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Female horses are more socially dependent than geldings kept in riding clubs

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Abstract

The effect of the sex of horses in personality studies seems an underestimated aspect, specifically
for the social dependence personality trait. A simple two-choice test could be a promising tool to
address the strength of social dependence in riding horses. The present study aimed, at exploring
the effect of sex on social dependence, hypothesising that females will react with more anxiety than
castrated males when exposed to social separation. We examined whether a two-choice test will
predict social dependence in horses. Twenty-four experienced leisure horses (7 mares and 17

geldings) were tested with a simple two-choice (food vs equine companion) preference and subsequent social dependence test in three sessions within the test (Session1: separation with a preferred companion; Session2: isolation, and Session3: social stimulus, i.e. conspecific passing nearby). During the test, six horses chose the companion (companion-motivated, CM), and 18 horses chose the treats (food-motivated, FM). The choice was skewed for mares, as significantly more mares than geldings preferred the companion over the food (CM: $N_{\text{mares}}=4$, $N_{\text{geldings}}=2$ and FM: $N_{\text{mares}}=3$, $N_{\text{geldings}}=15$). In isolation, CM horses displayed significantly reduced feeding duration and standing still towards increased arousal, i.e. locomotor activity: trotting, cantering, pawing and vocalizations, as compared to FM horses that were calmer and more comfortable without companions than CM horses. Our results indicate that mares, compared to geldings, showed weaker interest in feeding, and longer bouts of movement (walk and trot), which indicated the prevailing willingness to reunite with the companions and were more socially dependent compared to castrated male horses. The authors propose that this simple two-choice test can be used as another test to assess social dependence in horses.

Keywords horse, equitation, social dependence, sex differences, personality

1. Introduction

Recreational equitation is currently the most popular horse-related activity (Keaveney 2008, Dashper 2014; Janczarek and Wilk, 2017). Considering high speeds when riding and the instant reactivity of horses to sudden and unexpected events, this activity is accident-prone and can lead to injuries and fatal incidents (Hawson et al. 2010; Thompson et al. 2015; Starling et al. 2016). Often, extreme anxiety as a result of separation from the group is responsible for unsafe behaviour when handled and/or ridden, which may lead to accidents. The way in which horses respond to environments, i.e. their psychological construction, is crucial to riders (Visser et al. 2008; Górecka-

Bruzda et al. 2011; Graf et al. 2013; Suwała et al. 2016). For this reason, equine personality has been extensively studied since it contributes to riders' safety (Górecka-Bruzda et al. 2015a; Lansade et al. 2019), satisfaction (Graf et al. 2013; Górecka-Bruzda et al. 2016) and to horse performance (Lansade et al. 2016) and has been proposed as a goal of genetic selection (Graf et al. 2013; Lansade et al. 2016).

Several approaches to distinguish personality in horses have been adopted. Some concepts involve all psychological properties as a whole, calling them interchangeably either personality or temperament (e.g. Visser et al. 2001; Momozawa et al. 2007; Lloyd et al. 2007), while others consider temperament and character to be separate qualities of equine personality (e.g. König von Borstel 2013; Suwała et al. 2016). Several personality traits/dimensions have been distinguished in horses, including sociability/gregariousness (LeScolan et al. 1997; Lloyd et al. 2007; Lansade et al. 2008). However, sociability or gregariousness could be interpreted as a willingness of animals to gather in groups, while the cited authors also tested the reaction to social separation/isolation, i.e. social dependence. As horses are social animals that live in stable groups for life (Jaworska et al., 2020), the need to be in proximity of conspecifics, especially familiar ones, is usually very high (Søndergaard et al., 2011). This need is expressed in social separation anxiety, alertness, vocalization and often extreme motivation to re-join conspecifics. Therefore, we will use the term "social dependence" to define the mental feature of an animal exhibiting such anxiety response to social separation and following isolation. As it is an important component of equine herd cohesion, it is expected that horses will react negatively to social separation. The tests for the reactivity of the horse when isolated, separated and reunited (Lansade et al. 2008) were used for the assessment of social dependence. The two-choice preference tests for social companionship, outdoor space and activity, human touch or food were used to evaluate the motivation for specific resources important for equine wellbeing (e.g. Edouard et al., 2009; Lee et al., 2011; Mejdell et al., 2016; Kieson et al. 2020), so it was used in the present study as a preselection tool of horses as being less or more socially dependent.

The effect of sex on the reactivity of horses seems an underestimated aspect, specifically for the social dependence personality trait. This special aspect of the equine personality seems to be strongly affected by sex, as feral horses live in female-dominated social groups (Keiper 2005). Social bonds between semi-feral female horses have been reported to be lifelong (Jaworska et al., 2020). Although some studies confirm practical observations that females are more easily agitated (Jezierski et al., 1999; Wolff et al., 1997; Quberstein and Gilkenson, 2010; Schmidt et al., 2010; Sackman and Houpt, 2019), other studies have found the opposite (Roberts et al. 2016), very little experimental and field studies have approached the aspect of sex differences in equine personality. Rather, the sex/gender effect of the horse was avoided by using sex-balanced groups (Visser et al. 2001; Lansade et al. 2008) or was neglected when discussing the traits/dimensions of personality (reviewed by König von Borstel, 2013). Since many biomedical studies using animal models used only males to avoid the “confounding” effect of female sex hormones (e.g. Beery and Zucker 2011; Kokras and Dalla 2014), the present study addressed the differences in social dependence between mares and castrated males (geldings) to deepen the knowledge of the potential effect of sex on social dependence in horses.

The aim of the present study was to examine whether the sexes of horses differ in social dependence, hypothesising that females will react with more anxiety than castrated males when exposed to social separation. A simple two-choice behavioural test was administered to distinguish horses that would prefer the conspecific over the food reward and transient social separation to test the differences in behavioural responses in mares and geldings as indicators of the social dependence personality trait.

2. Material and methods

2.1. Animals

Twenty-four warmblood horses (17 geldings and seven mares), aged 9 – 20 years, were involved in testing. Routinely, the horses were daily paddocked in groups for at least 5h/day and were ridden on average for 5h/week. They were fed with three rations of rolled oats, barley and hay and water and salt blocks were available *ad libitum*. They were used for both riding lessons for groups of riders and for individual rides.

2.2. Two-choice test

Two tests characterizing the intensity of social dependence were performed: the two-choice test and the social isolation test. During both tests, the horses were visually separated from conspecifics present in the riding centre. First, the horses were tested in a two-choice preference test to classify them as “companion-motivated” (CM) or “food-motivated” (FM). Food was used in social motivation tests (Lansade et al. 2008, Lee et al. 2011), and feeding behaviour was a good indicator of social separation stress during weaning in the authors’ previous study (Górecka-Bruzda et al. 2015b). On the days of testing, the mares were not showing the signs of oestrus (e.g. tail raising, clitoral winking).

Twelve pairs of horses (one tested and one companion individual in a pair), previously selected by stable managers as being the most social with each other, were chosen. The selection of the pairs was based on observed allogrooming, grazing at close distances without mutual aggression as compared to other horses in the group. Before tests, the horses were conditioned to approach the hand-held bucket with treats (chopped carrots). All horses approached and were fed from the bucket. On the test day, two handlers led the horses into the familiar arena. The companion horse was held by one person while the tested horse was led 30m away from the companion horse. At a 15m distance from the tested animal, within the same line as the companion horse, stood the experimenter with a bucket (previously used for habituation) with treats. On a given signal, this person was shaking the bucket three times to emit a sound of moving carrots, and the tested horse was released to go to the chosen stimulus (companion horse or treats). The horse was allowed to

spend 10 seconds with its companion or take a mouthful of treats, and then it was taken back to its home box. According to the first choice of the horse, it was categorized as CM or FM. On the next testing, the roles of horses in pairs were switched. The testing of all 24 horses followed a semi-random design, assuring a minimum of three days apart between testing horses of the same pair.

2.3. Social dependence tests

The horses were subjected to social dependence tests two weeks later to validate the preliminary classification of the two-choice test using the social isolation test and the “passing” test with social stimuli with the conspecific (always the same gelding) passing nearby (adapted from Jezierski and Górecka, 1999 and Lansade et al., 2008). The protocol of the test is presented in Figure 1. The horses were led in pairs to a familiar arena (of about 250 m²) where feed was present within reach of the horses. They were released for 2 min, and one horse from the pair was then taken away. At the 40th minute, an audience horse (familiar, but not paired to the tested one) was led in sight of the tested horse (passing conspecifics test) within 1.5 m from the arena. The behaviour of the tested horse was recorded with a camera placed outside the arena and three observation periods (sessions) were analysed post-hoc. The behaviour during the first 10 min of social isolation was indicative of the intensity of reaction when the horse was deprived of the preferred companion (Session 1). The behaviour during 10 min after 30 min of isolation was informative of the possible habituation (Session 2). The behaviour during 10 min after the social stimulus (the horse passing nearby), was informative of the ease of re-evoking separation arousal by any other horse (Session 3, Fig. 1). Following this, the tested horse was taken back to its box. In total, each horse was observed for 30 min.

The behaviour representative of arousal and urge to join the conspecifics was recorded (e.g. Mal et al., 1991; Lansade et al., 2008). The durations (minutes) of standing still, walking, trotting, cantering, bucking, feeding, pawing and the occurrences of vocalizations (whinnies) and defecations (Table 1) were recorded and assessed in each 10-minute observation session.

154

155 Table 1. Behaviours observed during isolation test, sampling method and measurement units

Behaviour	Definition (measure)
Standing still	Standing motionless, including resting position; duration (minutes)
Walking	Slow four-beat gait; duration (minutes)
Trotting	Medium-speed two-beat gait; duration (minutes)
Cantering	Fast three-beat gait; duration (minutes)
Bucking	Throwing hind legs into the air (occurrence)
Whinnying	Vocalisation relative to calling; emitted to provoke a conspecific response; occurrence (occurrence)
Feeding	Eating the feed; duration (minutes)
Pawing	Repetitive movement of forelimb against the ground while staying in place; duration (minutes)
Defecation	Elimination; occurrence (occurrence)

156

157

158 2.4. Statistical analysis

159 Fisher's exact test was applied to compare the numbers of CM and FM horses in a two-choice test
160 and test the effect of sex of the tested horse and of the companion horse on the two-choice test
161 result (FREQ Procedure, SAS 9.4 Statistical system). Next, the effects of two-choice test result
162 (horse vs food), sex (gelding vs mare), session (1-3), and their interactions on behavioural variables
163 during social dependence tests were assessed. Due to the lack of normality of distribution of
164 behavioural variables, a generalized linear mixed model (GLIMMIX Procedure) with random effect
165 of the identity of the horse and Tukey's adjustment for repeatable measurements for a "session"
166 factor was applied. In two cases (cantering and pawing), the analysis did not converge when the

interactions were included in the model, so only the main effects were retained for these variables. The binomial distribution was assumed for feeding, standing and walking (duration related to session duration/ %), and a Poisson distribution was assumed for rare behaviours (whinnies and buckings). The results are presented as numbers and medians [Q1, Q3].

2.5. Ethics statement

The horses belonged to the University of Warmia and Mazury and to private owners who agreed to the study. They were under the care of two of the authors (MD and EJ). The procedures took place in a familiar environment and did not cause them any pain, suffering, or damage. The horses were clinically healthy, with any signs of sensory disorders, and no clinical signs of lameness or musculoskeletal injury. They demonstrated a comparable condition and athletic ability before and after the study. Since the horses were subjected to studies simulating circumstances regularly occurring in riding centres (transient separation for riding purposes) and only behavioural observations were carried out, non-invasive standardised experimentation imitating every-day practice was performed in accordance with European directive 2010/63/EU and Polish laws related to ethics in animal experimentation. All procedures were conducted following the Polish Animal Protection Act (21 August 1997).

3. Results

3.1. Two-choice test

During the two-choice test, six horses chose the companion (companion-motivated, CM), and 18 horses chose the treats (food-motivated, FM). The choice was skewed for mares as more mares than geldings preferred the companion over the food (CM: $N_{\text{mares}} = 4$, $N_{\text{geldings}} = 2$ and FM: $N_{\text{mares}} = 3$, $N_{\text{geldings}} = 15$, $P = 0.0381$). There was no effect of the sex of the companion horse on the choice

between the companion and the treats food (CM: $N_{\text{mares_companion}} = 1$, $N_{\text{geldings_companion}} = 5$ and FM: $N_{\text{mares_companion}} = 6$, $N_{\text{geldings_companion}} = 12$, $P = 0.6287$).

3.2. Social isolation test

The defecations occurred only occasionally and were excluded from analyses. The interaction of sex and session did not affect any of the studied behaviours during the social isolation test and was not further analysed. The effects of choice, sex, session, and the interaction of choice and sex, and choice and session are presented in Table 2.

Table 2. The effects (test statistics and P – values) of choice, sex and session and choice and the effects of interactions between sex and choice and choice and session on behaviours recorded during the social isolation test.

Effects		Choice	Sex	Session	Choice x Sex	Choice x Session
Variable (unit)		(CM vs. FM)	(mare vs. geldings)	(1 – 3)		
Feeding		$F = 13.7$	$F = 2.52$	$F = 1.77$	$F = 24.0$	$F = 4.71$
(minutes)		$P = 0.0004$	$P = 0.1177$	$P = 0.1794$	$P < 0.0001$	$P = 0.00124$
Standing	still	$F = 14.2$	$F = 2.58$	$F = 3.70$	$F = 0.00$	$F = 0.78$
(minutes)		$P = 0.004$	$P = 0.1133$	$P = 0.0305$	$P = 0.9677$	$P = 0.4627$
Walking		$F = 0.51$	$F = 0.04$	$F = 0.51$	$F = 5.72$	$F = 0.96$
(minutes)		$P = 0.4766$	$P = 0.8439$	$P = 0.6028$	$P = 0.0198$	$P = 0.3893$

Trotting (minutes)	F = 11.7 P = 0.0011	F = 3.87 P = 0.0535	F = 3.85 P = 0.0264	F = 2.05 P = 0.1574	F = 0.17 P = 0.8415
Cantering (minutes)	F = 25.38 P < 0.0001	F = 0.11 P = 0.7360	F = 1.36 P = 0.2631	Do not converge	Do not converge
Whinnies (occurrence)	F = 13.5 P = 0.0005	F = 0.31 P = 0.5808	F = 6.83 P = 0.0021	F = 0.04 P = 0.8951	F = 6.70 P = 0.0023
Bucking (occurrence)	F = 0.03 P = 0.8575	F = 5.01 P = 0.0288	F = 6.36 P = 0.0031	F = 1.22 P = 0.2735	F = 0.17 P = 0.8462
Pawing (minutes)	F = 9.25 P = 0.0034	F = 0.53 P = 0.4692	F = 0.19 P = 0.8321	Do not converge	Do not converge

204

205 3.2.1. Effect of choice

206 During the whole test, the behaviours of horses differed between CM and FM horses, the choice
207 has either the main effect or/and interaction effect was found (test statistics and P – values, Tab. 2
208 and 3). The CM horses were feeding (0 [0; 0.5] min) and standing (1 [0.5; 1.5] min) for less time
209 than FM horses (2.5 [0.5; 8.0] min and 2 [1.0; 4.0] min for feeding and standing, respectively).
210 Logically, they were more agitated than FM horses: they trotted (2.0 [1.5; 3.0] min) and cantered
211 1.5 [0.5, 2.0] min) longer (FM horses: 0 [0; 1.5] min and 0 [0; 0] min, for trot and canter,
212 respectively). They whinnied (2.0 [1.0; 3.0] times) and pawed (0.5 [0; 1.0] min) more than FM
213 horses (whinnies: 0 [0; 1.0] and pawing: 0 [0; 0] min). The time spent on feeding and walking, as
214 well as the occurrences of whinnies, also depended both on the sex of the horse (feeding, walking)
215 and the choice they made in the two-choice test (whinnies; described in sections 3.2.2 and 3.2.3).

216

217 Table 3. Behaviours shown by companion-motivated (CM) and food-motivated (FM) horses in
 218 three sessions following social separation (median [Q1; Q3] minimum, maximum).

	CM horses		FM horses			
Behaviour	Me [Q1; Q3]	Min – Max	Me [Q1; Q3]	Min – Max	t	P
Session 1						
Feeding ¹	0 [0; 0] A	0 – 2.0	3.5 [1.0; 8.0] BX	0 – 10.0	3.76	0.0004
Standing still ¹	0.25 [0. 1.0]	0 – 2.0	1.25 [0; 2.0]	0 – 5.0	2.24	0.0285
Walking ¹	2.75 [1.5; 4.0]	0.5 – 5.0	1.75 [0.5; 5.0]	0 – 7.0	0.30	0.9997
Trotting ¹	2.5 [2.0; 4.0]	0.5 – 5.0	0.5 [0 ; 1.5]	0 – 5.5	1.59	0.1179
Cantering ¹	1.75 [1.5; 3]	0 – 4.0	0 [0; 0]	0 – 1.0	n.e.	n.e.
Whinnying ²	2.5 [1.0; 4.0] A	1.0 – 5.0	0 [0; 0] B	0 – 1.0	3.84	0.0003
Bucking ²	0.5 [0; 2.0] A	0 – 3.0	0 [0; 0] B	0 - 12	0.69	0.4952
Pawing ¹	0.75 [0; 2.0]	0 – 3.0	0 [0; 0]	0 – 0.5	n.e.	n.e.
Session 2						
Feeding ¹	0.5 [0; 1.5]	0 – 6.0	2.75 [1.0; 8.0] Bx	0 – 10.0	2.30	0.0250
Standing still ¹	1.5 [1; 2.5] <i>a</i>	1.0 – 3 .0	2.25 [1.0; 4.5] <i>b</i>	0 – 7.0	1.93	0.0577
Walking ¹	3.25 [2.5; 6.0]	1.0 – 6.0	2.0 [0; 4.0]	0 – 7.0	1.46	0.1491
Trotting ¹	1.5 [1.0; 2.0]	0 – 3.0	0 [0; 0.5]	0 – 3.0	2.58	0.0121
Cantering ¹	0.75 [0.5; 1.5]	0 – 1.5	0 [0; 0]	0 – 2.0	n.e.	n.e.
Whinnying ²	1.5 [1.0; 2.0] <i>a</i>	1.0 – 2.0	0 [0; 1.0] <i>b</i>	0 – 3.0	1.99	<i>0.0510</i>
Bucking ²	0 [0; 0]	0 – 1.0	0 [0; 0]	0 – 3.0	0.03	1.0000
Pawing ¹	0.25 [0; 1.0] A	0 – 2.0	0 [0; 0] B	0 – 0.5	n.e.	n.e.
Session 3						
Feeding ¹	0 [0; 0.5]	0 – 9.0	1.0 [0 – 3.5] BYy	0 – 10.0	0.78	0.4394
Standing still ¹	0.5 [0.5; 1.5]	0 – 2.0	2.25 [1.0; 4.0]	0 – 6.5	2.86	0.0058
Walking ¹	3.0 [2.0; 4.0]	1 – 5.0	3.0 [2.0; 4.5]	0 – 6.0	0.78	0.9998
Trotting ¹	3.0 [2.0; 3.5]	0 – 3.5	0.5 [0; 2.0]	0 – 3.0	2.44	0.0176
Cantering ¹	1.25 [1.0; 2.0]	0 – 2.0	0 [0; 0]	0 – 1.0	n.e.	n.e.
Whinnying ²	2.0 [1.0; 3.0] A	1.0 – 4.0	1 [0; 3.0] B	0 – 5.0	0.13	0.8970
Bucking ²	0 [0; 1.0]	0 – 2.0	0 [0; 1.0]	0 – 7.0	0.04	1.0000
Pawing ¹	0.25 [0; 0.5]	0 – 1.5	0 [0; 0.5]	0 – 0.5	n.e.	n.e.

¹ minutes; ² occurrence; Session 1: 10 min following social separation; Session 2: 30-40 min following social separation; Session 3: 10 min following social stimulus in 40th min; A, B – values differ between CM and FM horses at $P < 0.05$; X, Y – values differ between sessions at $P < 0.05$; bolded values are significant at $P < 0.05$; values in italics are significant at $P < 0.1$ (tendency); n.e. values not estimated (no convergence when the interaction was included in the model)

3.2.1. Effect of sex

The sex of the horse had a significant effect on feeding, walking, trotting and bucking (Fig. 3). During the separation and passing of conspecific tests, the mares presented more arousal than geldings (Tab. 2).

CM mares fed very briefly during the test (0.5 [0; 0.5] min) compared to any other group of horses: CM geldings (1.25 [0; 6.0] min), FM geldings 1.5 [0.5; 7.0]) or FM mares (5.5 [3.5; 9.0] (Fig. 3A). CM mares also walked more (3.75 [2.5; 5.0] min) than FM mares (0.5 [0; 2.0] min, $t = 2.13$, $P = 0.0368$) or FM geldings (3.0 [1.0; 4.0] min, $t = 2.03$, $P = 0.0466$; Fig. 3B). Irrespective of the choice they made in two-choice test, the mares trotted more than geldings (mares: 1.0 [0; 2.0] min, geldings 0 [0; 1.0] min, Fig. 3C). In turn, the geldings were bucking (Fig. 3D) more often (0 [0; 1.0] times) than mares (0 [0; 0] times, Tab. 2).

3.2.2. Effect of session

Generally, the behaviour indicating the separation arousal decreased after 30 minutes of isolation (Session 2), indicating calming process/habituation when isolated from other horses. However, arousal was also shown when it concerned the horse from the pair (Session1, after separation with the preferred individual) or any other horse (Session 3, Figure 2). Social isolation and the conspecific passing by provoked the horses to spend a longer time on locomotor behaviours (trotting and cantering).

The horses differed in feeding behaviour between Sessions 1 – 3 (Tab. 2 and 3), but while CM horses were more aroused and only occasionally fed irrespective of the session (Session 1 vs. 2, $t = 2.09$, $P = 0.9605$; Session 1 vs. 3, $t = 2.19$, $P = 0.2575$ and Session 2 vs. 3, $t = 0.13$, $P = 1.0000$), FM horses ate willingly during the first two sessions (Session 1 vs. 2, $t = 0.41$, $P = 0.9605$), they then slightly reduced their interest in feed (Session 1 vs. 3, $t = 3.69$, $P = 0.0059$ and Session 2 vs. 3, $t = 2.90$, $P = 0.0551$; Tab. 3).

The horses stood still for a shorter time just after the separation with a companion horse in Session 1 (1.0 [0.25; 2.0] min) than after 30 minutes of isolation during Session 2 (1.75 [1.0; 4.5] min, $t = 2.62$, $P = 0.0290$), but not shorter than in Session 3, when again they experienced contact with an audience horse (1.75 [0.75; 3.35] min, $t = 1.14$, $P = 0.49$). The duration of standing still did not differ between Sessions 2 and 3 ($t = 1.52$, $P = 0.2878$). When the horses were exposed to social challenge, they were more locomotor active, they trotted more in Session 1 (1.0 [0; 2.0] min, $t = 2.62$, $P = 0.0293$) and Session 3 (1.0 [0; 2.75] min, $t = 2.42$, $P = 0.0477$) than in Session 2 (0 [0; 1.5] min), while sessions 1 and 3 did not differ ($t = 0.22$, $P = 0.9738$).

4. Discussion

A simple two-choice behavioural test determined that horses reacted with different intensities to social separation, as shown by a social separation and isolation test with an additional social stimulus (conspecific passing nearby). The horses presented alertness in isolation with increased locomotor behaviour, producing loud calls (whinnies) and reduced interest in available feed. The current results indicate that mares, showing long bouts of movement representing the intention to reunite social companions, are more socially dependent compared to castrated male horses.

4.1. Social dependence

The fact of a relationship between the reaction in the two-choice task and in the isolation test confirms the existence of a “social” temperamental trait that presents stability across situations. At a given time point of the tests, the horses could have been differently motivated to being in the company of another horse. They had different life experiences and types of use (individual or/and group riding) that could have played an important role in habituation to isolation. However, the variability of their responses to isolation was predictable by the two-choice test result, and it can be proposed that this simple test could be used for a preliminary assessment of the intensity of equine social dependence.

Social dependence has rarely been studied as a separate trait, although in many personality studies (e.g. LeScolan et al. 1997; Wolf et al. 1997; Visser et al. 2001; Seaman et al. 2002; Napolitano et al. 2008, Krueger et al. 2021) horses subjected to a battery of tests were also individually tested for “reactivity,” i.e. the intensity of arousal when separated from conspecifics. Thus, in many cases, the open field test measured, in fact, the cumulated effect of social isolation and the level of “reactivity”, which is a more general tendency engaged in any response causing arousal. In turn, in the studies of Lansade et al. (2008), the “reactivity to social isolation and association with conspecifics” or “gregariousness” temperamental trait was confirmed in four tests measuring the reaction of horses when isolated and separated from conspecifics, as well as the motivation to stay close to their mates (attraction towards and passing next to conspecifics). As in the present study, the locomotor activity and vocalizations, as well as the preference to stay close to another horse, were indicative of high social motivation in young horses. This trait was not related to other personality traits, such as fearfulness or reactivity to humans, so the authors suggested “gregariousness” was a separate temperamental trait related to social aspect of life in horses.

The current study confirms that while horses are socially dependent, they are dependent with different intensities. Following the isolation test, the CM horses showed the urge to re-join other horses and whinnied, which is behaviour specific to social behaviour, indicative of localization of conspecifics when at a far distance or out of view (Fraser 1992, Lemasson et al. 2009). However, it should be admitted that in the two-choice tests, the companion was in view of tested horses, so

its reassuring presence could prevent the full expression of feed vs companion choice as it would be when the companion was out of sight. It has been proven that social animals present different behavioural responses when alone and when in social groups (Schuett and Dall 2009, Webster and Ward 2011). In spite of this, the horses distinguished by the two-choice test also differed in arousal-indicative (increased locomotor activity and calls) and relaxation-indicative (feeding) behaviour when alone.

The present results are in line with a previous study on social companion vs feed motivation, which showed that most of the horses chose the feed (Lee et al. 2011). This shows that horses may have some tolerance to social deprivation when habituated to individual stabling or use and when offered an attractive resource. In turn, the studies of Boissy and Dumont (2000) showed that ewes, when offered grazing at a preferred site, will only leave the social group when accompanied by the conspecifics. Houpt and Houpt (1988) demonstrated that when devoid of social company, mares were three times more active and spent 10% less time eating than mares with visual contact with other horses. The sheep from the Dumont and Boissy (2002) study were more likely to leave the group when with familiar than when with non-familiar individuals. In the current study, both the separation from a preferred and a non-preferred (but familiar) horse was sufficient to provoke visible arousal in the tested individual. This clearly demonstrates that even a very short exposition to the conspecific is able to produce a similarly intense behavioural response even after a transient calm period (Session 2) in FM horses. Moreover, the fact that the CM horses were constantly more aroused during all sessions indicates that some horses may be more prone to social deprivation than others and more severely respond to individual stabling. This shows how important social company is to horses as well as for those that were feed-oriented.

4.2. Sex and social behaviour

The intensity of social dependence could be related to the sex of the horse since more females' first choice was another horse rather than food. In the equestrian milieu, the sex of the mount is

perceived as important for the performance, safety and satisfaction from the ride, and geldings are usually assessed as calm, trainable, reliable and predictable, while mares are assessed as the least suitable for trail-riding, dressage and show-jumping disciplines (Fenner et al. 2019). In high-level competitions, most equine athletes are male (Wulf et al. 2013). On the other hand, in a survey of a highly numerous population of equestrians, only two items relative to handling were revealed to be different between mares and geldings (Aune et al. 2020). Similarly, females were found to be more alert in isolation tests in Wolff et al.'s study (1997) of riding school horses. Moreover, young females (fillies) were found to be more reactive than castrated males in a study by Duberstein and Gilkeson (2010). However, while some studies have shown that females are more stress-prone (Jezierski et al. 1999; Schmidt et al. 2010; Górecka-Bruzda et al. 2015b), other studies did not confirm this but rather showed that sexes differ in how behavioural and physiological measures of stress are exhibited (Wulf et al. 2013, 2018). All of these studies addressed the sex effect on anxiety, fear and stress reactivity, but not the social dependence/sociability. While studies on the social aspect of personality in horses are scarce, the effect of sex on the strength of social dependence/sociability was even less studied. Although changes in personality across the main developmental phase, including sexual maturity, were found in several animal studies (reviewed by Cabrera et al. 2021), none were related to socially-related personality traits. However, the authors' studies on yearling foals indicate that juvenile post-weaned foals differ in social behaviour when reunited with their mothers and familiar mares, with females showing more bouts and durations of social behaviour (Lansade et al. in submission). The tendency to stay in close proximity to the dam and groom with her was also more typical behaviour of female foals (Rho et al., 2007; Heitor and Vicente, 2008). Moreover, the fillies were under the influence of their sire; they were playing more with the colts in the presence of their father, while they had more female playmates in herds without a stallion (Šandlová et al. 2021).

Therefore, our results correspond to the behavioural biology of horses, since in free-living equine groups, the mares form the core of the herd with adult males. Although they are engaged in social behaviour (Šandlová et al. 2020 and 2021), they have the guarding and reproductive functions

(Cameron et al. 2009; Kaseda et al. 1995). It was proven that, if possible, mares keep to each other for their whole lives (Jaworska et al. 2020), so equine social dependence must be strong enough to form and keep such long term bonding (Cameron et al. 2009; Stanley et al., 2018). It was also found that semi-feral and domestic mares form stable herds in the absence of stallions (Sigurjónsdóttir et al. 2003; Jaworska et al. 2020). Furthermore, the mares in familiar groups were observed to display specific social defence behaviour (spatial group formation) in response to the vocalisation of predators (Janczarek et al. 2020).

Finally, it has to be underlined that the equine population is very specific as per the sex bias of animals used for riding. The majority of individuals are either mares or castrated males, while intact males, due to sexual drive and resulting behaviour making recreational riding problematic, are used at a very small rate and, importantly, are practically denied social contact. This calls into question the welfare of socially isolated stallions that are instinctively engaged in (but practically denied) important social roles.

The present study, relating in part to castrated males, can be relative to true sex differences in the social behaviour of horses. While biomedical male-biased studies the results relates mostly to males, ironically, the equine studies, based on females and castrated males, very rarely consider stallions in comparisons. It can be concluded that the results on true sex differences in various traits of horses are still scarce.

5. Conclusions

It is proposed that the mares can, in general, present stronger social dependency. Considering, however, that there are also feed-oriented mares and that this orientation could be purportedly typical to dominant mares, more in-depth experimental studies are needed to confirm the preliminary observations. There is a need to consider sex in equine studies to avoid generalising the outcomes based on sex-biased results.

The use of this simple two-choice test as another method of assessing social dependence in horses is proposed. Since the present study involved horses experienced in recreational riding, it is hypothesised that this test could be used in practice when buying a horse of unknown strength of reaction to social separation.

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547

548 **Figure captions**

549

550 Fig.1. The protocol of the social isolation test.

551 Fig. 2. Time spent on feeding (A), walking (B), trotting (C) and the occurrence of bucking (D) in
552 CM and FM geldings and mares in the social isolation test. A, B values differ at $P < 0.01$; a, b
553 values differ at $P < 0.05$. The data are presented as medians with inter-quartile ranges, the whiskers
554 show the min-max range.