better in terms of manageability and demonstrated lower heart rates than individuals born in free-roaming herds, although they were held in a conventional stable with stable-born group mates after weaning. However, it is not known whether weaning itself and initial handling by humans are more stressful for horses that have been living for 10 generations as a semi-feral, free-roaming population than in horses of the same genetic background but that have been living for many generations under conventional stable conditions and are accustomed to the presence of humans. According to the best of our knowledge, no scientific report on behavioural and physiological responses to human handling during events around weaning in feral horses was published hitherto and such information could be referential for other feral equine populations.

Maternal separation, mixing with new pen mates, placing in novel pens etc., all which take place around weaning, are assumed to provoke "weaning stress" (Weary et al., 2008). There is no consensus on the definition of "stress" (Koolhaas et al., 2011). However, the most often used nomenclature defines environmental stimuli threatening homeostasis as "stressors" and the resulting defence responses (behavioural and/or physiological) of an animal as "stress responses" (Möstl and Palme, 2002). The incidence or latency of particular behaviours or behavioural scores were assumed to be indicative for the stress of weanlings in numerous studies (Heleski et al., 2002; Henry et al., 2012; Hoffman et al., 1995; McCall et al., 1985, 1987; Malinowski et al., 1989; Moons et al., 2005) and some behaviours were reported to correlate with the change in adrenocortical response to exogenous adrenocorticotropic hormone (ACTH, Hoffman et al., 1995; McCall et al., 1987). The neuroendocrine system is activated during exposure to aversive situations, resulting in the secretion of cortisol, which is traditionally measured in blood samples as a physiological parameter of stress (Mormède et al., 2007). In the case of untamed, feral animals, especially when sampling itself may increase defence reactions, non-invasive methods such as measuring faecal cortisol metabolites are better suited (Möstl and Palme, 2002; Sheriff et al., 2011). It has been proven that faecal cortisol metabolites (FCM) parallel those of cortisol in the blood (Möstl et al., 1999; Palme et al., 1999) and provide a useful measure of adrenocortical activity (Palme, 2012).

Behavioural and physiological responses of stabled weanlings are relatively well known thanks to numerous studies (Heleski et al., 2002; Henry et al., 2012; Hoffman et al., 1995; McCall et al., 1985, 1987; Malinowski et al., 1989; Moons et al., 2005). Forest-reared foals have limited contact with humans, no experience with restraint and freedom of movement. On the day of weaning, they undergo maternal separation, restraint, transportation and stabling. Our study on feral horses would thus contribute to the on-going discussion on welfare of horses kept in different housing systems.

The aim of our study was to evaluate physiological and behavioural reactions of weaned Konik polski foals reared in a forest reserve and compare them to those of stable-reared peers weaned in the traditional manner. As "weaning" we define the husbandry procedure related to all events following mare-foal separation, i.e. social and environmental challenges for weanlings in new environment with unknown pen-mates. We attempted to answer the following questions: (1) what are the physiological (changes in concentrations of faecal cortisol metabolites) and behavioural (occurrence/latency of particular behaviours) responses to the events around weaning in forest-reared foals as compared to stable-reared mates? And (2) what weanling behaviour is best related to the levels of cortisol metabolites in the faeces?

2. Materials and methods

All procedures were accepted by the 3rd Local Commission for Ethics in Animal Experimentation, Warsaw, Poland. No other than routine horse management procedures were applied.

2.1. Animals

Fifty-three Konik polski horses, born in 2010 and 2011 (pooled, 24 colts and 29 fillies) at the Research Station of Polish Academy of Sciences, located in northeastern Poland, were used in the study. For more than 10 generations, the breeding stock was maintained concurrently in two keeping systems – in a forest reserve in the form of semi-feral familial groups and in traditional stables, where horses had daily access to paddocks. Seasonally, stabled horses were pastured in close proximity to the forest reserve.

The forest-reared foals (FR, $N = 26$) were born in five different herds consisting of one stallion and one to seven adult mares. All herds were dispersed on 1600 ha of coniferous and partly deciduous forest located on a peninsula surrounded by three lakes. The horses only occasionally met in the forest, and no interactions between foals were noticed. No human intervention activities other than the annual withdrawing of the offspring, winter hay feeding during deep snow cover and herds monitoring every second day were carried out.

The stable-reared foals (SR, $N = 27$) were born in dams' boxes and reared in a traditional manner. The foals were turned out with mares on a paddock or pasture according to weather conditions, from one to few hours after birth to 10–24 h daily in the summer.

SR foals were subjected to daily contact with caretakers during feeding, displacing, watering and, from 3 months of age, hoof trimming. At 6 months of age, the foals were marked with microchips. Approximately one month before weaning, the foals were tied during feeding. The stables, paddocks and surrounding pastures for mares with foals were frequently visited by tourists; thus, the foals were accustomed to human contact and occasional petting. At the time of weaning, the foals ranged from seven to nine months of age (median 7.5).

2.2. Weaning procedure

The weaning and sampling protocols are shown in Fig. 1. The FR foals (FR weaning scheme) were weaned in winter (first half of January) when snow covered the forest. Two to three weeks before the weaning, the herds were gradually habituated to enter enclosures located throughout the forest to feed on hay. On the weaning day, the herds were enclosed in the enclosures and weanlings were separated from adult horses using the system of poles. Then, the foals were individually confined in the squeeze chute, being a part of enclosure's construction. They were fitted with different coloured halters and marked on the back with individual numbers using cattle markers. The foals were then loaded in pairs into a trailer and transported for 25–40 min (depending to the location of the enclosure with chute within the forest reserve) and placed in loose stables with deep straw bedding. The foals from different natal groups were placed together (5–8 weanlings) in 2 pens (from 25 to 40 m^2) and provided with hay, water and salt blocks ad libitum. Over the next few days, the foals were turned out in one group from 8:00 to 13:00 and upon their return to the stable were provided with oats. They were trained to accept human touch, to be tied during feeding and to follow human commands when moving to the paddocks. Basing on long-term experience, three weeks was the standard period when the FR foals reach a level of manageability for that stable-reared group, already accustomed to tying and moving by humans, could be safely introduced to FR group (Fig. 1).

Three weeks after weaning, FR foals were separated into sex groups and placed in new pens. On the same day, the SR foals were fitted with halters of different colours, marked with cattle markers and turned out in one group without their dams. Using the paddock system, SR weanlings were placed in the stable in four pens with FR foals (SR weaning scheme) according to their sex (two pens for colts and two pens for fillies). Over next days, all FR and SR foals were turned out together in sex groups. The weaning protocol assured similar social facilitation for FR and SR foals, as FR foals were familiar with the foals from their natal groups, but unfamiliar with the foals from other herds, and SR foals were familiar with each other, but unfamiliar with FR foals on the time of placement in common pens.

Thus, the weanlings were submitted to two weaning schemes differing in that FR foals were captured, manipulated before the transportation and were transported to the stable. Both groups were submitted similarly to maternal separation, social challenge (new pen mates: for FR those were foals from other herds and for SR those were FR weanlings) and environmental novelty (for FR foals this was a building; for SR foals this was a new pen).

2.3. Sampling of faeces and analysis of cortisol metabolites

Considering the feasibility of taking biological samples from untamed horses, a non-invasive method based on the

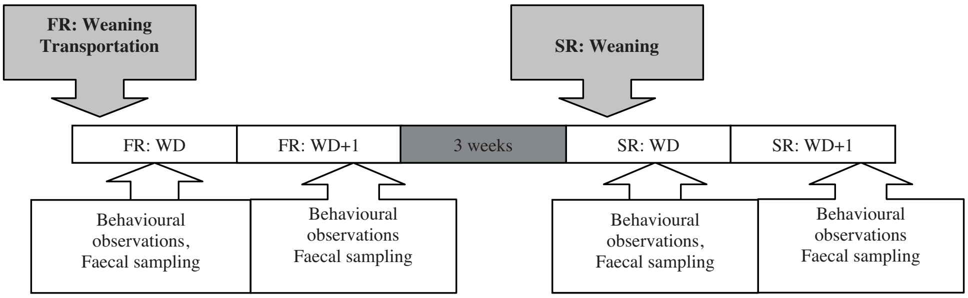

Fig. 1. Weaning and sampling schemes in forest-reared (FR) and stable-reared (SR) foals. WD: weaning day; WD – 1: pre-weaning day; WD + 1: post-weaning day.

measurement of glucocorticoids in faeces (faecal cortisol metabolites; FCM) was chosen to assess the physiological effects of weaning (Palme, 2012). The same method was used in SR horses for comparison. Assuming that FCM levels in horses reflect blood cortisol levels with a delay of 24 h (Möstl et al., 1999), faecal samples were taken on the day of weaning (FCM reflecting plasma adrenocortical activity the day before weaning (WD – 1, Fig. 1). FCM concentrations on WD – 1 were assumed as baseline levels. About 24 h later, on the day after weaning FCM levels reflected adrenocortical activity on the weaning day (WD). All samples were stored at −18 °C prior to being sent to the laboratory.

Faecal samples were extracted as described in detail elsewhere (Merl et al., 2000; Palme et al., 2013) and FCM levels were measured with a group-specific enzyme immunoassay (11-oxoaetiocholanolone EIA; for details, see Möstl et al. (1999) and Palme and Möstl (1997).

2.4. Behavioural observations

For FR foals, the observations began when weanlings transported from the forest were placed in the stable (approximately 4 p.m.). For SR horses, the observations began after the final placement of the horses in pens with FR foals (at the same time of day as for FR foals, Fig. 1). Because behavioural responses to the new conditions are expressed predominantly upon first contact and over the following hours (Hoffman et al., 1995; Nicol et al., 2005), the first six hours of the foals' stay in the new environment were observed. For this reason FR foals were not observed when SR weanlings were placed together with FR pen mates.

One of six experienced observers trained to use an equine ethogram (Appendix 1) remained immobile and quiet in the corner of each pen (foals from each pen were observed at the same time). The ethogram covered typical behaviours of loose stabled horses, involving locomotor activity, feeding, resting, exploration, eliminative behaviour and social interactions. Maintenance behaviours relative to body care (scratching) were classified in one group as they are generally related to the comfort of the animal (Fraser, 1992). Observers alternately (shifts every two or three hours) registered the behaviour of the horses from 4:00 to 10:00 pm. The behaviour of each horse in a pen was registered by instantaneous sampling every 5 s for 10 min of each of 6 consecutive hours, which resulted in one hour of observation per horse on the weaning day (WD). The observations were repeated the next day (post-weaning day, WD + 1) within the same time period, yielding a total of 720 samples per horse per day.

2.5. Statistical analyses

2.5.1. Faecal cortisol metabolites

We compared the concentrations of FCM related to WD – 1 and WD to examine the effect of weaning on adrenocortical activity. FCM levels (ng/g faeces) were log transformed using the natural logarithm. The effects of the day (WD – 1 and WD), rearing conditions, sex and the interactions between rearing conditions and sex on FCM levels were examined using PROC MIXED (SAS System 9.3), with individual horse incorporated as a random effect. The results are presented as means and standard deviations.

2.5.2. Time budget

First of all, we wanted to compare behaviours of FR and SR foals immediately after weaning. In order to see any potential adaptation to new conditions, we compared the occurrence of these behaviours on the day of weaning (WD) and on the post weaning day (WD + 1).

Behavioural observations were totalled for each behavioural category for all six hours for WD and WD + 1 separately. Each behaviour was characterised by its number of occurrences per 720 samples taken for each horse. The raw numbers were transformed into scores (Martin and Bateson, 1993) and, for clarity, were multiplied by 100; thus, the time budget was represented as the percentage of occurrence of each behaviour. Except for vocalisation, the short-lasting and infrequent behaviours (drinking, licking salt, urination, defecation, pawing, trotting and yawning) were not further analysed. The differences in the percentage of the occurrence of each behaviour between WD and WD + 1 were analysed using the Sign Rank test of PROC UNIVARIATE. The effect of rearing conditions and sex on each behavioural category recorded on WD and WD + 1 day was calculated using the Wilcoxon two-sample test within the NPAR1WAY procedure. The results (% of occurrence) are presented in means ± standard deviation.

2.5.3. Relationship between behaviour and FCM level

To examine which behaviours on WD were associated to the FCM levels related to the weaning day, non-parametric Spearman correlations were performed using the PROC

Table 1
The effects of rearing conditions and sex of foals on FCM levels (ng/g of faeces; means ± SD) related to WD and WD − 1.

	WD − 1				WD			
Rearing conditions	Stable		Forest		Stable		Forest	
Sex	Colts $N = 11$	Fillies $N = 16$	Colts $N = 13$	Fillies $N = 13$	Colts $N = 11$	Fillies $N = 16$	Colts $N = 13$	Fillies $N = 13$
	6.90 ± 2.70	5.72 ± 1.42A	6.20 ± 2.10	5.22 ± 2.08A	6.95 ± 1.23	8.62 ± 1.54B	8.10 ± 3.36	10.74 ± 5.65B
	WD − 1				WD			
Total for rearing conditions	Stable $N = 27$		Forest $N = 26$		Stable $N = 27$		Forest $N = 26$	
	6.20 ± 2.08A		5.75 ± 2.11A		7.95 ± 1.23B		9.33 ± 4.66B	
	WD − 1				WD			
Total for sex	Colts $N = 24$		Fillies $N = 29$		Colts $N = 24$		Fillies $N = 29$	
	6.51 ± 2.35a		5.50 ± 1.72Ab		7.60 ± 2.66b		9.53 ± 3.93aB	
	WD − 1 $N = 53$				WD $N = 53$			
Grand total	5.98 ± 2.08A				8.62 ± 3.50B			

FCM – faecal cortisol metabolites related to WD − 1: pre-weaning day and WD: weaning day, A, B – values with different letters in rows differ at $P < 0.01$.
a, b – values with different letters in rows differ at $P < 0.05$.
Comparisons between sexes between WD − 1 and WD (e.g. between colts on WD − 1 and fillies on WD) were omitted.

CORR procedure for all horses as well as for rearing conditions and sex groups separately.

To answer the question which behaviour of the horse reflects the physiological response to weaning best, the studied population was divided in four groups based on their relative FCM levels in the interquartile intervals (IQI: <Q3; Q3–Q2; Q2–Q1, >Q1). The behaviours of horses significantly differing in the occurrence between IQI were classified as the candidate markers for the behavioural profile of less or more “stressed” weanlings. To enable standardisation of the profile, the results are presented in mean rank scores (Wilcoxon two-sample test). Within each IQI, the distribution of individuals according to the rearing place and sex was tested with Chi-square test (PROC FREQ).

3. Results

3.1. Faecal cortisol metabolites

A significant increase in FCM levels in all horses after weaning was observed ($P < 0.0001$, Table 1), but FR foals did not differ from SR mates ($P = 0.1542$). However, an effect of the sex was observed ($P = 0.0064$). Before the weaning, FCM levels tended to be higher in colts than in fillies ($P = 0.0543$), but on WD the opposite was observed ($P = 0.0506$). Thus, FCM levels increased more expressed in fillies compared to colts ($P < 0.001$ vs. $P = 0.0493$).

3.2. Time budget

For all animals the adaptation to new conditions could be seen, as the occurrence of 7 out of 13 recorded behaviours decreased significantly on the post-weaning day (WD + 1) compared to that on the weaning day (Fig. 2). These behaviours included stepping ($P = 0.006$), walking ($P < 0.0001$), sniffing the surroundings ($P < 0.0001$), adopting an alert posture ($P < 0.0001$), neutral interactions with pen-mates ($P < 0.0001$), agonistic interactions with pen-mates ($P = 0.0404$) and vocalisation ($P < 0.0001$). In turn, behaviours indicative for resuming normal functioning and the relaxation increased in duration on the post-weaning day. Those included lying ($P < 0.0001$) and resting (standing) ($P < 0.0001$, Fig. 2). The durations of feeding and standing in a non-resting position did not differ significantly between WD and WD + 1 (Fig. 2).

3.3. Effect of the rearing condition

FR foals showed a different behavioural repertoire as the SR weanlings. On the weaning day, longer episodes of standing ($P = 0.0013$) and standing in a relaxed position (resting standing, $P = 0.041$) were observed in FR foals than in SR foals, as the SR foals were more active than the FR foals (Fig. 3). The SR foals walked more frequently ($P < 0.0001$), sniffed their surroundings more ($P < 0.0001$), adopted an alert position for longer periods ($P < 0.0001$) and engaged in neutral ($P < 0.0001$) and agonistic ($P = 0.002$) interactions with their pen-mates more often (Fig. 3).

On the post-weaning day, the SR foals still stood in a non-relaxed position less often than their FR peers ($P = 0.0004$) and exhibited alert behaviour ($P = 0.0013$), vocalised ($P = 0.0099$), walked ($P < 0.0001$) and socialised (neutral interactions; $P = 0.0058$, Fig. 3) more often.

3.4. Effect of sex

On the weaning day, fillies seemed to be more active than colts as they walked and vocalised more often ($P = 0.0405$ for walking and $P = 0.0018$ for vocalisation, Fig. 4). On the post-weaning day, the colts sniffed their

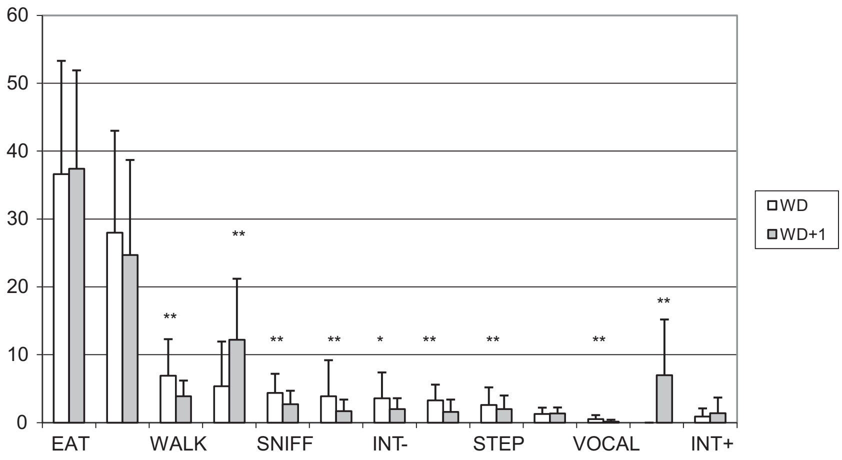

Fig. 2. Behaviours of all foals on weaning and post-weaning days.

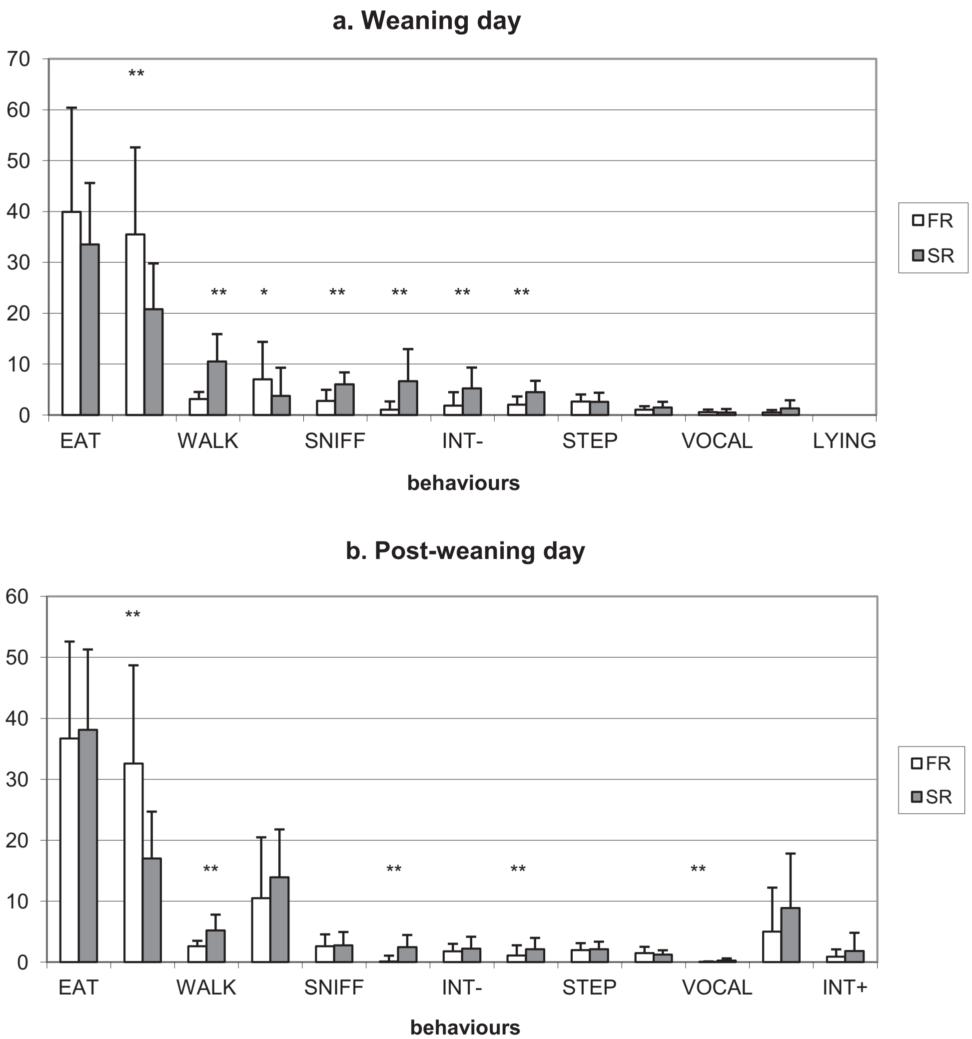

Fig. 3. Behaviours of forest-reared (FR) and stable-reared (SR) foals on weaning and post-weaning days.

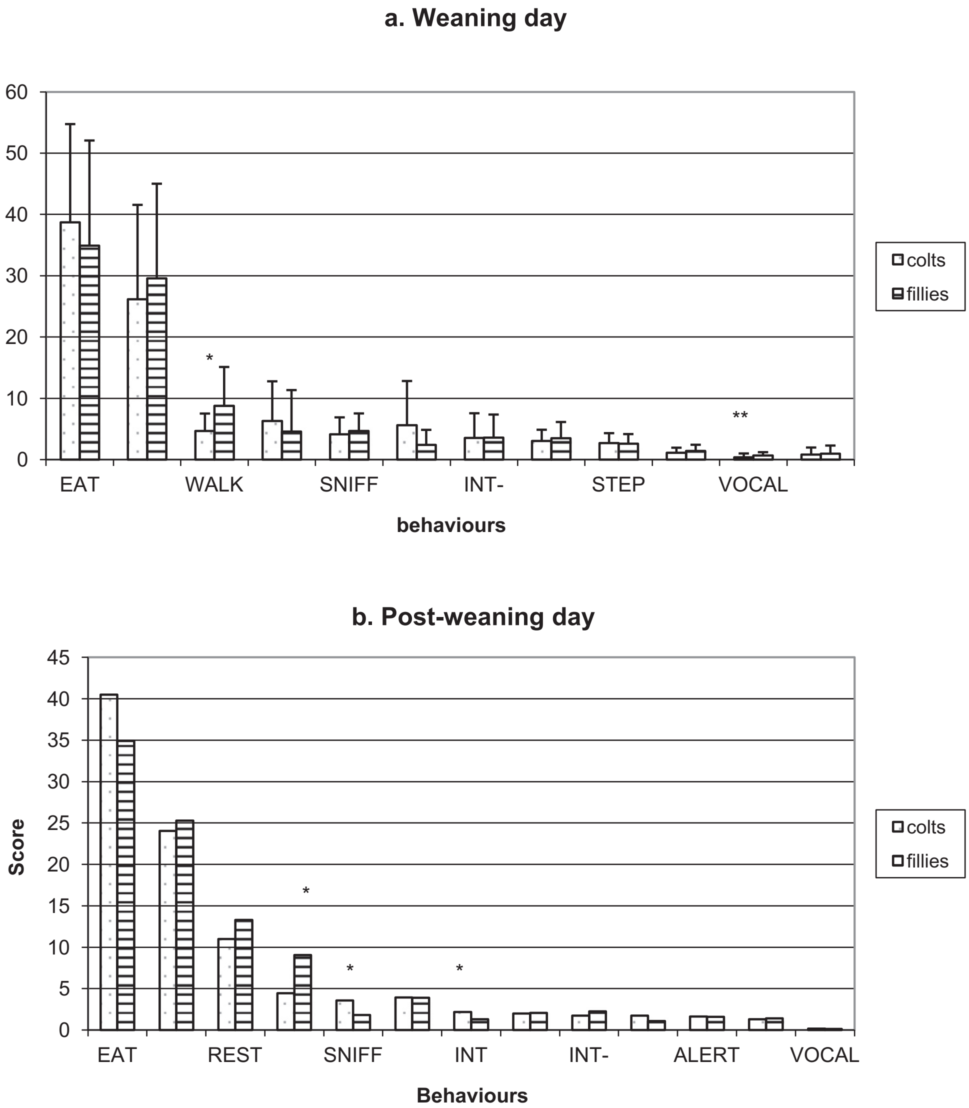

Fig. 4. Behaviours of colts and fillies on weaning and post-weaning days.

surroundings and engaged in neutral social interactions more often than fillies ($P = 0.0148$ and $P = 0.0052$ for sniffing and neutral social interactions, respectively, Fig. 4). On the other hand, fillies lay down more often than colts ($P = 0.0331$, Fig. 4).

3.5. *Effect of interaction between rearing conditions and sex*

As shown in Fig. 5, the SR and FR colts and fillies differed in their behaviours on WD and WD + 1 (all $P < 0.05$, exact P-values omitted in the text for better clarity of results). Descriptive statistics is presented in Appendix 2.

On WD, the SR colts walked more, sniffed their surroundings more and demonstrated more neutral social interactions than the FR colts, whereas FR colts spent less time resting (Fig. 5A). Similarly, on WD, higher alert, locomotor and investigative activity was observed for SR fillies than for FR fillies (Fig. 5B). Moreover, the SR fillies engaged in more neutral and agonistic social interactions than the FR fillies (Fig. 5B).

On the post-weaning day, the SR colts were still more active, as they spent more time walking, in an alert posture and engaging in neutral social interactions than FR colts (Fig. 5C). The FR colts showed passive behaviour (standing) more often than did the SR colts (Fig. 5C). The FR and SR fillies differed significantly on the post-weaning day only in their locomotor behaviour, as the SR fillies walked more and stood less than the FR fillies (Fig. 5D).

When comparing sexes within the same rearing conditions, only the SR colts and fillies differed significantly in their behaviour on WD, as the SR fillies spent less time eating, demonstrated less neutral social interaction and vocalised less. However, the SR fillies spent more time standing and walking than the SR colts (Fig. 5E). On WD + 1, the SR fillies were less socially interested (less neutral

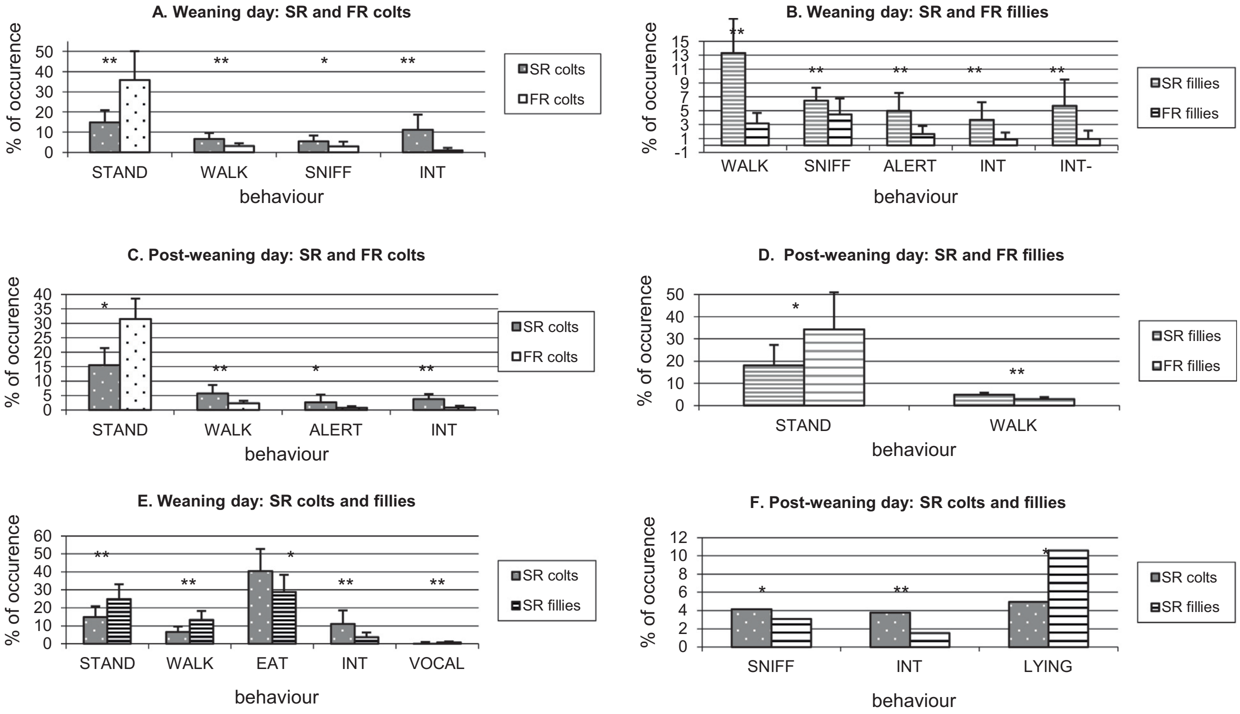

Fig. 5. Behaviours of forest-reared (FR) and stable-reared (SR) colts and fillies on weaning and post-weaning days.

interactions) and sniffed less but lay down more than the colts (Fig. 5F).

3.6. *Behaviours indicative of high cortisol levels*

In total, increased FCM levels recorded on the weaning day were related to horses spending longer periods standing (r_s = 0.32, P = 0.0149) and shortened feeding bouts (r_s = −0.42, P = 0.0017, Table 2). These two behavioural patterns were correlated with one another (r_s = −0.47, P = 0.0004, data not tabulated).

When groups were considered separately, the behaviour was significantly correlated with FCM levels according to weaning conditions and sex (Table 2). SR foals with higher FCM levels interacted neutrally (r_s = −0.48, P = 0.0118) and ate (r_s = −0.41, P = 0.0201) less. In turn, they walked more (r_s = 0.44, P = 0.0201) and showed more comfort behaviours (r_s = 0.41, P = 0.0327) as their FCM level increased. For FR horses, only eating was negatively correlated with FCM (r_s = −0.41, 0.0107).

With respect to sex-based differences on WD, significant correlations were found only in fillies. Fillies with higher FCM concentrations stood more (r_s = 0.47, P = 0.0105) and ate less (r_s = −0.55, P = 0.0018).

In the search for behaviours that could describe the behavioural profile of the foals that were less or more physiologically afflicted by all events around weaning, eight distinct behaviours significantly differing between IQI were found. They were as follows: standing (Chi-square = 16.02, P = 0.0011), walking (Chi-square = 18.14, P = 0.0004), feeding (Chi-square = 10.07, P = 0.0180), sniffing (Chi-square = 8.07, P = 0.0445), alertness (Chi-square = 8.30, P = 0.0402), agonistic interactions (Chi-square = 8.58, P = 0.0353) and comfort behaviours (Chi-square = 9.69, P = 0.0214). When plotted against the IQI, almost all of them showed a non-linear relationship (Fig. 6A and B). Actually, only the eating was characterised by a linear (negative) relationship with FCM (Fig. 6A).

It should be noted here, that although the numbers of colts and fillies were similar within each interquartile interval (P > 0.05), the groups of the highest FCM (Q2–Q1 and >Q1) differed in the distribution of foals from different rearing conditions (Fig. 6). There were more SR foals in IQI = Q2–Q1 (Chi-square = 7.3; P = 0.0023; Fig. 6) but FR horses outnumbered stable weanlings in IQI > Q1 (Chi-square = 7.1; P = 0.0075; Fig. 6).

4. Discussion

According to the best of our knowledge, this is the first scientific report on behavioural and physiological responses to events around weaning in feral horses. The results of the present study show an increase in FCM levels after weaning, indicating that these events provoked an adrenocortical response, which is in line with the results of previous studies using different cortisol measures (Erber et al., 2012; Henry et al., 2012; Malinowski et al., 1989; McCall et al., 1987; Moons et al., 2005). No differences in FCM levels were detected between the SR weanlings that were "only" separated from their dams and FR foals undergoing maternal separation, first manipulations by humans and the transportation. However, forest-reared weanlings outnumbered their stable reared counterparts in the upper quartile of FCM levels. Quick behavioural adaptation to new

Table 2
Spearman correlation coefficients[a] between FCM levels on WD and behaviours observed on WD as related to rearing conditions and sex of horses.

Sex/rearing conditions	FCM				
	SR foals $N=27$	FR foals $N=26$	Colts $N=11$	Fillies $N=16$	Grand total
Behaviour	r_s; P				
STAND	–	–	–	0.47; 0.0105	0.32; 0.0149
WALK	0.44; 0.0201	–	–	–	–
EAT	−0.41; 0.0336	−0.41; 0.0107	–	−0.55; 0.0018	−0.42; 0.0017
INT	−0.48; 0.0118	–	–	–	–
COMFORT	0.41; 0.0327	–	–	–	–

FCM – faecal cortisol metabolites, WD – weaning day, WD + 1 – post-weaning day, FR – forest reared foals, SR – stable-reared foals.
[a] Only the behaviours significantly correlated with FCM are shown.
– correlation non-significant.

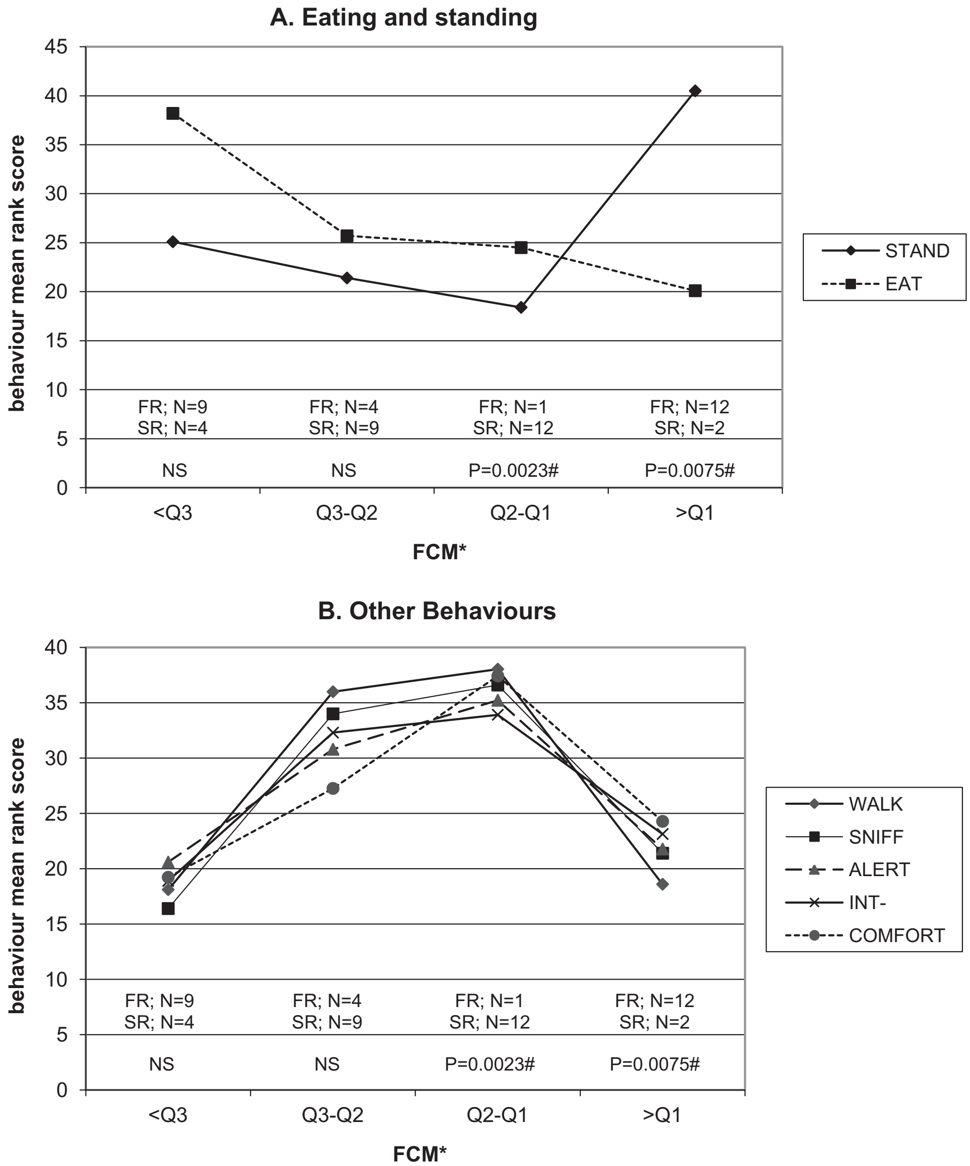

Fig. 6. Profile of behaviours differing between interquartile intervals (IQI) of FCM. * on *X*-axis is the ranges of IQI's are presented as related to Q1 (9.58 ng/g), Q2 (7.6 ng/g) and Q3 (6.2 ng/g) values. # Number of SR and FR foals in each IQI differs significantly according to Chi-square test.

conditions was observed as the general arousal decreased and resuming typical activities increased in weaned foals. However, clear differences between the FR and SR foals in terms of the behavioural responses to the two weaning schemes were observed. The occurrence of some observed behaviours was non-linearly linked to the increase of faecal cortisol metabolites. In general, the horses with high adrenocortical activity (FCM levels) on the weaning day ate less and stood more.

4.1. Behavioural response

On the day of weaning, all horses were predominantly engaged in feeding and standing (approximately 60% of time) but also showed a high occurrence of alertness, locomotion, vocalisation and frequent engagement in social interactions, mostly agonistic ones. No horse was observed to lie down over the 6-h period after weaning. High locomotor activity was especially manifested by stable-reared weanlings, in line with the results of previous studies on stabled horses (Heleski et al., 2002; Henry et al., 2012; Hoffman et al., 1995; Houpt et al., 1984; McCall et al., 1985, 1987; Malinowski et al., 1989; Moons et al., 2005). FR foals clearly showed more passive behaviour by walking, interacting and exploring less frequently but standing and resting (standing) more often. Basing on this behavioural response it is likely that FR weanlings were overwhelmed by the procedures they experienced during weaning and transportation and were more uncertain and fearful of the novel environment. Nonetheless, it was observed that after being placed in completely unknown surroundings, FR foals rather quickly adapted to the novel conditions; at least, they fed for the same amount of time as their stable born counterparts. Together with standing, these two behaviours of FR and SR horses prevailed among all other behaviours.

In contrast, stable horses were more aroused, explorative and socially active. Because SR horses were already accustomed to stable conditions, they walked and explored the pens, as well as other, unknown stable-mates with lower caution. Stable-reared weanlings occasionally heard the mares kept in the stable located within the same building, which may explain their frequent vocalisation. It appears that familiarity with stabling enabled the expression of typical behaviours in response to separation from the dam (vocalisations, restlessness) and made the resulting arousal more noticeable in SR than in FR foals. It is likely that the overwhelming effect of transportation and novelty in part mimicked that of maternal separation in FR horses.

Although the majority of alert behaviours decreased on the day following weaning, some differences in activity and social behaviour between groups persisted. The decrease in alert behaviours exhibited on the day following weaning, as well as the increase in resting behaviour at that day indicate a decrease in emotional tension and are indicative of all of the horses' adaptation to new social and environmental conditions, which is in line with the results of most previous studies (Heleski et al., 2002; Henry et al., 2012; Hoffman et al., 1995; Moons et al., 2005).

4.2. Validity of behavioural markers of weaning stress

In addition to a greater number of vocalisations (Weary et al., 2008), increased locomotor activity is often accepted as a behavioural marker of the intensity with which animals respond to weaning (Hoffman et al., 1995; Nicol et al., 2005). These two behavioural patterns are manifested in any case in which socially bonded horses are separated, regardless of age and conditions (Jezierski et al., 1999; Lansade et al., 2008), and are not specific to the case of weaning alone. To the best of our knowledge, the frequency of vocalisation has not been proved to be a valid indicator of weaning stress, by any reference to physiological measures in horses. In our study, only feeding on the weaning day was directly and linearly related to FCM levels in all horses, in agreement with the results of previous studies (Hoffman et al., 1995; McCall et al., 1987). Our results agree with those of the latter authors, who observed a relationship between eating time or feed intake (negative) and standing and walking (positive) with pre-ACTH challenge serum cortisol levels, which demonstrated that more "stressed" horses were less willing to forage and were more engaged in resting or locomotor activities. Such discrepancy regarding locomotor activity could be explained by considering the fact that stress responses are very complex and may not always involve the same typical behavioural patterns, or they can fluctuate as the stress reaction changes. Hoffman et al. (1995) adopted Selye's (1976) definition of stress to develop a subjective scale involving distress and eustress categories, each represented by qualitatively and quantitatively different behavioural responses. The authors have observed that studied weanlings exhibiting more signs of distress (apathy, standing with head lowered, immobility, refusal to eat, silence) exhibit lower ACTH challenge responses, indicating a lower adrenal reserve and thus a higher stress response following weaning (Hoffman et al., 1995). Our results are in line with arbitral classification of the behaviour in the latter study. Some specific behaviours (walking, sniffing, alert behaviour, negative social interactions, as well as comfort behaviours) were found to be related to the measured marker of adrenocortical activity (FCM). A non-linear character of the relationship between above listed behaviours and FCM was noted. Since the distribution of horses showing these behaviours followed an inverted U-shape as FCM raised, it would be difficult to distinguish between "not stressed" and "very stressed" horses based only on the frequency of above behaviours. The behavioural profile of weaned horses in our study involved increasing frequency of walking, sniffing, social interactions, comfort behaviours and the alertness as their physiological "stress" reaction increased. However, when all of these behaviours were relatively low, but the foal refused to feed and in most of the time remained in a standing posture, it may had been suffering acute stress. It should be noted here, that due to different distributions of horses from the two rearing conditions, the behaviour of horses in Q2–Q1 were characteristic mostly for SR foals. Similarly, the behaviour of horses from the upper quartile was typical to forest foals. Although no difference in FCM was found, it may be supposed that forest foals were also more physiologically afflicted as they outnumbered SR foals in the upper quartile.

It seems that forest foals were more disturbed by all events around weaning. They presented apathy and more of them showed highest values of FCM concentrations. However, as the FCM did not differed between groups from different rearing conditions, the claims of a higher "stress" response to weaning scheme in forest foals cannot be unequivocally supported based on behavioural and physiological measures in our study.

4.3. Sex differences

Interestingly, we observed sex to have an effect on cortisol levels, in line with the results of previous studies (Hoffman et al., 1995; Moons et al., 2005). The fillies showed higher increases in FCM levels in response to weaning, which suggests that female horses are more susceptible to challenging situations. Similarly, the highest FCM levels in female horses have been observed during their first month of training (Gorgasser et al., 2007) and a more prolonged FCM increase in female llamas after confinement (Arias et al., 2013) has been reported. In the study by Hoffman et al. (1995), serum cortisol concentrations before and after an ACTH challenge were observed to be higher in fillies; however, there were no differences in the serum cortisol response after the ACTH challenge. The authors stated that this observed does not provide evidence that males cope better with stress, as serum cortisol levels are affected by sex in most mammals (Gray, 1971). Based on studies on the binding capacity and affinity of corticoid receptors, Horst et al. (2012) suggest that there is a sex-specific regulation of the stress response in brain areas that are crucial for emotional reactions. Thus, sex-based differences in response to the challenge in horses should be crucial for studying welfare in the field of equitation and horse training. Because other studies on cortisol levels at rest (Muñoz et al., 2012) or under stressful conditions (laminitis, acute or chronic disease (Ayala et al., 2012); weaning (McCall et al., 1987)) have not confirmed sex-based differences, further research on sex effects on behavioural and physiological stress measures in horses is required.

As a final remark concerning the welfare of weaned foals, we would like to point out that Konik foals are refused to feed by receiving aggressive threats from their dams, which take place in both forest and stable groups around 8–9 months of age of the foal (Jaworski, personal communication). In the forest reserve, the stallions maintain a stable herd composition by expelling their maturing offspring. This natural dispersion by the father involves chasing and biting the young horse (occasionally leading to injuries; Jaworski, personal communication) for a substantial period of time, until complete separation from the natal herd occurs. Although weaning itself is considered a stressful event for stabled foals (Waran et al., 2008), it is a natural and necessary biological mechanism of preventing inbreeding (Boyd, 1991; Jezierski et al., 1999). It appears that complete eradication of a foal's discomfort in the case of separation from the mare is not possible because horses remain socially dependent throughout their entire life (Jezierski and Górecka, 1999; Lansade et al., 2008).

5. Conclusions

Both forest and stable-reared foals showed similar physiological stress responses, as demonstrated by an increase in faecal cortisol metabolites following weaning. However, because forest-reared foals differed from stable horses in their behavioural responses, we propose that further research is needed to understand the basis of those differences and their importance as indicators of stressful conditions which would be beneficial for a more accurate assessment of equine welfare.

Finally, considering the differences in behaviour and physiological response between colts and fillies, further research on sex effects in stressed horses may contribute to the development of better sex-adjusted horse management schemes.

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Appendix 1. Ethogram used in behavioural sampling

Variable	Description
STAND	Remaining standing position with no other activity
STEP	Stepping, taking no more than one step forward, backward or aside
WALK	Displacing in walk
EAT	Eating hay or straw
DRINK	Drinking water
SALT	Licking salt blocks
LICK	Licking other than salt (stable walls, crib)
SNIFF	Sniffing the surroundings (not the other horses)
ALERT	Remaining alert posture, with observation and attentive listening
INT	Neutral interaction with another horse (e.g. sniffing)
INT+	Positive interaction with another horse (e.g. allogrooming, resting the head on another horse's croup or back)
INT−	Negative (agonistic) interaction with another horse (e.g. threats, chasing, biting, kicking)
URINE	Urinating
DEFEC	Defecation
VOCAL	Vocalisation (whinnying)
PAW	Pawing the litter
LIE	Lying down
COMFORT	Comfort behaviours: scratching against solid parts of the stable, scratching with a hoof or incisors, 'wet-dog' or head shaking, rolling
TROT	Displacing in trot/canter
REST	Standing in relaxed position with flexed hind leg, lowered head and one pelvis
YAWN	Yawning

Appendix 2. Time budget (behaviours, % of occurrence) of stable- and forest-reared colts and fillies on weaning day (WD) and post-weaning day (WD + 1)

	WD N = 53				WD + 1 N = 53			
Rearing conditions	Stable N = 27		Forest N = 26		Stable N = 27		Forest N = 26	
Sex	Colts, N = 11	Fillies, N = 16	Colts, N = 13	Fillies, N = 13	Colts, N = 11	Fillies, N = 16	Colts, N = 13	Fillies, N = 13
Behaviour score	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.	Mean ± s.d.
STAND	14.8 ± 6.10	24.9 ± 8.21	35.8 ± 14.3	35.3 ± 20.2	15.5 ± 5.94	18.1 ± 9.14	31.2 ± 7.08	34.0 ± 16.8
STEP	2.61 ± 1.91	2.55 ± 1.80	2.74 ± 1.45	2.60 ± 1.38	2.35 ± 1.56	1.94 ± 1.04	1.74 ± 0.59	2.20 ± 1.51
WALK	6.55 ± 3.05	13.3 ± 4.96	3.09 ± 1.34	3.19 ± 1.48	5.74 ± 2.93	4.80 ± 0.97	2.40 ± 0.76	2.80 ± 0.97
EAT	40.3 ± 12.5	28.8 ± 9.52	37.4 ± 18.9	42.5 ± 22.4	40.2 ± 14.3	36.7 ± 12.7	40.7 ± 13.1	32.7 ± 17.8
SNIFF	5.45 ± 2.88	6.45 ± 1.86	3.01 ± 2.18	2.47 ± 2.31	4.12 ± 2.69	3.06 ± 2.14	3.06 ± 2.15	1.65 ± 1.21
ALERT	3.79 ± 1.39	4.99 ± 2.58	2.39 ± 1.98	1.64 ± 1.16	2.68 ± 2.64	1.74 ± 1.03	0.76 ± 0.55	1.45 ± 2.70
INT	11.1 ± 7 .55	3.68 ± 2.56	1.02 ± 1.16	0.85 ± 1.00	3.79 ± 1.77	1.53 ± 1.64	0.85 ± 0.64	1.01 ± 1.22
INT+	1.28 ± 1.54	1.34 ± 1.64	0.45 ± 0.42	0.54 ± 0.54	2.51 ± 3.09	1.37 ± 2.91	1.09 ± 1.51	0.75 ± 0.75
INT−	4.52 ± 4.61	5.70 ± 3.79	2.73 ± 3.40	0.92 ± 1.21	1.99 ± 1.61	2.42 ± 2.18	1.54 ± 0.86	2.04 ± 1.50
VOCAL	0.25 ± 0.70	0.69 ± 0.60	0.48 ± 0.56	0.61 ± 0.50	0.35 ± 0.53	0.17 ± 0.21	0.04 ± 0.09	0.04 ± 0.07
LYING	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.93 ± 10.6	4.05 ± 6.64	4.05 ± 6.64	5.96 ± 7.92
COMFORT	1.11 ± 0.97	1.78 ± 1.11	1.07 ± 0.78	0.99 ± 0.62	1.93 ± 0.72	1.29 ± 0.84	1.29 ± 0.83	1.65 ± 1.21
REST	4.75 ± 6.02	0.07 ± 0.24	7.61 ± 6.79	6.43 ± 8.10	13.1 ± 8.98	0.11 ± 0.18	9.26 ± 7.63	11.8 ± 34.4

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Corrigendum

Corrigendum to "Events around weaning in semi-feral and stable-reared Konik polski foals: Evaluation of short-term physiological and behavioural responses" [Appl. Anim. Behav. Sci. 163 (2015) 122–134]

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The authors would like to correct Figs. 2–4. The appropriate figures are displayed below.
The authors would like to apologise for any inconvenience caused.

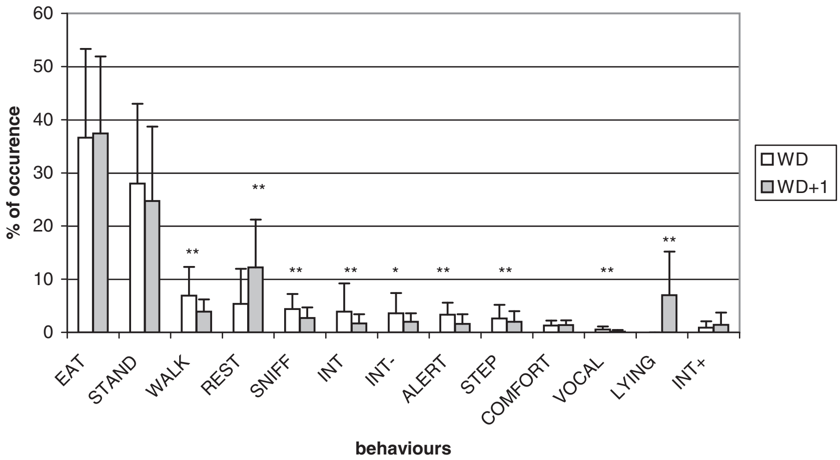

Fig. 2. Behaviours of all foals on weaning and post-weaning days.

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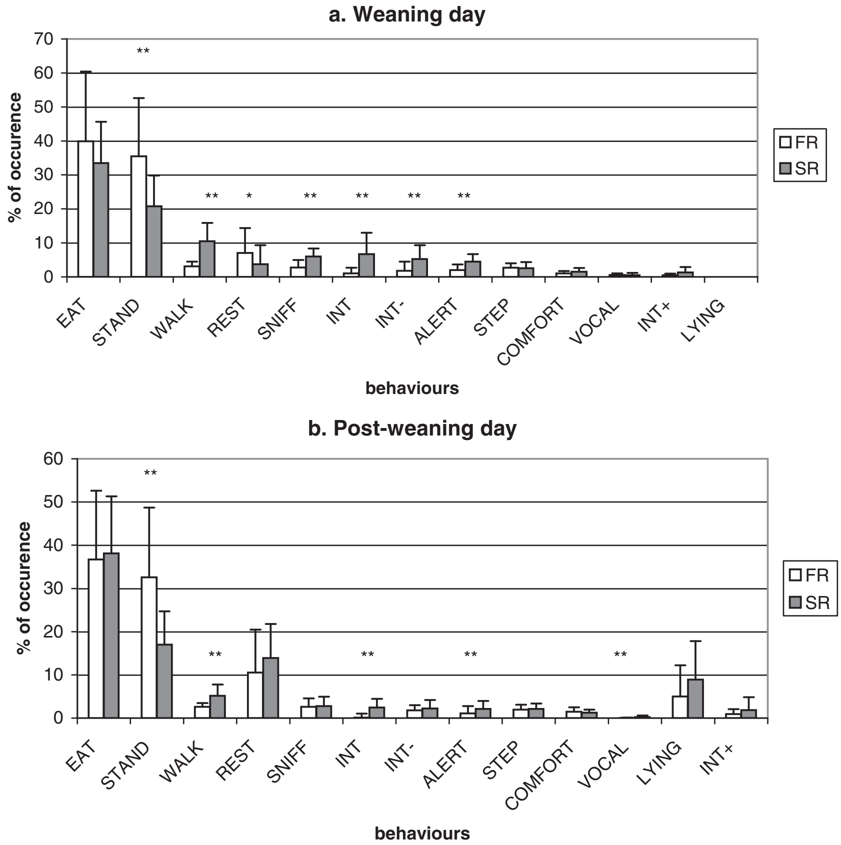

Fig. 3. Behaviours of forest-reared (FR) and stable-reared (SR) foals on weaning and post-weaning days.

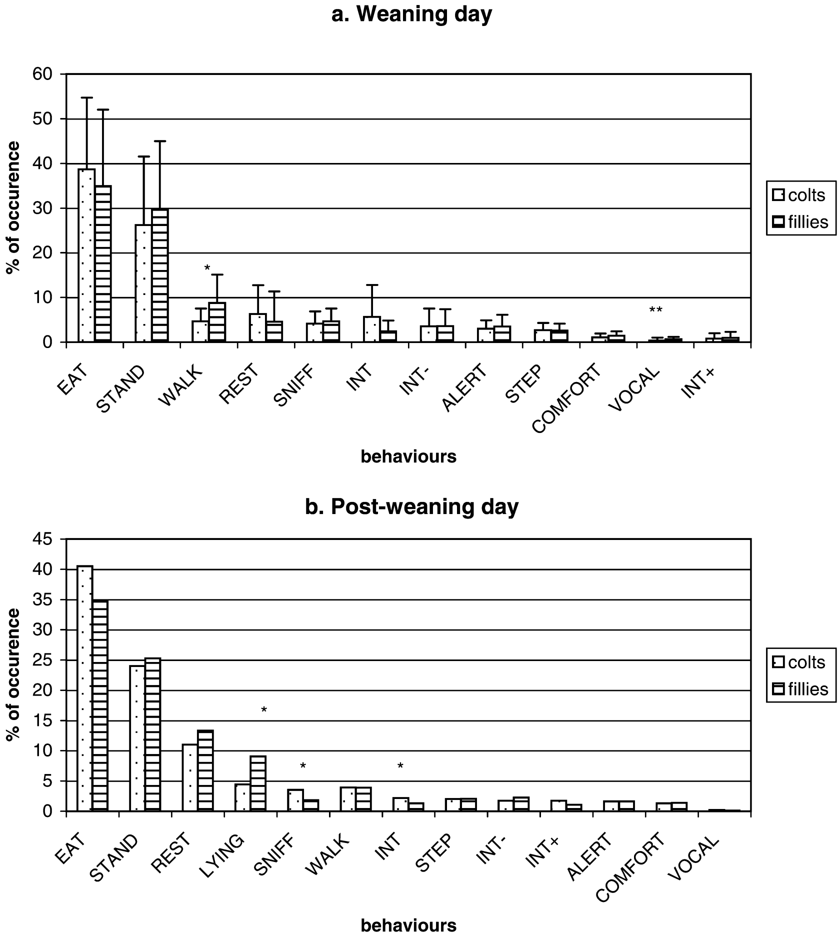

Fig. 4. Behaviours of colts and fillies on weaning and post-weaning days.