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Abstract

Camel management has been changing in recent years from an extensive to a semi-intensive or intensive system, particularly for breeding bulls and dairy dromedary camels. Captivity may affect animal welfare, and low libido is the major complaint for housed breeding bulls. Since welfare status could also affect reproductive performance, the aim of this study was to evaluate different management practices on behavior, particularly on sexual behavior, and to identify some behavioral needs of male dromedary camels reared for semen collection. The effects of the following management systems on their behavior were compared: i) traditional: housing in a single stall for 24 hours (H24), ii) housing in a single stall for 23 hours with one hour free in the paddock (H23) and iii) housing in a single stall for 22 hours and 30 min with 1 h paddock time and 30 min exposure to a female camel herd (ExF). During the trial, blood cortisol concentrations were assessed and camels werefilmed dailyfor thirty minutes in the mornings and during a female passage in the evenings. Videos were analyzed in order to fill out a focal sampling ethogram and to score sexual behavior. As a result, there were no differencesbetweenthe H24 and H23 systems, whereas ExF had a significant positive impact ontheir sexual behavior score and behavioral repertoire, further reducing cortisol levels. Overall, it seems that male dromedary camel welfare status improves when theirbehavioral needs for social interaction and movement are satisfied.

Keywords: dromedary camel, husbandry, sexual behavior, cortisol, welfare.

Introduction

Camels (*Camelus dromedarius*) are seasonal breeders, with mating occurringduring the coolest months of the year; indeed,thisshort breeding season is one of the main factors limiting reproductive performance (Vyas et al. 2001). Consequently, male camel husbandry has recently been changing towards a more intensive system where they are kept isolated and used for programmed mating or artificial insemination (Skidmore et al. 2013).

During the breeding season, male camels exhibit morphological, behavioral and endocrinological peculiarities, increasing pacing and anxiety and becoming extremely restless and aggressive (Deen 2008). Due to this aggressiveness toward other males and humans, rutting camels are traditionally reared tied with ropes in little pensand/or kept in single stalls (Abu-Zidan et al. 2012).

Captivity imposes different kind of constraints on the animal (e.g. limited space, social isolation) that could affectits behavioral repertoire and welfare status (Dawkins 2003; Christie et al. 2006). Under intensive management, animals are in fact confronted with a wide range of potentially provocative environmental challenges (potential stressors) that may adversely affect the animal's life (Morgan and Tromborg 2007). During stress conditions, blood glucocorticoid levels increase and this could impair reproductive aspectssuch as libido and fertility (Phillips et al. 1989, Orr et al. 1994, Wingfield and Sapolsky 2003).

A feral camel bull would naturally roam wide areas of land, moving over pastures with his female herd (Dörges et al. 1992), by contrast,a camel bull, kept in isolation during intensive management, could show abnormal behaviors (Padalino et al. 2014). In addition, male camels show earlier sexual behavior and higher mating ability under field conditions, compared with housed males (Abdel Rahim & Nazier, 1992). Consequently, the major complaints for housed camels bred for semen collection are low libido and mating desire, short breeding season and high variability in terms of semen quality and quantity (El-Hassanein 2003; El-Bahrawy 2005).

Low sexual libido is also a common complaint in breeding stallions, if they are bred in poor welfare conditions (Stout 2005). Davies Morel (2003) stated that suchlow reproductive performances could be overcome by alleviating boredom, providing social interaction and exercise. Indeed, it had already been proved that basic fitness and muscle tone aids in maintaining the physical and psychological well-being of a stallion, with positive effects on libido, performance and behavior (Dinger and Noiles 1986). Moreover, McDonnel (2000) suggested that placing the stallion under natural light, with the chance to get both exercise and social interaction with mares, could improve reproductive efficiency and fertility, overcoming specific breeding problems.

The effect of management on sexual behavior has been poorly investigated in camels, except by Vyas et al. (2001) and Bhakat et al. (2005) who first proposed a method for augmenting the rut of male camels, out of the breeding season, byparading the male sire in front of the female herd.

Hypothesizing that management practices and sexual arousal of bulls could influence their welfare and libido, the aim of this study was to evaluate the effect of three different management practices on behavior, particularly on sexual behavior, and to begin the process of identifying the behavioral needs of Maghrebi male dromedary camels reared for semen collection.

Material and Methods

Animals and management systems

Four clinically healthy male dromedary camels (*Camelus dromedarius*), ranging in age from 5 to 8 years, with a mean body weight (BW) of 526 ± 25 kg and good body condition score (BCS = 3.5 ± 0.3 arbitrary units; from 0 to 5 according to Faye et al. (2001)) and one non-pregnant female camel (10 years old, 420 kg BW, and 3.0 BCS) were used for this study. All animals were identified by ear tags. The camels had been reared at the Arid Lands Institute's experimental station in Médenine, Tunisia (33° 30' N, 10° 40' E), 18 m above sea level.

In summer, the bulls were kept in a single open-air paddock shaded by trees whereas, starting fromOctober, before the beginning of the breeding season, they were put into single stalls (Height = 3 m, Length = 5 m and Width = 3 m) with sand floors. They were tethered with a rope on the fetlock of the foreleg and were able to walk around inside the stall. The stalls were located far from the females' pen, preventing them from seeing and touching any dams; the gates of the stable pointed eastwards, facing an open-air paddock and with small windows on the opposite wall.

During this trial, the male dromedary camels were randomly tested in three different management systems: i) traditional: housed in single stalls for the whole day (H24), ii) housed in the same stall for 23 hours, plus1 hour free in a paddock (H23) or iii) housed in the same stall for 22 hours and 30 min, with 1 hour of paddock time and 30 min from 8:00 to 8:30 a.m. in a little pen adjacent to the female herd's pen (ExF). The paddock lies in front of the stable where the stalls are located and measures 250 m², whereas the little pen adjacent to the female herd's pen measures 150 m² and is bordered by a 130 cm-high wall dividing the two pens. Females were free to move and to touchthe males with their heads.

One hour of paddock time was chosen, as set out in a study by Freire et al. (2009) who proved that one hour or regular exercise in a paddock has positive effects on horse welfare. Moreover, 30 minutes of exposureto females had already been adopted by Bhakat et al. (2005) to enhance the male camel breeding season.

Each experimental condition lasted 7 days and was preceded by a habituation week, so the whole trial lasted six weeks (three weeks for the habituation period and three weeks of experimental situations), from the middle to the end of the breeding season.

The camels were fed daily with 5 kg oat hay at 9:00 a.m. and 3 kg concentrate supplement based on barley (60.0%), wheat bran (17.5%), olive cake (17.5%) and a mineral and vitamin complex (5.0%) at 3:00 p.m. (Table 1). Feeding quantity and quality remained constant throughout the experiment. The diet met the maintenance requirements as set by Laudadio et al. (2009), and water was made available once every two days.

During the trial, the bulls were used for semen collection twice weekly. They were well accustomed to this practice and to the traditional husbandry system, so we only changed the management system in accordance with the experimental protocol.

The entire study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. No special permission for behavioral research on wild animals such as this study is required in Italy.

Behavioral parameters and processing

In each management system, the four males were filmed by a video-camera (Sony Camcorder digital video) for30 min every morning at the same time from 8:00 to 8:30 a.m. for 7 days in each experimental condition, without being disturbed by the operator. Moreover, during each experimental condition every day (Monday to Sunday) at 6:30 p.m., the female camel was used to evaluate the sexual behavior of captive males according to Padalino et al. (2013); the female camel was brought into the paddock in front of the males'stalls and placed with her rear end attached to the gate of each sire. During the parade, the male was able to reach and smell/touch the female's perineumwith his nose. The female was presented to each male, sire by sire, for 4 min; the first parade lasted 16 min in overall, and three parades were performed daily for a total of 48 min.

The videos were recorded every morning for 30 min, and then analyzed by two expert ethologists, who filled out a focal animal sampling ethogram, defined as the sampling method whereby the recorder chooses one individual and records all behaviors performed by the individual in a specified time window (Altmann 1974). The duration of the following behavioral states was noted down: feeding, rumination, resting, standing, walking and looking outside. The occurrence of the following behavioral events was also recorded: leg movements, number of steps, sound emission, defecation, urination, tail flapping, opened legs, yawning, teeth grinding, *blatering*, sniffing and flehmen.

Moreover, while watching the videos of the female parades, the two experts scored the male camel sexual behavior (Table. 2) in accordance with the method proposed by Padalino et al. (2013) and subsequentlyused by Fatnassi et al. (2013).

Blood sampling and analysis

On Monday and Thursday of each experimental week, blood samples were collected by the same operator to evaluate cortisol concentration, while camels were in resting condition in their stalls. Blood samples were collected from the jugular vein under aseptic conditions directly into Venoject® tubes (Terumo Europe N. V. 3001 Leuven, Belgium) with lithium heparin and were kept in ice until plasma was separated within 2 h of collection by centrifugation at 4°C for 15 min at 3000 rpm. Plasma samples were stored at -20°C until analyzed.The plasma cortisol concentration was assessed in duplicate by Liquid chromatography-mass spectrometry (LC/MS).

Statistical analyses

Data were normally distributed. Cortisol concentrations were statistically analyzed through an analysis of variance using the Generalized Linear Model (GLM) procedure (SAS, version 9, 1999), where the independent variable was the management system (H24, H23, ExF). By contrast, the data related to behavioral parameters (both duration and occurrence) and sexual behavioral score were subjected to repeat measures of analysis of variance using the GLM procedure (SAS, version 9, 1999). The independent variables were the management system (H24, H23, ExF), the period of observation (from Monday to Sunday) and the interaction between those variables. Tukey's post hoc test was used to perform statistical multiple comparison. The *p*-level was set at 0.05. All data were expressed as quadratic mean and mean standard error.

Results

Cortisol concentration slightly decreased among the three different management systems (H24: 18.8 ± 2.0; H23: 15.9 ± 2.0; ExF: 14.1 ± 2.0 ng/ml), but there was no statistical difference (df = 2; F = 1.27; P = 0.3) (Fig. 1).

No significant difference was observed in any parameter for day (from the first to the seventh day of the week) (df = 6; F = 1.53; P = 0.18) and in the interaction between day and experimental condition (df = 12; F = 1.82; P = 0.06). On the other hand, the effect of the experimental condition was significant (df = 2; F = 116.30; P < 0.0001). Concerning the duration of the behavioral pattern, there was no difference among the first and the second management system, but both were significantly different from the third (feeding P = 0.0004; rumination P < 0.0001; standing P < 0.0001; walking P < 0.0001), except for resting that was not significant (H24: 10.0 ± 5.9; H23: 13.8 ± 5.9; ExF: 0.1 ± 5.9 sec; P = 1.0).

Under ExF, during morning observation, males spent most of the time looking outside at the females (P < 0.0001) (Fig. 2).

Regarding the frequency of behavioral patterns studied (Table 3),as camels were more active and stimulated in ExF, the occurrence of all the events was significantly higher in ExF compared with H24 and H23 (P<0.01).

Finally, the sexual behavior score (SBS) was also affected by management system; during the ExF week, the average SBS was 3.2 ± 0.1, significantly higher than for H24 (2.7 ± 0.1; P = 0.02) and H23 (2.8 ± 0.1; P = 0.02) (Fig. 3).

Discussion

In the present study, we compared the effects of three housing systems on male camel physiology and sexual behavior. It is well documented that welfare status can be assessed by four broad criteria: behavior, physiology, health and production (Dawkins 2003), while it is also known that animals housed in artificial habitats are confronted by a wide range of potentially provocative environmental challenges that could affect their endocrine system (Morgan and Tromborg, 2007). Camels housed all daylong in single stalls (H24) reported a blood cortisol concentration twice the normal value range, as previously reported in Indian male dromedary camelsby Kataria and Kataria (2010) and Kataria et al. (2010). This is likely due to the traditional husbandry system (H24) being more stressful for the animals. Nevertheless, cortisol concentration showed a downward trend from H24 to ExF, indicating that both freedom of movement in an open paddock (H23) and the freedom to express normal social behavior,interacting with females (ExF), had a positive impact, alsoon the animals' well-being. This is in accordance with McGlone et al. (2004) who reported that cortisol concentrations were higher in gestating sows kept in stalls compared with those housed in pens. It is also noteworthy that the level of blood cortisol in male camels reared in the ExF system became more similar to those reported by Bhakat et al. (2005) for male dromedary camels exposed to females during the Indian rutting season.

Underthe H24 and H23 systems, the animals spent the majority of their time ruminating, standing and feeding. Rumination is an important health indicator and thus it is important for welfare (Schwan 2011) but it was also defined by Hoyer (2013) as an anti-boredom activity caused by captivity and social isolation. In ExF, a significant change in behavior repertoire was observed: the camels showed a reduction in feeding and rumination duration and an increase in walking and looking at females. This is in agreement with Bhakat et al. (2005), who reported that, compared with unexposed one,a male camel regularly exposed to a female showed a significant reduction in dry matter and water intake and a decrease in body weight. Due to the feed intake reduction and the energy spent in mating activities, male camels have usually become underweight by the end of the breeding season (El-Wishy 1988; Skidmore 2000; Khan et al. 2003).

The behavioral repertoire changed significantly among the three different management systems, which could be explained by the presence of the females, stimulating the natural sexual behavior of the rutting males. During the rutting season, feral bulls show an increase in anxiety and restlessness and spend most of their time guarding the herd and surveying the fence line to prevent intrusion by other males. Similar behaviors were observed in harem stallions which spend most of their time guarding the harem fence line area, with their attention apparently focused on the mares in nearby pastures, aggressively evicting intruders, and periodically harassing bachelor stallions (McDonnell 2000).

The increased mobility caused the significant difference in the occurrence of leg movements and number of steps between the different rearing systems. It should be underlined, in any case, that the frequency of all recorded events was

lower in H24 and H23 than in ExF, likely because the presence of the female induced and stimulated the males to express their normal sexual behavior.Male dromedary camel sexual behavior is characterized by species-specific behaviors, such as open leg standing, tail flapping, *blatering*, *dulaa* extrusion and emission of metallic sounds(Skidmore 2000). In camels, pheromones are present in rutting male urine, and Skidmore (2000) observed that urine spraying generally increases in the presence of another male or when females are passing nearby. Thus, as expected, urination and defecation occurrence was higher in ExF, becausethey are markers indicating the male's presence to establish his territoriality.McDonnell (1986) stated that in stallions repeated defecation, particularly near the fence line, is a common behavior of the stud and that it results in accumulation of fecal matter into large mounds, known as "stud piles". Tail flapping frequency also increased in ExF, because this behavior usually accompanies urination, and is also used as a sign of marking, dominance and sexual excitability.

Underthe ExF system, camels also frequentlydisplayed teethgrinding, associated with an increase in salivary gland secretionandsound emissions, as the male's excitement increased and more auditory signals were producedto attract the females (Bhakat et al. 2005).Flehmen occurrence also increased, as observed in other species. Indeed, sniffing, licking and nuzzling of the female's genitalia are the most frequent patterns in sexually active stallions (McDonnell 2000), but flehmen is also normal behavior in cow-bull courtship (Bailey et al. 2005).

The lower expression of mentionedsexual behaviorsin H24 and H23 systems could indicate a lack of sexual stimuli. The traditional husbandry system (H24) seems not to meet the animal's behavioral needs, and this could be the reason for the low mating desire and reproductive performance lamented by El-Hassanein (2003) and El-Bahrawy (2005) in their male camels bred for semen collectionin H24.

Nevertheless, it is often assumed that for welfare purposes, farm animals should be given "the freedom to express their natural behaviors" (Spinka 2006). Haskell et al. (2013) showed that when cows are housed in open areas they are able to express their natural behaviors more easily than in confined spaces, andour data reported a similar situation also in camels.Thus, when the housing condition metan animal's needs, itspsychological and physiological status improved, with a positive knock-on effect on sexual behavior; in fact,the camel's sexual behavior score was significantly higher in ExF than in H24 and H23.

The rise in the sexual behavioral score demonstrates that improved management, more compliant with the camel's behavioral needs, exerts a long-term effect on the animal's physiological status. The interactionsbetween the hypothalamic–pituitary–adrenal (HPA) and the hypothalamic–pituitary–gonadal (HPG) are well noted: stress response negatively affected sexual behavior (Rivier and Riviest, 1991; Retana-Márquez et al., 2003; Kirby et al., 2009). Therefore, we can suppose that, since stress activity decreased in H23 and ExF, HPG activity was stimulated, leading to more intense expression of the male camels' rutting signs.

Conclusion

Overall, this study demonstrated that traditional management (H24) negatively affects male camels'sexual behavior, also leading to increased production of cortisol. From our preliminary data, it seems that the management system that allows movements and interaction with females (ExF) had a positive impact on camel rutting behavior and welfare. We therefore hypothesize that freedom to move and to express social behavior are behavioral needs in camels reared for semen collection, ensuring their well-being. However, further studies are needed to investigate the effects of the ExF system on camel libido and semen quality.

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Disclosure

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Conflict of interest

The authors declare that they have no conflict of interest.

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Fig. 1 Average plasma cortisol concentration (ng/ml)in housed male dromedary camel (n=4) in the three different management systems: housed in a single stall for 24 hours (H24), housed in a single stall for 23 hours and one hour in a paddock (H23), housed in a single stall for 22 hours and 30 minutes, one hour in a paddock and 30 minutes of female exposure(ExF)

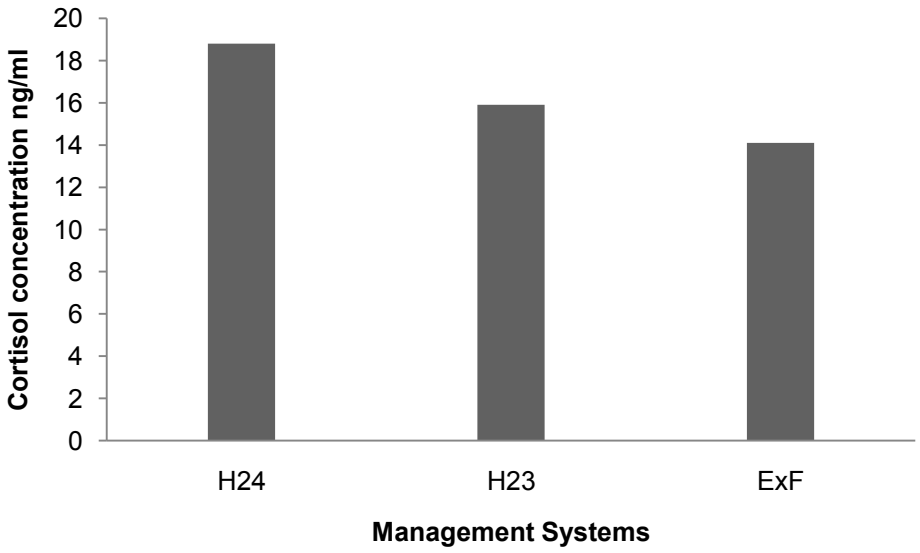
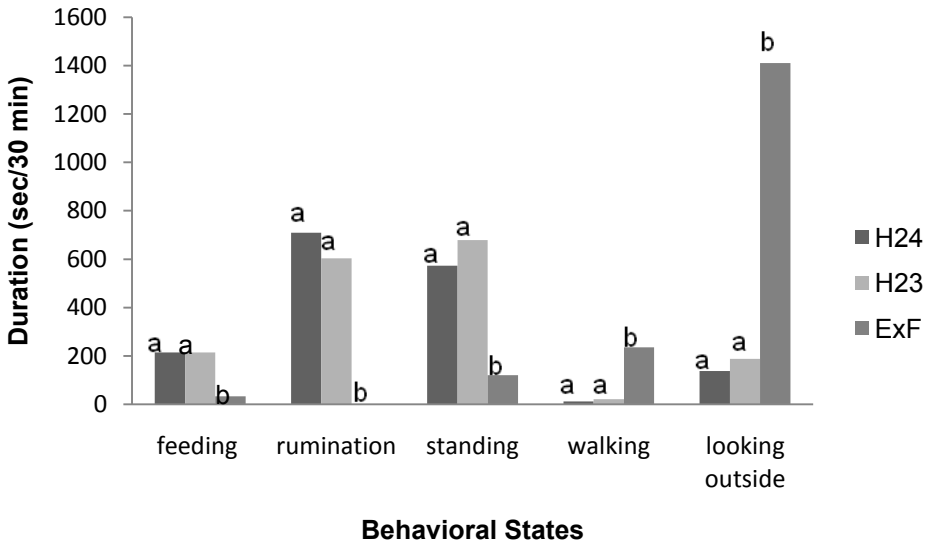
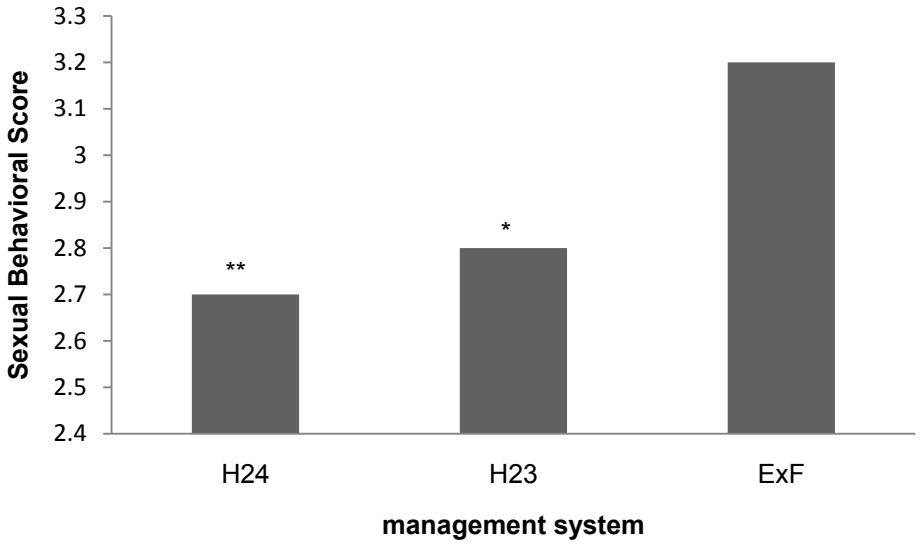


Fig. 2 Behavioral states recorded during 30 minutes of observation period mornings in housed male dromedary camels (n = 4) in three different management systems:: housed in a single stall for 24 hours (H24), housed in a single stall for 23 hours and one hour in a paddock (H23), housed in a single stall for 22 hours and 30 minutes, one hour in a paddock and 30 minutes of female exposure(ExF)



[a,b]differ significantly (P< 0.01)

Fig. 3 Sexual behavioral scored (from 1 to 5, by Padalino et al. (2013)) after female passage in the evening in housed male dromedary camels (n=4) in three different management systems: housed in a single stall for 24 hours (H24), housed in a single stall for 23 hours and one hour in a paddock (H23), housed in a single stall for 22 hours and 30 minutes, one hour in a paddock and 30 minutes of female exposure(ExF)



**differ significantly (P< 0.01); *differ significantly (P< 0.05)

Table 1 Chemical composition (% on dry matter basis) of oat hay and concentrate fed to camels during the experimental period.

	Oat Hay	Concentrate
Dry Matter	90.0	90.9
Crude Protein	6.8	11.4
Crude Fiber	48.9	20.5
Neutral detergent Fibre	55.8	31.6
Acid detergent Fiber	35.1	16.3
Lignin	5.7	3.2
Ether Extract	2.0	4.5
Ash	7.9	8.1

Table 2 "Sexual Behavioral Score" for housed male dromedary camels during female passage (Padalino et al. 2013)

Behavioral Score	Criteria
1: not interested	The male does not show any sexual behavior parameters
2: low interested	The male goes near the female and shows a low frequency of sniffing and flehmen
3: interested	The male goes near the female and shows sniffing, flehmen, grinding of teeth/ whistling, yawning
4: high interested	The male goes near the female and shows sniffing, flehmen, grinding of teeth/ whistling, yawning (more than 3), urination and tail raising. He is very agitated, stands with open legs, poll gland secretion and neck rubbing are observed
5: excited	As in 4, but the male shows *blatering* and *dulaa* extrusion, is very excited, stands with open legs, high poll gland secretion and neck rubbing are observed

Table 3 Least square means (± SE) of behavioral parameters occurrence ((n/30 min) in housed male dromedary camels (n=4) in three different management systems:housed in a single stall for 24 hours (H24), housed in a single stall for 23 hours and one hour in a paddock (H23), housed in a single stall for 22 hours and 30 minutes, one hour in a paddock and 30 minutes of female exposure (ExF)

Behavior	H24	H23	ExF
Leg movement frequency	36.7 ± 4.0^{b}	35.0 ± 4.0^{b}	92.5 ± 4.0^{a}
Number of steps	47.5 ± 15.5^{b}	27.1 ± 15.5^{b}	240.2 ± 15.5^{a}
Sound emission frequency	0.1 ± 2.5^{b}	0.1 ± 2.5^{b}	11.5 ± 2.5^{a}
Defecation frequency	0.5 ± 0.2^{b}	0.2 ± 0.2^{b}	2.3 ± 0.2^{a}
Urination frequency	0.1 ± 0.3^{b}	0.1 ± 0.3^{b}	2.5 ± 0.3^{a}
Tail Flapping	0.5 ± 0.2^{b}	0.5 ± 0.2^{b}	9.2 ± 3.0^{a}
Open leg frequency	1.4 ± 0.6^{b}	1.2 ± 0.6^{b}	5.5 ± 0.6^{a}
Yawning	0.8 ± 0.5^{b}	0.5 ± 0.5^{b}	4.4 ± 0.5^{a}
Teeth Grinding	3.6 ± 2.4^{b}	2.7 ± 2.4^{b}	$37.3 \pm 2.^{a}$
Blatering	0.1 ± 1.1^{b}	0.1 ± 1.1^{b}	11.5 ± 1.1^{a}
Sniffing	0.2 ± 0.5^{b}	0.1 ± 0.1^{b}	14.9 ± 1.4^{a}
Flehmen	0.1 ± 0.7^{b}	0.1 ± 0.7^{b}	6.6 ± 0.7^{a}

[a,b]Least square means (± SE) in each column with no common superscript differ significantly (P< 0.01)