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

2

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17

18 **Abstract**

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

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

39

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

41

## 42 **1. Introduction**

43

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

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

56 Several approaches to distinguish personality in horses have been adopted. Some concepts involve  
57 all psychological properties as a whole, calling them interchangeably either personality or  
58 temperament (e.g. Visser et al. 2001; Momozawa et al. 2007; Lloyd et al. 2007), while others  
59 consider temperament and character to be separate qualities of equine personality (e.g. König von  
60 Borstel 2013; Suwała et al. 2016). Several personality traits/dimensions have been distinguished in  
61 horses, including sociability/gregariousness (LeScolan et al. 1997; Lloyd et al. 2007; Lansade et al.  
62 2008). However, sociability or gregariousness could be interpreted as a willingness of animals to  
63 gather in groups, while the cited authors also tested the reaction to social separation/isolation, i.e.  
64 social dependence. As horses are social animals that live in stable groups for life (Jaworska et al.,  
65 2020), the need to be in proximity of conspecifics, especially familiar ones, is usually very high  
66 (Søndergaard et al., 2011). This need is expressed in social separation anxiety, alertness,  
67 vocalization and often extreme motivation to re-join conspecifics. Therefore, we will use the term  
68 "social dependence" to define the mental feature of an animal exhibiting such anxiety response to  
69 social separation and following isolation. As it is an important component of equine herd cohesion,  
70 it is expected that horses will react negatively to social separation. The tests for the reactivity of the  
71 horse when isolated, separated and reunited (Lansade et al. 2008) were used for the assessment of  
72 social dependence. The two-choice preference tests for social companionship, outdoor space and  
73 activity, human touch or food were used to evaluate the motivation for specific resources important  
74 for equine wellbeing (e.g. Edouard et al., 2009; Lee et al., 2011; Mejdell et al., 2016; Kieson et al.  
75 2020), so it was used in the present study as a preselection tool of horses as being less or more  
76 socially dependent.

77 The effect of sex on the reactivity of horses seems an underestimated aspect, specifically for the  
78 social dependence personality trait. This special aspect of the equine personality seems to be  
79 strongly affected by sex, as feral horses live in female-dominated social groups (Keiper 2005).  
80 Social bonds between semi-feral female horses have been reported to be lifelong (Jaworska et al.,  
81 2020). Although some studies confirm practical observations that females are more easily agitated  
82 (Jeziarski et al., 1999; Wolff et al., 1997; Quberstein and Gilkenson, 2010; Schmidt et al., 2010;  
83 Sackman and Houpt, 2019), other studies have found the opposite (Roberts et al. 2016), very little  
84 experimental and field studies have approached the aspect of sex differences in equine personality.  
85 Rather, the sex/gender effect of the horse was avoided by using sex-balanced groups (Visser et al.  
86 2001; Lansade et al. 2008) or was neglected when discussing the traits/dimensions of personality  
87 (reviewed by König von Borstel, 2013). Since many biomedical studies using animal models used  
88 only males to avoid the “confounding” effect of female sex hormones (e.g. Beery and Zucker 2011;  
89 Kokras and Dalla 2014), the present study addressed the differences in social dependence between  
90 mares and castrated males (geldings) to deepen the knowledge of the potential effect of sex on  
91 social dependence in horses.

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

98

## 99 **2. Material and methods**

100

### 101 **2.1. Animals**

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

107

## 108 2.2. Two-choice test

109 Two tests characterizing the intensity of social dependence were performed: the two-choice test  
110 and the social isolation test. During both tests, the horses were visually separated from conspecifics  
111 present in the riding centre. First, the horses were tested in a two-choice preference test to classify  
112 them as “companion-motivated” (CM) or “food-motivated” (FM). Food was used in social  
113 motivation tests (Lansade et al. 2008, Lee et al. 2011), and feeding behaviour was a good indicator  
114 of social separation stress during weaning in the authors’ previous study (Górecka-Bruzda et al.  
115 2015b). On the days of testing, the mares were not showing the signs of oestrus (e.g. tail raising,  
116 clitoral winking).

117 Twelve pairs of horses (one tested and one companion individual in a pair), previously selected by  
118 stable managers as being the most social with each other, were chosen. The selection of the pairs  
119 was based on observed allogrooming, grazing at close distances without mutual aggression as  
120 compared to other horses in the group. Before tests, the horses were conditioned to approach the  
121 hand-held bucket with treats (chopped carrots). All horses approached and were fed from the  
122 bucket. On the test day, two handlers led the horses into the familiar arena. The companion horse  
123 was held by one person while the tested horse was led 30m away from the companion horse. At a  
124 15m distance from the tested animal, within the same line as the companion horse, stood the  
125 experimenter with a bucket (previously used for habituation) with treats. On a given signal, this  
126 person was shaking the bucket three times to emit a sound of moving carrots, and the tested horse  
127 was released to go to the chosen stimulus (companion horse or treats). The horse was allowed to

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

132

### 133 2.3. Social dependence tests

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

150 The behaviour representative of arousal and urge to join the conspecifics was recorded (e.g. Mal et  
151 al., 1991; Lansade et al., 2008). The durations (minutes) of standing still, walking, trotting,  
152 cantering, bucking, feeding, pawing and the occurrences of vocalizations (whinnies) and  
153 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

<b>Behaviour</b>	<b>Definition (measure)</b>
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

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

171

## 172 2.5. Ethics statement

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

184

## 185 **3. Results**

186

### 187 3.1. Two-choice test

188 During the two-choice test, six horses chose the companion (companion-motivated, CM), and 18  
189 horses chose the treats (food-motivated, FM). The choice was skewed for mares as more mares  
190 than geldings preferred the companion over the food (CM:  $N_{\text{mares}} = 4$ ,  $N_{\text{geldings}} = 2$  and FM:  $N_{\text{mares}} =$   
191  $3$ ,  $N_{\text{geldings}} = 15$ ,  $P = 0.0381$ ). There was no effect of the sex of the companion horse on the choice

192 between the companion and the treats food (CM:  $N_{\text{mares\_companion}} = 1$ ,  $N_{\text{geldings\_companion}} = 5$  and FM:  
 193  $N_{\text{mares\_companion}} = 6$ ,  $N_{\text{geldings\_companion}} = 12$ ,  $P = 0.6287$ ).

194

195 3.2. Social isolation test

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

200

201 Table 2. The effects (test statistics and P – values) of choice, sex and session and choice and the  
 202 effects of interactions between sex and choice and choice and session on behaviours recorded  
 203 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)	<b>P = 0.0004</b>	P = 0.1177	P = 0.1794	<b>P &lt; 0.0001</b>	<b>P=0.00124</b>
Standing still	F = 14.2	F = 2.58	F = 3.70	F = 0.00	F = 0.78
(minutes)	<b>P = 0.004</b>	P = 0.1133	<b>P = 0.0305</b>	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	<b>P = 0.0198</b>	P = 0.3893

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

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

219

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

225

### 226 3.2.1. Effect of sex

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

230 CM mares fed very briefly during the test (0.5 [0; 0.5] min) compared to any other group of horses:  
231 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).  
232 CM mares also walked more (3.75 [2.5; 5.0] min) than FM mares (0.5 [0; 2.0] min,  $t = 2.13$ ,  $P =$   
233  $0.0368$ ) or FM geldings (3.0 [1.0; 4.0] min,  $t = 2.03$ ,  $P = 0.0466$ ; Fig. 3B). Irrespective of the choice  
234 they made in two-choice test, the mares trotted more than geldings (mares: 1.0 [ 0; 2.0] min,  
235 geldings 0 [0; 1.0] min, Fig. 3C). In turn, the geldings were bucking (Fig. 3D) more often (0 [0;  
236 1.0] times] than mares (0 [0; 0] times, Tab. 2).

237

### 238 3.2.2. Effect of session

239

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

246 The horses differed in feeding behaviour between Sessions 1 – 3 (Tab. 2 and 3), but while CM  
247 horses were more aroused and only occasionally fed irrespective of the session (Session 1 vs. 2,  $t$   
248 = 2.09,  $P = 0.9605$ ; Session 1 vs. 3,  $t = 2.19$ ,  $P = 0.2575$  and Session 2 vs. 3,  $t = 0.13$ ,  $P = 1.0000$ ),  
249 FM horses ate willingly during the first two sessions (Session 1 vs. 2,  $t = 0.41$ ,  $P = 0.9605$ ), they  
250 then slightly reduced their interest in feed (Session 1 vs. 3,  $t = 3.69$ ,  $P = 0.0059$  and Session 2 vs.  
251 3,  $t = 2.90$ ,  $P = 0.0551$ ; Tab. 3).

252 The horses stood still for a shorter time just after the separation with a companion horse in Session  
253 1 (1.0 [0.25; 2.0] min) than after 30 minutes of isolation during Session 2 (1.75 [1.0; 4.5] min,  $t =$   
254 2.62,  $P = 0.0290$ ), but not shorter than in Session 3, when again they experienced contact with an  
255 audience horse (1.75 [0.75; 3.35] min,  $t = 1.14$ ,  $P = 0.49$ ). The duration of standing still did not  
256 differ between Sessions 2 and 3 ( $t = 1.52$ ,  $P = 0.2878$ ). When the horses were exposed to social  
257 challenge, they were more locomotor active, they trotted more in Session 1 (1.0 [0; 2.0] min,  $t =$   
258 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;  
259 1.5] min), while sessions 1 and 3 did not differ ( $t = 0.22$ ,  $P = 0.9738$ ).

260

#### 261 **4. Discussion**

262

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

269

##### 270 4.1. Social dependence

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

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

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

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

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

320

#### 321 4.2. Sex and social behaviour

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

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

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

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

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

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

369

## 370 **5. Conclusions**

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

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

380

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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.