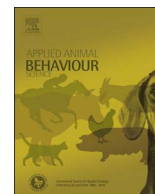




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## Behavioural changes induced by handling at different timeframes in Lusitano yearling horses

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### ABSTRACT

Recent interest has focused on the advantage of intensely handling young horses, as a good tool for improving emotional reactivity and thence trainability. Its effectiveness remains controversial, suggesting it might depend on the right timeframe to do so. The present study aimed to evaluate the effects of 3-handling sessions in foals, during eventual optimal periods – the day of birth (2–3 h after it) and the day after weaning – to be compared to handling in an unspecific period (at  $50 \pm 7$  days old), or no handling. Lusitano breed foals ( $n = 50$ ) were divided into five experimental groups with three being submitted to handling (consisting in desensitization, socialization and sensitization): at birth (Imp-H), at weaning (Wean-H), or at  $50 \pm 7$  days old (Ex-H); and two not-handled groups: one not-handled and submitted to a 4-sessions training schedule (Not-H); and another left undisturbed (Control) until the end of the study (that finished when the foals reached  $12 \pm 1$  months old). Training, consisted in four 12-min sessions, where foals were taught to be lead-in-hand finishing with exhibition of trot. Handling effects, regarding skills to face new situations and environments in the presence of and on human demands, were evaluated twice: two months after weaning, just before starting the training; and one month after it ended, when they were submitted to a final performance test. Previous to training, handling effects were not found in the foals' behavioural responses when facing a motionless person. When approached, differences were shown: animals handled at birth or at weaning displayed less fearfulness, showed less locomotor activity and tolerated a closer approach, when compared to Controls.

Moreover, the Wean-H foals were those that learned faster at training and the only group that passed all aims at the final test; the Imp-H foals were the less reactive, but did not learn better than Ex-H foals and were more troublesome when trained. In summary, our data suggest that 3-handling sessions in young horses are enough to act on human-animals' relationship, learning performance and, and may be improved if performed in the specific period of weaning.

### 1. Introduction

Nowadays it is very clear that good or bad experiences during early life may have a strong influence on adult behaviour (Alleva and Francia, 2009; Pereira-Figueiredo et al., 2014). Bearing this in mind, early life is suggested to be a good period to shape social and performance skills. In horse rearing, recent interest has focused on the effects of early positive handling as it may define temperament and future response to environment, including to human demands (Houpt, 1981; McCall et al., 2006). With horses, good handling or gentling experiences are likely to affect fearfulness (Simpson, 2002; Lansade et al., 2005) and relationship with humans (Krohn et al., 2001; Fureix et al.,

2009; Sankey et al., 2010) and positively influence future behaviour (Nicol, 2002; Ligout et al., 2008). Some of these authors have questioned the existence of “sensitive” or “optimal” periods.

According to literature, there are specific developmental phase periods when animals are more prompt to respond to environmental stimuli or when a kind of sensitivity occurs (Hess, 1973). In these sensitive times a particular Central Nervous System (CNS) plasticity occurs, which appears to help socialization, and making learning faster and easier (Mal and McCall, 1996; Simpson, 2002). Any reorganizational period, with stress added, can become one of these special sensitivity periods. Miller (1991), that became renowned for his work on “imprint training”, and some other authors (Simpson, 2002; Spier et al.,

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2004; Lansade et al., 2005), favor the neonatal period as the ideal handling period. Indeed, the foal is an extremely precocious animal, born with highly developed perception and motor skills (Spier et al., 2004).

However, despite neonatal period being suggested as an important time for establishing a good relationship between man and horse, previous studies reported marginal effectiveness or little practical effect (Williams et al., 2002; Lansade et al., 2005), and even some risks and problems, such as upsetting mother-foal relationship (Henry et al., 2009). Moreover, safety risks associated with handling foals at this early age should be considered. So, an alternative optimal period to consider is at weaning (Lansade et al., 2004; Ligout et al., 2008).

In animal production, it is common practice, to wean artificially and abruptly (mother and young are separated suddenly at once). This abrupt separation is usually associated with social and physical environment changes, that induce further stress (Houpt et al., 1984; Nicol, 2002; Moons et al., 2005).

Lansade et al. (2004) reported the beneficial effects of handling just after weaning but, in their work, foals were intensely handled for 12 days. In the present work, we aimed to define optimal timeframes to handle young horses, in order to control fearfulness and create a positive bonding with humans, allowing learning performances improvement. We hypothesized if three handling sessions, during the suggested “sensitive” moments (just after birth and the day after weaning) might be enough to reach the same results.

The horse, being a prey animal (McGreevy, 2007; Keeling et al., 2009), is strongly driven by survival instincts. Some studies developed in the past, intending to determine the handling effects, did not take into consideration such sensibility (Brubaker and Udell, 2016) and few took into consideration the role of human-horse interactions in their behavioural responses (Sankey et al., 2010). Furthermore, studies quite often were performed using horses of different gender, age, and even breed, thus having great phenotypic and temperament differences (Simpson, 2002; Nicol, 2002; McCall et al., 2006; Merckies et al., 2014). Thence, previous studies have led to contradictory results, although using similar methodology. Some reported that there was a decrease in reactivity and subsequent response to novelty or manageability, in animals handled at any of these moments (Mal and McCall, 1996), while others reported lack of differences (Williams et al., 2002; Sondergaard and Jago, 2010); or only a temporary effect (Lansade et al., 2005). Bearing this concern in mind, in the present study, the behavioural outcomes of handled/or non-handled Lusitano foals, born at the same farm and with the same age ( $\pm 2$  months), were evaluated until they were one year old, regarding their response to novelty and stressful situations, when being forced to human contact.

## 2. Material and methods

### 2.1. Animals

The Lusitano foals, chosen to take part of the present study ( $N = 50$ , within a total of 91), were born at the stud farm of Alter (Alter-do-Chão, Portugal) from March to May 2014.

Both sexes foals (24 females and 26 males), were individually identified with a numbered collar and were assigned to one of five groups. Sex, age and sire were taken into account when allocating the animals to each group. As depicted in Fig. 1, three of these groups were human handled: the first, from  $4 \pm 1$  h after birth, i.e. handling did not begin until the foals had stood up, nursed and bonded with their dams (Spier et al., 2004) ( $N = 8$ , Imp-H); the second, the day after weaning ( $N = 9$ , Wean-H); and the third, at the age of  $50 \pm 7$  days ( $N = 11$ , Ex-H), a time considered to be out of a critical period (Mal and McCall, 1996). Two groups of unhandled foals were formed, one submitted to a 4-training sessions procedure ( $N = 10$ , Not-H), and the last group was left undisturbed and served as control ( $N = 12$ , Control).

All experiments and training were carried out at the same facility

where the animals were born. Each dam-foal was individually penned for the first week of life. All foals remained with their dams until approximately 7 months age ( $\pm 1$  month), when they were abruptly weaned. Then, they were allowed to stay at pasture all day and housed at night when they were supplemented with a concentrate feed. During the present study, foals remained always with their conspecifics, and spent all the time standing in close contact with each other, before and also after weaning, when they were paddock-housed.

The study was based exclusively on behaviour observation and all handling was routinely performed at the stud farm. The authors confirmed that animal welfare was never at risk and that the experiments complied with the policy relating to animal ethics and welfare.

### 2.2. Handling sessions

The handled groups' foals (Imp-H, Wean-H and Ex-H) were subjected to individual contact by two experienced handlers, consisting of three sessions. The handling procedure was positive but forced, and followed the one described by Miller (1991), with minor modifications, based on other authors suggestions (Williams et al., 2002; Henry et al., 2009), regarding the moment it was performed and being done with the foals standing. The first handling session lasted 40 min. The two other sessions were performed in the subsequent mornings, starting at 9:00 am and lasting 20 min.

During handling (3 days long), the handled foals remained penned (in a  $5 \times 4$  m box), in the presence of their mothers, in the case of the Imp-H and the Ex-H ones; or in groups of three, in the case of the Wean-H. In these, 20 min previous starting the handling sessions, each foal was extracted from its group into a similar pen (Lansade et al., 2004), with the help of a barren mare, known to the foals.

During handling procedure, the mother/or the known mare, was constrained at approximately 3-m distance from the foal, with food and water available, and let quiet. Each session started once both handlers (two experienced male persons) entered the pen. One immediately began approaching the foal, gently fitting a halter, while the second handler approached the mare and only helped in restraining the young one if it tried to step away. Once it was haltered, the first man persistently started touching it throughout all body except the belly (*desensitization*: Spier et al., 2004): successively the head, including gently rubbing ears, nostrils and mouth behind the tongue, then the shoulders, back, hindquarters and legs (it lasted a total of 10 min in the first session and 3 min in the next ones); afterwards, he repeated it by rubbing all over the foal's body, with plastic and paper (for another 3 min). Then, the handler gently tried to flex the neck, and flex and stretch each foot (for approximately 5 min). It was attempted to get the foal to interact with the handler, by making gentle sounds and calling him (*socialization*); finally, in the last 5 min, it was tried to get the young to remain motionless in close proximity to both handlers, even when they stepped back and/or approached it (*sensitization*).

### 2.3. Behavioural tests

#### 2.3.1. Tests in an unfamiliar environment

Two months after weaning (at  $9 \pm 1$  months), all foals ( $N = 50$ ) were subjected to the first behavioural tests. These were performed in an open, unfamiliar,  $7 \times 6$  m pen, with walls covered by a 1.6 m high net, where each foal was isolated and forced to remain in the presence of an unfamiliar handler. During three days, from 10:00 am until 14:00 pm, all foals were led together and freely, to a  $16 \times 20$  m outdoor paddock; and, when being tested, each animal was surrounded by its peers and by hidden observers on all sides. In order to be isolated and enter the pen, the foal had to pass through a stalk, that was only closed after being crossed.

In the first part of the experiment a “*motionless person*” test was performed (Ligout et al., 2008): the handler was already inside the pen, and observations began once the foal entered it, free willing. The man

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