#### RESEARCH PAPER

### LIVESTOCK STUDIES

# The Effect of Different Management Systems and Racing on the Stress Level of Arabian Horses

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#### Abstract

Considering the stud farms and hippodromes, there are differences between them both in terms of management systems. The study population consisted of 40 Arabian mares. The breeding systems of horses at the stud farm and hippodrome, the physical dimensions of their boxes, agonistic behaviors, stereotypic behaviors, and intra/interspecies interactions were examined and evaluated comparatively by considering their physiological stress and behavioral responses. The salivary cortisol analysis was performed at rest on the horses at the stud farm and pre-post race on the horses at the Hippodrome. Statistical difference was not found between the stereotypic and agonistic behaviors of the horses but the longer-term effects of the absence of intraspecies interaction should be investigated. There was no statistical difference in the resting horse's saliva cortisol value housed at the stud farm (0,84 ng/ml) and hippodrome (0,52 ng/ml). It was determined that the pre-race cortisol value (0,52 ng/ml) was significantly lower than the post-race (3,82 ng/ml) value. In order for the horses to have a long and healthy sports life, the welfare of the horses should be structured by considering the training, behavior, and management systems with a holistic approach, and intra-species interactions should not be overlooked.

#### Introduction

Stress is defined as a response to environmental stimuli that threaten an organism's homeostasis (Ramos and Mormède, 1998). The decrease in welfare also causes stress in the horses, which may cause the horses to not be able to benefit from the desired efficiency as a result of stereotypical behaviors. However, as a result of the increase in the frequency of agonistic behaviors, accidents and injuries may occur. Long-term exposure to stressors or short-term exposure to high-intensity stressors can lead to reduced animal welfare. In the case of chronic stress, it can cause some health problems. The organism of the animal under stress cannot renew its biological resources, so this situation affects the animal in a negative way (Moberg and Mench, 2000; Etim et al., 2013). Minimizing stress in horses used for riding is necessary for animal health and welfare (Jung

et al., 2019). Because competition is a mix of various stress factors, simply being in the competition arena before the competition can cause a classic physiological stress response in horses that can affect racing performance. Healthy sport horses recover rapidly at the end of the competition season when the stimuli that cause stress have gone (Negroa et al., 2018).

Horses are herd animals in their natural life; they do not live alone (Klingel, H., 1967). However horses need daily interaction with intraspecifics to lead a normal life and for their mental health (Landsberg, 2013). Although housing the horses in individual boxes limits their natural behavior (especially locomotor and social behavior), this housing system is widely preferred, especially for racehorses used for flat racing. Although the free presence of horses in paddocks and pastures has a positive effect on animal welfare, this system is not preferred by many horse owners because it causes sports horses to get injured (Houpt, 2005; McGreevy et al., 1995; Christensen et al., 2002; Houpt and McDonnell, 1993). However, the presence of paddocks where horses can socialize with other horses and show their natural behavior is critical for horse welfare. Domestic horses in a herd interact socially with other horses in their paddocks and get the opportunity to exercise by moving throughout the day (Erber et al., 2013). In socially organized animals, the absence of their conspecifics causes stress responses (Schmidt et al., 2010). Although daily riding or training reduces the need for additional physical activity in horses (Alexander and Irvine, 1998), it cannot fully meet the need for free exercise (Werhahn et al., 2011).

A positive horse-human relationship is a key factor in establishing reliable interaction with animals (Hausberger et al., 2008). When judged on the basis of animal welfare, the familiar (friendly) response of horses to humans is the most desirable behavior for establishing a safe human-horse relationship. Despite the long history of horse-human interaction, both professional and amateur people who come into contact with horses are exposed to horse-related accidents (Mills et al., 2000). Studies show that there are problems related to horse-human interaction in administrative areas such as care, management, and training (Hausberger et al., 2008). Undesirable horse behaviors (agonistic and stereotypic behaviors) are considered inappropriate responses to the current situation and may pose a danger to both animals and humans (Cooper and Albentosa, 2005). However, they may also display normal but undesirable behaviors in response to certain environmental deficiencies (Mills et al., 2000). These are agonistic behaviors such as rearing, kicking, and biting (Hausberger et al., 2008). Stereotypical or agonistic behaviors play an important role as an indicator of poor welfare and chronic stress (Cooper and Albentosa, 2005; Nicol, 1999; Mason and Latham, 2004). Stereotypical behaviors are defined as repetitive, unvarying, and nonfunctional behavior patterns (Mason, 1991). The most important way to ensure a horse-human dyad for the welfare of both horses and humans is to create and develop a strong positive interaction (Hausberger et al., 2008).

The relationship between stress and the animal's metabolic system has been demonstrated by many studies (Minton, 1994; Salak-Johnson and McGlone, 2007), and different methods have been developed to assess stress levels (Valera et al., 2012). Most of the techniques used to measure stress in animals include invasive procedures such as blood sampling that can induce a stress response (Stewart et al., 2005). One of these techniques is the measurement of cortisol in saliva, which has been highlighted as a more useful method than plasma or urine cortisol for stress assessment in horses (Schmidt et al., 2009; Bohák et al., 2013; Cordero et al., 2012). Cortisol in the saliva is a direct reflection of free cortisol concentration. It is a much more sensitive indicator of blood and

activity than plasma "total" adrenocortical concentrations. Since cortisol is rapidly diffused into saliva, salivary cortisol concentrations reliably reflect blood cortisol concentrations (Peeters et al., 2011; Schaefer et al., 2002). Changes in cortisol hormone levels in horses can be symptoms of both acute and chronic stress (Hada et al., 2001; Nunez et al., 2014). An animal's response to negative stimuli includes the release of cortisol and the immediate response of the sympathetic-adrenomedullary system. During shortterm stress, cortisol can increase vitality with energy mobilization (Raynaert et al., 1976) and cause changes in behavior (Korte et al., 1993). However, cortisol is also released when the stressor persists, emphasizing the presence of chronic stress (Schaefer et al., 2002). These chronic stress symptoms may include reproductive problems, ulcers, an increased incidence of disease, decreased body weight, and the development of abnormal behaviors such as stereotypes (Mills and Nankervis, 1999).

Evaluation of factors affecting animal welfare has been the basis of many scientific studies in recent years. Most of them focus on a specific potential impact factor (e.g., management; Casamassima et al., 2001; Meunier-Salaün et al., 1987; social behaviors; Grignard et al., 2000; Van Reenen et al., 2000; Bouissou et al., 2001; Wechsler et al., 1997; feeding; Freire et al., 2009). However, only a multifactorial approach with maximum management parameters would be beneficial in terms of an overall assessment and thus an improvement of the actual results on animal welfare.

Arabian horses are bred in stud farms where they live together with their conspecifics until they are 3 years old, and then they are taken to hippodromes where they are socially isolated from their conspecifics for flat races. The aim of this research is to examine the housing conditions, stereotypic behaviors, agonistic behaviors, and intra- and interspecies interactions of horses used for breeding and flat racing with a multifunctional approach and to use salivary cortisol concentration as a physiological stress parameter in determining the stress caused by race in flat races. As a result of the research, the welfare of Arabian horses in hippodrome and farm conditions was evaluated by considering physiological and behavioral stress parameters, and suggestions were made to increase welfare. At the same time, by determining the stress levels of horses used in different fields (for breeding and racing) and horses competing in hippodromes during the racing season and out of season, problems and solution suggestions were put forward from a scientific perspective.

#### **Materials and Methods**

This study was carried out within the scope of the permission of Eskişehir Osmangazi University Animal Experiments Local Ethics Committee 17/02/2021 date, 839/2021 number of decision.

#### **Experimental Animals**

Within the scope of the research, a total of 40 Arabian mares were used, 20 of which were breeding at a stud farm in Mahmudiye and 20 of which were in the hippodrome for flat racing. Both groups were housed using the boxing system, but while the horses had access to the paddock at the stud farm, there was no paddock at the Hippodrome. Horses at the stud farm were in the paddock for an average of 12 hours per day during the breeding season. The mares at the stud farm kept in social contact with the other mares and foals in the paddock. At the same time, they could see each other in their box in the stable. The mares in the hippodrome could not see other horses in their box in the stable.

Wheat straw was used as litter material in the boxes at the stud farm and wood shavings in the hippodrome. While the horses at the stud farm always had access to water, they were watered by hand at the hippodrome. Horses in both groups were fed meadow grass and alfalfa hay as roughage and barley paste as concentrate feed. Horses in the stud farm consumed an average of 5-6 kg of feed per day, and horses in the hippodrome consumed 6-8 kg of concentrate feed in two parts a day, in the morning and in the evening. Meadow grass was given ad libitum to both groups of horses. Horses at the stud farm were given 4 kg of alfalfa grass daily in the paddock between 10:00 and 17:00. The horses in the hippodrome were given 1 kg of alfalfa grass after training.

#### **Experimental Design**

Stereotypical behaviors, agonistic behaviors, intraspecies and inter-species interactions of the horses, and physical parameters of the stable were recorded with the help of observations and information received from the researchers. The agonistic behavior of horses has been studied on the basis of horse-human interaction. In addition, pre- and post-race salivary cortisol levels were compared in order to determine the stress on Arabian horses from flat races. A total of 20 Arabian mares were bred in the stud farm and hippodrome (10 horses were housed in the stud farm and 10 horses in the hippodrome); saliva samples were taken twice for the horses in the hippodrome, in the racing season and out of the racing season, and once for the mares at the stud farm, in the resting time, between 9:00 and 11:00 in the morning. The salivary cortisol concentrations in the samples taken were analyzed and compared with the other data in the study. Other data (stereotypic behaviors, agonistic behaviors, intra-species and inter-species interactions, physical parameters of the stable) were obtained from all horses in the study (40 Arabian mares).

Within the scope of the research, the physical parameters of the stable in the stud farm and hippodrome were recorded by observing them with the help of "Table 1: Physical Parameters" were presented below, and obtaining information from the researchers.

The "Stereotypic Behaviors of Horses" presented in Table 2 within the scope of the research were created in the light of the data revealed in the research of Mills (2005) and Lesimple and Hausberger (2014). The stereotypical behavior of the horses was recorded with the information and observations taken from the researchers.

By the researcher's observations, the agonistic behavior of the horses was determined when the groom entered the box and the horse was being led and as presented in Table 3 "Agonistic Behaviors of Horses". Within the scope of the research, the presence of each agonistic behavior in the "Agonistic Behavior of the Horses" table was scored with "1". Agonistic behaviors of horses are scored between 0-17 within the scope of the table.

Within the scope of intraspecies interactions between horses and humans, their socialization status and interaction duration with other horses were evaluated with the help of the "Intraspecies and interspecies interaction table" presented in Table 4. Socialization and interaction durations with humans were determined within the scope of interaction between species.

Physical Parameters	Measurement
Box Dimensions	(width x length x height)
Window Dimensions	(width x length x height)
Height of boxing window from ground	meter
The time the boxing window stays open during the day	hour a day
Height of boxing door window from ground	meter
The time the door window stays open during the day	hour a day
Paddock area per horse	m²

Table 1. Physical Parameters of the Stable

Description	Incidence
	Frequency
	(day/week)
The horse grasps a fixed object with its incisors, pulls backward and	
draws air into its esophagus	
Obvious lateral movement of head, neck, forequarters, and sometimes	
hindquarters	
The horse hits the door or wall with one of its forelegs	
Vertical movements of head and neck,	
Repetitive tracing a route within the stable	
	The horse grasps a fixed object with its incisors, pulls backward and draws air into its esophagus Obvious lateral movement of head, neck, forequarters, and sometimes hindquarters The horse hits the door or wall with one of its forelegs Vertical movements of head and neck,

Table 2. Stereotypic Behaviors of Horses (Mills, 2005; Lesimple ve Hausberger, 2014)

Cortisol analysis from saliva is a non-invasive method. The cortisol test was performed with the Roche Cobas device by ELISA method. Salivette Tube (Salivette Cortisol; Sarstedt, Nümbrecht-Rommelsdorf, Germany) was used to collect saliva. The roll of cotton was removed from the inner tube, and it was brought to a saturated state by moving it between the teeth and cheek of the horse for about 2 minutes. After the roll of cotton was placed back into the inner tube, the tube was closed. The Salivatte instrument was centrifuged at 1000 g for 2 minutes to separate the saliva from the inner tube and exit to the outer tube.

The SPSS 25 package program was used in the analysis of the data. After determining whether the data were suitable or not for normal distribution, the Wilcoxon Signed Rank Test was used to determine whether there was a significant difference between the variables.

#### Results

When the agonistic behaviors of the horses housed in the stud farm were evaluated, it was seen that the score (the score value of each behavior was "1" within the scope of the research) was in the range of 0-5. When the distribution of agonistic behaviors was examined, it was seen in Table 5. Agonistic Behaviour of Horses in Stud Farms/Hippodromes; 0 at the rate of 17.64%, 1 at the rate of 11.76%, 2 at the rate of 11.76%, 3 at the rate of 11.76%, and 5 at the rate of 5.88%. When the agonistic behaviors of the horses housed in the hippodrome were evaluated, it was seen that the score was in the range of 0-6. When the distribution of agonistic behaviors was examined, it was seen that it was 0 at the rate of 17.64%, 1 at the rate of 11.76%, 2 at the rate of 11.76%, 3 at the rate of 11.76%, and 5 at the rate of 5.88%. While stereotypic

Horse-horse interaction	Horse-human interaction	
(McDonnell, 2003)	(McGreevy et al., 2009)	
Alert	Staring at horse while standing within its visual field	
Ears laid back/pinned	Ear threat towards human	
Avoidance/retreat	Horse avoiding being caught	
Balk	Horse ceasing forward movement while being led	
Bite threat	Horse threatening to bite handler	
Rearing	Rearing towards handler	
Chase	Horse chasing human out of the stable, paddock or roundpen	
Head bump	Head-to-head contact with human	
Head on neck, back or rump	Head-to-neck, back or rump contact with human	
Head-bowing	Horse bowing towards handler	
Herding and driving	Horse causing human to move in one direction	
Kick	Horse kicking handler	
Kick threat	Horse threatening to kick handler	
Nip	Horse nipping handler	
Parallel prance	Prancing alongside handler	
Push	Barging	
Stomp	Stomping at handler	

Table 3. Agonistic Behavior of the Horses.

Table 4. Intraspecies and interspecies interaction table.

Intra/interspecies interaction	Stud Farm Duration	Hippodrome Duration
Duration of horses in the paddock	12 hour/day	0 hour/day
Frequency of horses in the paddock	7 day/week	0 day/week
Duration of see other horses	24 hour/day	3 hour/day
Duration of hear other horses	24 hour/day	24 hour/day
Socialization time of the horse with other horses	12 hour/day	3 hour/day (during training)
Frequency of socialization of horse with humans	7 day/week	7 day/week
Socialization time of the horse with humans	0,5 hour/day	4 hour/day

Table 5. Agonistic Behaviour of Horses in Stud Farm/Hippodrome

Horses in	Agonistic	Horses in	Agonistic behaviour(%)
Studfarm	behaviour(%)	Hippodrome	
S1	5.88	H1	11.76
S2	29.4	H2	0
S3	0	H3	17.64
S4	0	H4	17.64
S5	5.88	H5	5.88
S6	11.76	H6	0
S7	11.76	H7	5.88
S8	17.64	H8	0
S9	0	H9	11.76
S10	17.64	H10	35.29

behavior was not observed in the horses housed in the stud farm; one stereotypic behavior was observed in 11.76% of the horses housed in the hippodrome.

Inter-species interaction durations between groups; while the horses housed in the stud farm were 0,5 hour/day, and the horses housed in the hippodrome were determined as 4 hours/day. When the intra-species interaction times between the groups were evaluated, it was determined that the horses housed in the stud farm were 12 hours/day, and the horses housed in the hippodrome were 3 hours/day. While the horses housed in the stud farms could show their natural behavior as they were free in the paddock during interspecies interaction, the horses in the hippodrome stayed together with their conspecifics during training, riding, or exercises.

When the paddock area per horse between the groups was evaluated, while the horses housed in the stud farm have an area of 2250 m<sup>2</sup>/horse, there was no paddock for the horses housed in the hippodrome. In both systems, the box size was 16 m<sup>2</sup>. The window area of the boxes was 0.5 m<sup>2</sup> at the stud farm and 0.6 m<sup>2</sup> at the Hippodrome.

When the pre-race (at rest) and post-race cortisol values were compared in the hippodrome, it was determined that the pre-race cortisol value (0.52 ng/ml) was significantly lower than the post-race (3.82 ng/ml) value (P<0.01). At rest, the cortisol determined from the saliva of the horses housed in the stud farm was 0.84 ng/ml on average, while the average cortisol determined from the saliva of the horses housed in

the hippodrome was 0.52 ng/ml. There was no statistical difference in the resting horses housed at the stud farm and hippodrome (P > 0.05).

#### Discussion

In Turkey, Arabian horses are bred by the government (Anatolian Agricultural Enterprise, Cifteler Stud Farm, Sultansuyu Stud Farm, Karacabey Stud Farm) or in private stud farms, and most of them are used for flat racing and breeding in stud farms. Considering the stud farms and hippodromes, it is seen that there are differences between them both in terms of the physical conditions and management systems of horses.

Horses are generally transferred from the places where they are housed in groups (stud farms) to the areas where they are housed individually (hippodromes) for their training and for flat races. Erber et al., (2013), based on physiological parameters, revealed that the restriction of living space in individual housing and keeping horses separate from other horses are potential sources of stress and that mares with access to the paddock have higher movement activities than those in individual boxing. However, it was stated that horses with access to the paddock showed less stressrelated behavior than those without. Studies have shown that horses kept in social groups were easier to manage (Søndergaard and Ladewig, 2004) and required less time to reach a certain level of training

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Table 6. Intra/interspecies Interaction		
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Duration of hear other horses	24 hour/day	24 hour/day
Socialization time of the horse with other horses	12 hour/day	3 hour/day (during training)
Frequency of socialization of horse with humans	7 day/week	7 day/week
Socialization time of the horse with humans	0,5 hour/day	4 hour/day

than horses housed individually (Rivera et al., 2002). In a study demonstrating the positive effect of the presence of their conspecifics on horses, it was stated that the stallions housed individually showed higher levels of aggression than the stallions housed in the herd (Christensen et al., 2002). Löcken et al. (2016) revealed in their research that after being kept alone in horseboxes for 6 months, a positive cognitive perception was formed in horses that were grazing for ten days and in contact with their conspecifics. As a result of the research, they mentioned the importance of social behaviors such as exploration, social interaction, play, and grooming for the welfare of horses. In another study examining the behavior and stress levels of horses with and without paddock access during training, they stated that the individual housing of the horses greatly restricted their natural behavior and placed stress on the horses throughout the day. For this reason, they drew attention to the importance of free exercise and social interaction in terms of horse welfare (Werhahn et al., 2012). Stereotypic behaviors in horses are considered an indicator of chronic stress rather than a coping mechanism

#### Table 7. Pre-race/post-race cortisol value

Horses in Hippodrome	Pre-race Cortisol Value	Post-race Cortisol Value	Р
	(ng/mL)	(ng/mL)	
H1	<0.5	6.40	
H2	<0.5	1.72	
H3	<0.5	5.31	
H4	0.70	2.49	
H5	<0.5	9.54	
H6	<0.5	3.55	
H7	<0.5	3.81	
H8	<0.5	1.85	
H9	<0.5	3.12	
H10	<0.5	0.40	**
Mean	0.52±0.02	3.82±0.83	

\*\*: P<0,01

(Broom, 1983). In the meantime, stress can adversely affect the welfare and health of animals. Hovey et al. (2021), revealed that there is a clear relationship between behavior and cortisol concentrations in horses in high-stress environments. In a study by Bachmann et al., (2003), it was suggested that woodchewing horses were more sensitive to stress and less physiologically and psychologically adaptable than control horses. In a study by Löckener et al., (2016), it was found that stereotypic mares had a lower mating success than non-stereotypic mares. In Turkey, Arabian horses are bred by the state or in private stud farms, and most of them are used for competition on flat running and for breeding in stud farms. Considering the stud farms and hippodromes, it is seen that there are differences between them both in terms of the physical conditions and management systems. With the research, agonistic and stereotypic behaviors of horses were determined and examined in relation to stress. In this study, the effect of two different housing types (with/without paddock), on the welfare of horses was examined. Within the scope of the research, there was no statistical difference in the agonistic and stereotypic behaviors of the horses

#### Table 8. Cortisol Value at Rest in Stud farm/Hippodrome

Horses in Studfar m	Cortisol Value at Rest (ng/ml)	Horses in Hippodrome	Cortisol Value at Rest (ng/ml)	Р
S1	2.88	H1	<0.5	
S2	0.99	H2	<0.5	
S3	0.5	H3	<0.5	
S4	0.99	H4	<0.7	
S5	0.5	H5	<0.5	
S6	0.63	H6	<0.5	
S7	0.5	H7	<0.5	
S8	0.5	H8	<0.5	
S9	0.5	H9	<0.5	
S10	0.5	H10	<0.5	-
Mean	0.84±0.23		0.52±0.02	

P>0.0

housed in the stud farm and hippodrome. The reason for this could be explained by the fact that the horses in the hippodrome within the scope of the research have been in the hippodrome for less than 6 months. While the percentage of stereotypical behavior in horses at the stud farm was 0, it was determined as 11.76% in the hippodrome. It is thought that the inability of horses to show their social behaviors in the long term would be increased the frequency of agonistic and stereotypic behaviors.

A study revealed that 216 veterinarians from Switzerland experience an accident with horses at least once a year (Jaeggin et al., 2005). In the USA 65% (Kriss and Kriss, 1997; McCrory and Turner, 2005), 66% in Australia (Abu-Zidan and Rao, 2003), and 75% in England (Chitnavis et al., 1996) riders had an accident related to the fall. In France, according to the report of "Mutualite' Sociale Agricole" (agricultural social insurance), while 2057 cases were evaluated in fields such as horse grooming and stable cleaning, it has been revealed that the proportion of horse-related accidents was %51. These data draw attention to the management of the unexpected reactions of the horses (Newton and Nielsen, 2005) and the choice and timing of the training method (Weeks and Beck, 1996). For this reason, it is important to create positive horse-human interaction, especially from the early age of horses. With the research, agonistic behaviors, which are the basic behavior in experiencing accidents and injuries in human-horse interaction, were examined. At the same time, the durations and types of socialization of horses with other horses and humans were also investigated. Within the scope of the research, the horses were not trained at the stud farm, and when the horses reached at the age of 2, the training started at the Hippodrome. It was thought that not giving training to horses in the early age period and when starting the training in the adult period cut off the intraspecies interactions was compulsive in terms of the adaptation of the horse to the training processes. For this reason, it is thought that starting the training processes with imprinting in the stud farm when the foal is born will have positive results in the training and management practices of the horse. It was seen that humans and horses on the stud farm interact for about 30 minutes a day. In the hippodrome, it was observed that this duration increased to 240 minutes a day. Although there was no paddock system in the hippodrome, it was thought that the increase in the interaction time of the horse with the human gives positive results in terms of the absence of agonistic and/or stereotypic behaviors in the 0-6 months period. Within the scope of the research, it was thought that the increase in the interaction time between the interspecies had positive results on the welfare of the horses. However, considering that horses are herd animals, the importance of intraspecies interactions for equine welfare should be considered. In this research, the training processes of the horses in the hippodrome were carried out by their trainers at the stud farm. It was

thought that the establishment of a trusting relationship between horses and humans at an early age had a positive effect on low stereotypic behavior in horses. However, it was thought that the inability of the horses in the hippodrome to interact with intraspecies might cause stress in the long term, and this situation could increase the frequency of agonistic and stereotypic behavior.

Budzyinska (2012) investigated the relationship between the level of reactivity to short-term stressful stimuli and behavioral and physiological stress indicators in Arabian mares. As a result of the research, it was revealed that there was a significant relationship between the salivary cortisol level and the behavioral score as a result of the effects of stress. Cortisol concentration in saliva was affected by various environmental factors, such as time of day, time of feeding, or welfare of the horses (Irvine and Alexander, 1994). Bohak et al. (2013), stated that salivary cortisol concentration had a daily circadian rhythm. Considering this, saliva cortisol samples were taken from mares on the same day and at the same time of the day, and they were examined in relation to the behavioral responses of the horses as a physiological stress parameter. Within the scope of the research, the stress levels of horses were examined, there was no significant difference between the horses housed in the stud farm and the hippodrome at rest. It was thought that the fact that the horses housed in the hippodrome were housed for less than 6 months had an effect. It was an important issue in terms of animal welfare to investigate the effect of long-term intraspecies social isolation on horses with future studies. It was stated that this situation should be investigated in future studies for horses kept in the hippodrome for more than 6 months. However, in our research, it was determined that the salivary cortisol levels of the horses after the race in the hippodrome increased approximately 7.5 times compared to the pre-race. The increase in plasma cortisol levels in horses may occur as a result of stress and exercise. Boucher and Plusquellec (2019) and Mahmood et al. (2020), suggested that the cortisol increase during an experience is directly related to the acute stress level. In the study of Keidzierski et al., (2014) in which they examined the changes in saliva and plasma cortisol levels during race training, they revealed that lactic acid in the blood, cortisol levels in saliva and plasma increased significantly after the exercise. In the study of Cengiz (2011), it was determined that the preexercise cortisol level in horses increased 1.8 times compared to post-exercise. Schmidt et al., (2009) demonstrated a positive correlation between plasma and salivary cortisol concentrations in horses exposed to different stress factors. In a study by Hovey et al. (2021), evaluating the differences in stress-related behaviors and serum cortisol concentrations in horses used in a therapeutic riding program and a university riding program, it was revealed that horses in both groups were exposed to low stressors. They emphasized that serum cortisol is an objective and easy-to-use method for assessing the stress levels of horses. Janczarek et al. (2023) revealed that increased salivary cortisol in horses represents a measure of fear-related stress. Research results reveal that flat races cause stress in horses. In this context, salivary cortisol is a reliable tool for measuring the stress caused by flat races in horses.

#### Conclusion

Hippodromes are designed for the organizations of races. Because of not include paddocks they are not suitable for horses to live in. As a result of being housed in management systems without paddocks, horses cannot show their social and locomotor behaviors. As a result of the horses' inability to show their natural behavior, one of the 5 freedoms that form the basis of animal welfare is not realized. This may adversely affect horse welfare and health. For this reason, it is a traumatic situation for horses to move from stud farms where the welfare conditions are suitable and where they can show their natural behaviors to hippodromes where they cannot show their intra-species social behaviors. It is recommended that the horses be kept only during the race period, instead of being housed in the hippodrome for a long time, in order to ensure their welfare.

Positive human-animal interaction plays an important role in the training and management system of horses. Horses are herd animals and the presence of people with whom they interact positively helps them feel safe. When horses move to a different management system, the presence of grooms/trainers with whom they have previously established a trusting relationship may prevent the increase in the frequency of agonistic and/or stereotypic behavior. It can also reduce accident and injury rates.

The inability of the horses housed in the hippodrome to exhibit their natural behaviors in intraspecies interactions has the potential to increase the frequency and intensity of agonistic and stereotypical behaviors. Within the scope of the research, it is seen that keeping the box windows of the horses housed in the hippodrome constantly closed, minimizes both intra-species and inter-species interaction. However, considering that horses are both herd animals and owe their lives to their timidity, seeing and hearing other horses and people is important for their welfare.

It is seen that flat-running races have an increasing effect on the cortisol levels of horses. In order for the horses to have a long and healthy sports life, the welfare of the horses should be structured by considering the training, behavior, and management systems with a holistic approach, and intra-species interactions should not be overlooked.

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#### **Conflict of Interest**

The author declare no conflicts of interest.

#### References

- Abu-Zidan FM, Rao S (2003): Factors affecting the severity of horse-related injuries. Injury, 34, 897–900
- Alexander SL, Irvine CHG (1998): The effect of social stress on adrenal axis activity in horses: The impertence of monitoring corticosteroid binding globülin capacity. J Endocrinol., 157,425-432
- Bachmann I, Bernasconi P, Herrmann R, Weishaupt MA, Stauffacher M (2003): Behavioural and Physiological Responses to an Acute Stressor in Crib-biting and Control Horses. Appl. Anim. Behav. Sci., 82, 297-311
- Bohák Z, Szabo F, Beckers JF, Melo de Sousa N, Kutasi O, Nagy K, Szenci O (2013): Monitoring the circadian rhythm of serum and salivary cortisol concentration in the horse. Domest. Anim. Endocrinol., 45, 38–42
- Boucher P, Plusquellec P (2019): Acute stress assesment from excess cortisol secretion: Fundamentals and perspectives. Front. Endocrinol., 10, 749.
- Bouissou MF, Boissy A, Veissier I (2001): The social behaviour of cattle: Social Behaviour in Farm Animals. Editör: Keeling LJ, Gonyou HW, CABI Publishing, Wallingford, UK
- Broom DM (1983): Stereotypies as animal welfare indicators: Indicators Relevant to Farm Animal welfare. Editör: Schmidt D, Nijhoff M, The Hague
- Budzyinska M (2012): Behavioural and physiological mechanisms of reactions to stressful stimuli in Arab horses. University of Life Sciences in Lublin Publishing, 361
- Casamassima D, Sevi A, Palazzo M, Ramacciato R, Collela GE, Bellitti A (2001): Effects of two different housing systems on behaviour, physiology and milk yield of Comisana wews. Small Rumin. Res., 41, 151–161
- Cengiz F (2011): Hayvanlarda Zorlanım (Stres) Oluşturan Etkenler. J. Fac. Vet. Med., 20, 147-153
- Chitnavis JP, Gibbons CLMH, Hirigoyen M, Lloyd Parry J, Simpson AHRW (1996): Accidents with horses: what has changed in 20 years? Injury, 27, 103–105.

- Christensen JW, Ladewig J, Søndergaard E, Malmkvist J (2002): Effects of individual versus group stabling on social behaviour in domestic stallions. Appl. Anim. Behav. Sci., 75, 233-48
- Cooper JJ, Albentosa MJ (2005): Behavioural adaptation in the domestic horse: potential role of apparently abnormal responses including stereotypic behaviour. Livest. Prod. Sci., 92, 177– 182
- Cordero M, Brorsen BW, McFarlane N (2012): Circadian and circannual rhythms of cortisol, ACTH, and a-melanocyte-stimulating hormone in healthy horses. Domest. Anim. Endocrinol., 43, 317–24
- Erber R, Wulf M, Aurich J, Rose-Meierhöfer S, Hoffmann G, Lewinski M, Möstl E, Aurich C (2013): Stress Response of Three-year-old Horse Mares to Changes in Husbandry System During Initial Equestrian Training. Journal of Equine Veterinary Science, 33, 1088-1094
- Freire R, Clegg H, Buckley P, Friend M, McGreevy P (2009): The effects of two different amounts of dietary grain on the digestibility of the diet and behavior of intensively managed horses. Appl. Anim. Behav. Sci., 117, 69–73
- Grignard L, Boissy A, Boivin X, Garel JP, Le neindre P (2000): The social environment influences the behavioural repsonses of beef cattle to handling. Appl. Anim. Behav. Sci., 68, 1–11
- Hada T, Onaka T, Kusunose R, Yagi K (2001): Effects of novel environmental stimuli on neuroendocrine activity in Thoroughbred horses. J. Equine Sci.,12, 33–8
- Hausberger M, Roche H, Henry S, Visser KE (2008): A Review of the Human-Horse Relationship. Appl. Anim. Behav. Sci., 109, 1-24
- Houpt KA (2005): Domestic animal behaviour for veterinarians and animal scientists. Ames: Iowa: Blackwell Publishing Professional
- Houpt KA, McDonnell SM (1993): Equine stereotypies. Comp. Cont. Educ. Pract. Vet., 15, 1265-72
- Irvine CH, Alexander SL (1994): Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. Domest. Anim. Endocrinol, 11, 227–38
- Hovey, M.R., Davis, A., Chen, S., Godwin, P., Shea Porr, C.A. 2021. Evaluating stress in riding horses: part one-behavior assessment and serum cortisol. Journal of Equine Veterinary Science. 96:103297.
- Jaeggin S, Furst A, Auer J (2005): Kick injuries of veterinarians during examination and treatment of horses: a retrospective study in Switzerland. Schweiz. Arch. Tierheilkd., 147, 289–295
- Janczarek, I., Stachurska, A., Pieszka, M., Dracz, K., Tkaczyk, W., Luszczynksi, J. 2023. Effect of fearfulness and cortisol reactivity to stress on the spatial learning performance in mountain primitive horses. Journal of Veterinary Bahavior. 60:10-17.

- Jung A, Jung H, Choi Y, Colee J, Wickens C, Lee JW, Yoon M (2019): Frequent riding sessions daily elevate stress, blood lactic acid, and heart rate of thoroughbred riding horses. Journal of Veterinary Behavior, 32,1-5
- Keidzierkski W, Cywinska A, Strzelec K, Kowalik S (2013): Chnges in salivary and plasma cortisol levels in Purebred Arabian horses during race training session. Animal Science Journal, 85, 313–317
- Klingel H (1967): Social Orgenization and Behavior in Free-ranging plains Zebras. Z. Tierpsyhol., 24, 580-624
- Korte SM, Bouws GAH, Bohus B (1993): Cental actions of corticotropin releasing hormone (CH-R) on behavioral, neuroendocrine and cardiovascular regulation: Brain corticoid receptor involvement. Horm. Behav., 27, 167-183
- Kriss T, Kriss V (1997): Equine related neurosurgical trauma: a prospective series of 30 patients. J. Trauma, 43, 97–99
- Landsberg GM (2013): Behavioral Problems of Horses. Editör: Kahn, C. M., Line, S. The Merck Veterinary Manual, tenth ed. Whitehouse Station, N. J.
- Lesimple C, Hausberger M (2014): How accurate are we at assessing others' well-being? The example of welfare assessment in horses. Front. Psychol., 5, 1–6
- Löckener S, Reese S, Erhard M, Wöhr AC (2016): Pasturing in herds after housing in horseboxes induces a positive cognitive bias in horses. Journal of Veterinary Behavior, 11, 50-55
- Mahmood Z, Davidsson A, Olsson E, Leanderson P, Lundberg AK, Jonasson L (2020): The effect of acute execises on interleukin-6 and hypothalamic-pituitary-adrenal axis responses in patients with coronary artery disease. Sci.Rep., 10 (1), 21390.
- Mason GJ, Latham NR (2004): Can't stop, won't stop: Is stereotypy a reliable animal welfare indicator? Anim. Welfare, 13, 57–69
- Mason GJ (1991): Stereotypies: a critical review. Anim. Behav., 41,1015–1037
- McCrory P, Turner M (2005): Equestrian injuries. Med. Sport Sci., 48, 8–17
- McDonnell SM (2013): The Equid Ethogram. A Practical Field Guide to Horse Behavior. The Blood-Horse Inc., Lexington, K.Y., USA.
- McGreevy PD, French NP, Nicol CJ (1995): The prevalence of abnormal behaviours in dressage, eventing and endurance horses in relation to stabling. Vet. Rec., 137, 36-7
- McGreevy PD, Oddie C, Burton FL, McLean AN (2009): The horse-human dyad: Can we align horse training and handling activities with the equid social ethogram? The Veterinary Journal, 181, 12–18

- Meunier-Salaün MC, Vantrimponte MN, Raab A, Dantzer R (1987): Effect of floor area restriction upon performance, behavior and physiology of growingfinishing pigs. J. Anim. Sci., 64, 1371–1377
- Mills DS, Eckley S, Cooper JJ (2000): Thoroughbred bedding preferences, associated behaviour differences and their Implications for Equine Welfare. Anim. Sci., 70, 99-124
- Mills DS, Nankervis KJ (1999): Equine Behaviour: Principles and Practice. Blackwell Science, 12, 33-8
- Mills DS (2005) Repetitive movement problems in the horse: The Domestic Horse, The Origins, Development and Management of Its Behaviour. Editör: Mills, D.S., McDonnell, S.M., Cambridge University Press, Cambridge
- Minton JE (1994) Function of the hypothalamicpituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. Journal of Animal Science, 72,1891–1898
- Moberg G, Mench J (2000): The Biology of Animal Stress: Basic Priciples and Implications fo Animal Welfare. CABI Publishing, Wallingford
- Negroa S, Bartoloméa E, Molina A, Soléc M, Gómeza D, Valera M (2018): Stress level effects on sport performance during trotting races in Spanish Trotter Horses. Research in Veterinary Science, 118, 86–90
- Newton A, Nielsen AM (2005): A review of horse-related injuries in a rural Colorado hospital: implications for outreach education. J. Emerg. Nurs., 31, 442– 446
- Nicol CJ (1999): Understanding equine stereotypies. Equine Vet. J. Suppl., 28, 20–5
- Nunez CNM, Adelman JS, Smith J, Gesquire LR, Rubenstein DL (2014): Linking Social Environment and Stress Physiology in Feral Mares (Equus caballus): Group Transfer Elevate Fecal Cortisol Levels. Gen. Comp. Endocrinol., 196, 26-33
- Peeters M, Sulon J, Becjers JF (2011): Comparison between Blood Serum and Salivary Cortisol Concentrations in horses using and adrenocorticotropic hormone challance. Equine Vet. J., 43, 487-93
- Ramos A, Mormède P (1998): Stress and emotionality: a multidimensional and genetic approach. Neurosci. Biobehav. Rev., 22, 33–57
- Raynaert R, De Paepe M, Peeters G (1976): Infkuence of stress, age and sex on serum growth hormone and free fatty acids in cattle. Horm. Metab. Res., 8, 109-114
- Rivera E, Benjamin S, Nielsen B, Shelle J, Zanella AJ (2002): Behavioral and physiological responses of horses to initial training: The comparison between pastured versus stalled horses. Appl. Anim. Behav. Sci., 78, 235-52
- Salak-Johnson JL, McGlone JJ (2007): Making sense of apparently conflicting data: stress and immunity in swine and cattle. J. Anim. Sci., 85, 81-8

- Schaefer AL, Matthews LR, Cook NJ, Webster J, Scott SL (2002): Novel noninvasive measures of animal welfare. NAWAC/ISAE Conference, Hamilton, New Zealand
- Schmidt A, Aurich C, Neuhauser S, Aurich J, Möstl E (2009): Comparison of cortisol levels in blood plasma, saliva and faeces of horses submitted to different stressors or treated with ACTH. Proceedings, 5th Internationla Symposium Equitation Science, Sydney
- Schmidt A, Biau S, Möstl E, Becker-Birck M, Morillon B, Aurich J, Faure JM, Aurich C (2010) Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. Domest. Anim. Endocrinol., 38,179–89
- Schmidt A, Möstl E, Aurich J, Neuhauser S, Aurich C (2009): Comparison of cortisol and cortisone levels in blood plasma and saliva and cortisol metabolite concentrations in faeces for stress analysis in horses. Proceedings 5th International Equitation Science Conference (ISES), Sydney, Australia
- Sondergaard E, Ladewig J (2004): Group housing exerts a positive effect on the behaviour of young horses during training. Appl. Anim. Behav. Sci., 87, 105-118.
- Stewart M, Webster JR, Schaefer AL, Cook NJ, Scott SL (2005): Infrared thermography as a non-invasive tool to study animal welfare. Anim. Welfare, 14,319-25
- Valera M, Bartolomé E, José Sánchez M, Molina A, Cook N, Schaefer A (2012): Changes in Eye Temperature and Stress Assessment in Horses During Show Jumping Competitions. Journal of Equine Veterinary Science, 32,827-830
- Van Reenen CG, Mars MH, Leushuis IE, Rijsewijk FAM, van Oirschot JT, Blokhuis HJ (2000): Social isolation may influence responsiveness to infection with bovine herpesvirus 1 in veal calves. Vet. Microbiol., 75, 135–143
- Wechsler B, Fröhlich E, Oester H, Oswald T, Troxler J, Weber R, Schmid H (1997): The contribution of applied athology in judging animal welfare in farm animal housing systems. Appl. Anim. Behav. Sci., 53, 33–43
- Weeks J, Beck A (1996): Equine agitation behaviors. Vet. Clin. North Am. Equine Pract., 18, 23–24
- Werhahn H, Hessel EF, Van den Weghe, HFA (2012):
  Competition Horses Housed in Single Stalls (II):
  Effects of Free Exercise on the Behavior in the
  Stable, the Behavior during Training, and the
  Degree of Stress. Journal of Equine Veterinary
  Science, 32, 22-31
- Werhahn H, Hessel EF, Schulze H, van den Weghe HFA (2011): Temporary turnout for free exercise in groups: Effects on the behavior of competitio horses housed in single stalls. J. Equine Vet. Sci., 31, 417-425.