UNIVERSIDADE FEDERAL DO PARANÁ

JÉSSICA CARVALHO SEABRA

FACTORS ASSOCIATED WITH THE DEVELOPMENT AND PREVALENCE OF ABNORMAL AND STEREOTYPIC BEHAVIORS IN HORSES

CURITIBA

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Tese apresentada ao Programa de Pós-Graduação em Zootecnia, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Zootecnia.

Orientador: Prof. Dr. João Ricardo Dittrich

Coorientadora: Prof.ª Drª. Tanja Hess

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CURITIBA, 31 de Janeiro de 2023.

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"Love of animals is a universal impulse, a common ground on which all of us may meet. By loving and understanding animals, perhaps we humans shall come to understand each other."

Louis J Camuti

RESUMO

Cavalos são animais gregários e quando estão livres em seu ambiente natural, passam a maior parte do tempo pastando, percorrendo longas distâncias e se socializando. Cavalos domesticados são utilizados para trabalho ou lazer, sendo mantidos em diferentes condições de manejo. A locomoção restrita, diminuição do tempo de alimentação e isolamento social podem alterar drasticamente o comportamento natural desta espécie, podendo levar ao desenvolvimento de comportamentos anormais. Esses comportamentos afetam estabelecimentos equestres em todo o mundo há várias décadas. O objetivo desta dissertação foi aumentar o conhecimento sobre fatores associados à prevalência de comportamentos anormais em cavalos, além de desenvolver e implementar novas estratégias para sua prevenção. Três estudos foram conduzidos. O objetivo do primeiro estudo foi realizar uma revisão sistemática e meta-análise da literatura científica, resumindo os principais fatores de risco associados ao desenvolvimento de comportamentos anormais em cavalos. Houve grande variação na prevalência média de comportamentos anormais, e foi identificado que o manejo nutricional incorreto pode ser o principal fator que influencia no desenvolvimento e prevalência de comportamentos anormais em equinos. O segundo estudo teve o objetivo de identificar como três diferentes arquiteturas de baias podem influenciar o repertório diário de comportamentos e os parâmetros fisiológicos em cavalos estabulados em tempo integral. Todos os cavalos observados apresentaram comportamentos anormais e estereotipados. A restrição de alimentos teve impacto direto na distribuição do tempo, criando longos períodos de ócio e jejum. O teor de forragem abaixo do ideal pode ter contribuído com desenvolvimento de comportamentos anormais (comer cama, coprofagia e mastigar madeira) e os cavalos gastaram mais tempo com essas práticas do que ingerindo alimentos adequados. Seis cavalos apresentaram alterações leucocitárias e nove apresentaram níveis de cortisol acima dos valores de referência. Os dados indicam que os cavalos estavam em estado de estresse crônico e a arquitetura das baias não alterou significativamente o comportamento. O terceiro estudo teve o objetivo de comparar os efeitos da alimentação com feno ilimitado (FC), um *“slow feeder*” (SF) e um comedouro automático (BF) no repertório diário de comportamentos de cavalos mantidos em piquetes. Cavalos com feno ilimitado usaram 64,5% mais feno do que a recomendação diária, enquanto cavalos no comedouro automático e *slow feeder* tiveram a menor taxa de utilização de feno e menor ganho de peso e ambos os tratamentos podem ser considerados equivalentes em eficiência alimentar e controle de ingestão. Cavalos em ambos os tratamentos de feno ilimitado e *slow feeder* passaram mais de 50% do repertório diário de comportamentos forrageando, gerando uma distribuição de tempo semelhante aos cavalos em seu ambiente natural. No entanto, o tratamento com comedouro automático diminuiu o tempo de alimentação para 25,81% do repertório diário de comportamentos e aumentou significativamente o tempo que os cavalos passaram em outras atividades (parado atento, cheirar o chão e praticar coprofagia). O nível de agressão entre cavalos tornou-se maior com a diminuição da área por comedouro e diminuição da acessibilidade da fonte de alimento, com o comedouro automático apresentando níveis mais altos de agressão.

Palavras-chave: Comportamentos anormais; Necessidades comportamentais; Nutrição de Equinos; Instalações; Time-budget.

ABSTRACT

Horses are a gregarious species, and when they are free in their natural environment, they spend most of their time grazing, covering long distances and socializing with conspecifics. Domesticated horses are used for work or recreation, and kept in different management conditions, according to their use. Restricted locomotion, decreased feeding time and social isolation can drastically change the natural behavior of this species, becoming a risk factor that may lead to the development abnormal behaviors and stereotypies. Stereotypies and other abnormal behaviors have been affecting the equestrian establishments worldwide for several decades. The objective of this dissertation was to advance in the knowledge of factors associated with the prevalence of abnormal behaviors in horses, as well as the development and implementation of new strategies to prevent those behaviors. Three studies were performed. The aim of the first study was to conduct a systematic review and meta-analysis of the scientific literature, summarizing the main risk factors associated with the development of abnormal behaviors in horses. The results showed a great variation in the mean prevalence of abnormal behaviors, and the data mining technique identified that incorrect nutritional management may be the main factor influencing the development and prevalence of abnormal behaviors in horses. The second study aimed to identify how three different stall architectures can influence the time-budgets, and physiological parameters in full-time stabled horses. All horses observed in this study presented abnormal and stereotypic behaviors. The restricted availability of food had a direct impact on the horses' time-budget, creating long periods of idleness and fasting. The forage content below ideal values may have led horses to develop abnormal behaviors (bedding-eating, coprophagy and wood-chewing) and to spend more time with those practices compared to eating proper food. Six horses in the studied population also presented alterations in immune cell numbers, and nine horses had cortisol levels above the reference range. These findings indicate that the stabled horses were in a state of chronic stress and the stalls' architecture did not significantly change the horses' capability to cope with such housing conditions. The third study aimed to compare the effects of feeding free choice hay (FC), a slow feeder (SF) and a box feeder (BF) on the time-budget of horses kept in dry lots. Horses in the free choice treatment used 64.5% more hay than the daily recommendation, whereas horses in the box and slow feeders had the lower hay utilization rate and lower weight gain and both treatments can be considered equivalent in food efficiency and intake control. Horses in both free choice and slow feeder treatments were able to spend more than 50% of the time-budget foraging, generating a time distribution similar to horses in their natural environment. However, the box feeder treatment decreased the time that horses spent eating to 25.81% of their time-budget, and significantly increasing the time horses spent in other activities (standing attentive, sniffing the ground, and practicing coprophagy). The level of aggression between conspecifics became significantly higher as the area per feeder became smaller and the food source became less accessible, with horses in the box feeder treatment showing higher levels of aggression.

Key words: Abnormal behaviors; Behavioral needs; Equine nutrition; Housing conditions; Time-budget.

LIST OF FIGURES

LIST OF TABLES

INDEX

1. GENERAL INTRODUCTION

1.1 JUSTIFICATION AND SIGNIFICANCE

Under natural conditions, horses are gregarious herbivores (BOYD; BANDI, 2002). They evolved to use structural carbohydrates as the primary source of energy in the diet, traveling great distances to find various forages and water (BOYD; CARBONARO; HOUPT, 1988; GEOR; HARRIS, 2007; HAMPSON *et al.*, 2010). Natural forages are poor in energy and rich in fiber, requiring the ingestion of great volumes to satisfy the energy demands and ingestive behavior of a horse.

When horses are free in their natural environment, they can spend around 16 hours per day grazing (MAYES; DUNCAN, 1986) Studies with horses suggest that offering restricted amounts of forage, as well as introducing concentrate in the diet can drastically change the natural behavior of this species, becoming a risk factor that may lead to the development abnormal behaviors and stereotypies. This can occur due to the reduction of feeding time, visceral discomfort, and also the inability to express the grazing behavior, for which the species is highly motivated. Keeping horses in individual stalls is also a management practice that can lead to the development of abnormal behaviors due to social isolation and restricted movement (MCBRIDE; HEMMINGS, 2009).

Stereotypies are invariant and repetitive behavior patterns that seemingly have no function and other abnormal behaviors are practices that do not fit this definition but are not commonly observed in horses living in wild environments (MASON, 2006). The prevalence of stereotypies and other abnormal behaviors in domesticated horses have been studied for decades (HANIS *et al.,* 2020; LUESCHER; MCKEOWN; DEAN, 1998), and researchers believe that several environmental and biological factors may be involved in the development of these behaviors. Abnormal behaviors and stereotypies are commonly recognized as indicators of poor welfare (RUET *et al.,* 2019) and may directly impair athletic performance, resulting in great economic losses to horse owners.

The prevalence of stereotypies in horses is commonly measured through behavioral assessment, which is a simple method that does not require specific equipment. According to authors, scan or focal sampling does not provide a continuous assessment of behavior and thus potentially misses or underrepresents important, infrequent behaviors (AUER *et al.,* 2021). Therefore, continuous observation is believed to be the most accurate method for behavioral measurements (MITLÖHNER et al., 2001).

Time-budget is a term that can be defined as the daily amount of time an animal engages in behavioral activities. Researchers used direct observations of feral horse populations to construct the natural "time-budget" of this species and calculated the percentage of time horses would spend in different activities. domestic horses will display time budgets similar to those of feral horses when given the opportunity. Therefore, domesticated horses are often compared to feral individuals from the same species. With that in mind, differences in the time-budgets of domesticated horses compared to feral conspecifics are currently used to reveal welfare impairment (AUER et al., 2021; LESIMPLE, 2020).

Management practices used in the horse sector have changed very little over the years. Therefore, studies aiming to identify factors associated with the prevalence of abnormal behaviors in horses, as well as the development and implementation of new strategies to prevent those behaviors are extremely necessary to improve this species' welfare.

1.2 OBJECTIVES

1.2.1 General objectives

- Conduct a systematic review and a meta-analysis of scientific literature about factors associated with the development of abnormal behaviors in horses.
- Identify how three different stall architectures can influence the daily time distribution of horses (time-budget).
- Compare the effects of feeding free choice hay (FC), a slow feeder (SF) and a box feeder (BF) on the equine behavior by 24-hour continuous sampling.

1.2.2 Specific objectives

- Identify and summarize the main risk factors associated with the development of abnormal behaviors in horses.

- Determine the prevalence of stereotypic and abnormal behaviors in a stabled horse population.
- Evaluate how three different stall architectures can influence the cortisol circadian rhythm (CCR) of stabled horses.
- Evaluate how three different stall architectures can influence the leukocyte profile and neutrophil/lymphocyte ratio in stabled horses.
- Determine the prevalence of stereotypic and abnormal behaviors in horses kept in dry lots using three different feeders.
- Determine how three different feeders can influence the body weight and body condition score of horses kept in dry lots.
- Determine which one of the three feeders can offer better values regarding food efficiency and intake control.
- Determine which one of the three feeders can offer more natural time-budgets in horses kept in dry lots.
- Determine which one of the three feeders can decrease agonistic behavior in horses kept in dry lots.

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2. FACTORS ASSOCIATED WITH THE DEVELOPMENT AND PREVALENCE OF ABNORMAL BEHAVIORS IN HORSES: SYSTEMATIC REVIEW WITH META-ANALYSIS

ABSTRACT

Researchers believe that the development and prevalence of abnormal behaviors in horses may be caused by several environmental and biological factors, and the literature offers numerous reports that discuss the causes and effects of stereotypies in these animals. In this light, this study aimed to conduct a systematic review and meta-analysis of the scientific literature, summarizing the main risk factors associated with the development of abnormal behaviors in horses. The searches were conducted over the course of four years in Portuguese, Spanish, and English. The publications reviewed were full text research thesis or articles that addressed issues within the following criteria: (1) presentation of epidemiological information on the studied population; (2) prevalence of abnormal behaviors in equine populations; (3) factors associated with the development or prevalence of stereotypies. Data were extracted from each study and inserted into an Excel spreadsheet to be analyzed through descriptive statistics. In addition, the Mann- Whitney U test was used to verify the existence of significant differences between the methodologies (direct observation/questionnaires). The dataset was also analyzed through data mining to identify the main factors that influence the prevalence of abnormal behaviors in the studied population. The results showed a great variation in the mean prevalence of abnormal behaviors, with no significant difference between research conducted through questionnaires or direct observation, and the data mining technique identified that incorrect nutritional management may be the main factor influencing the development and prevalence of abnormal behaviors in horses.

Key words: Data mining; Equine nutrition; Management techniques; Stable; Stereotypies.

2.1 INTRODUCTION

Under natural conditions, horses are gregarious herbivores (BOYD; BANDI, 2002), traveling great distances to find water and the various forages (BOYD; CARBONARO; HOUPT, 1988; HAMPSON *et al*., 2010) they are well adapted to, that are poor in energy and rich in fiber. However, today domesticated horses are used for work or as athletes in equestrian competitions, and kept in different management conditions, according to their use (DITTRICH *et al.*, 2010). Most often they are housed in stables with small individual stalls or at properties with restricted grazing areas and reduced forage diversity, requiring the inclusion of concentrated meals to support all the nutritional needs increased by their intense physical activity (HOTHERSALL; NICOL, 2009; MÉTAYER *et al*., 2004).

The cereal-based meals, typically administered twice or three times a day, are rich in non-structural carbohydrates. However, the equine gastrointestinal tract is not adapted to high grain intake and, according to Geor and Harris (2007, p. 178), this feeding practice is linked to “the development of gastrointestinal problems, particularly gastric ulcer disease and colic associated with disturbances to hindgut function”. In addition, research on horses suggests that animals on low fiber diets are at higher risk of developing abnormal behaviors, such as stereotypies, possibly due to reduced feeding time and visceral discomfort. Abnormal behaviors in stabled horses can also be attributed to the inability to express highly motivated behaviors, such as social interaction, foraging, or locomotor activity (MCBRIDE; HEMMINGS, 2009).

Abnormal behaviors and stereotypies are commonly recognized as indicators of poor welfare (RUET *et al.*, 2019) and may directly impair athletic performance, resulting in great economic loss to horse owners. The prevalence of stereotypies in domestic horses have been studied for decades, and researchers believe that several environmental and biological factors may be involved in the development of these behaviors. The literature also offers numerous reports that discuss the causes and effects of stereotypies in horses (BACHMANN; AUDIGÉ; STAUFFACHER, 2003; CHRISTIE *et al.,* 2006; HANIS *et al.*, 2020; HOUPT *et al.*, 2001; LEME *et al.*, 2014; LUESCHER; MCKEOWN; DEAN, 1998; MCBRIDE; LONG, 2001; MILLS *et al*., 2002; MUÑOZ ALONZO; CRUCES LEAL; BRIONES LUENGO, 2016; MUÑOZ *et al.*, 2009; REZENDE *et al.*, 2006; RUET *et al.,* 2019, 2020; SACKMAN; HOUPT, 2019; SEABRA, 2017; TADICH *et al.*, 2012; VISSER; ELLIS; VAN REENEN, 2008; WATERS; NICOL;

FRENCH, 2002). Therefore, the aim of this study was to conduct a systematic review and a meta-analysis of scientific literature, summarizing the main risk factors associated with the development of abnormal behaviors in these animals.

2.2 MATERIAL AND METHODS

The literature review and meta-analysis procedures (search strategy, inclusion/exclusion criteria, and data extraction) were established and conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines (MOHER et al., 2009). Approval of the Ethics Committee was not required for this study type.

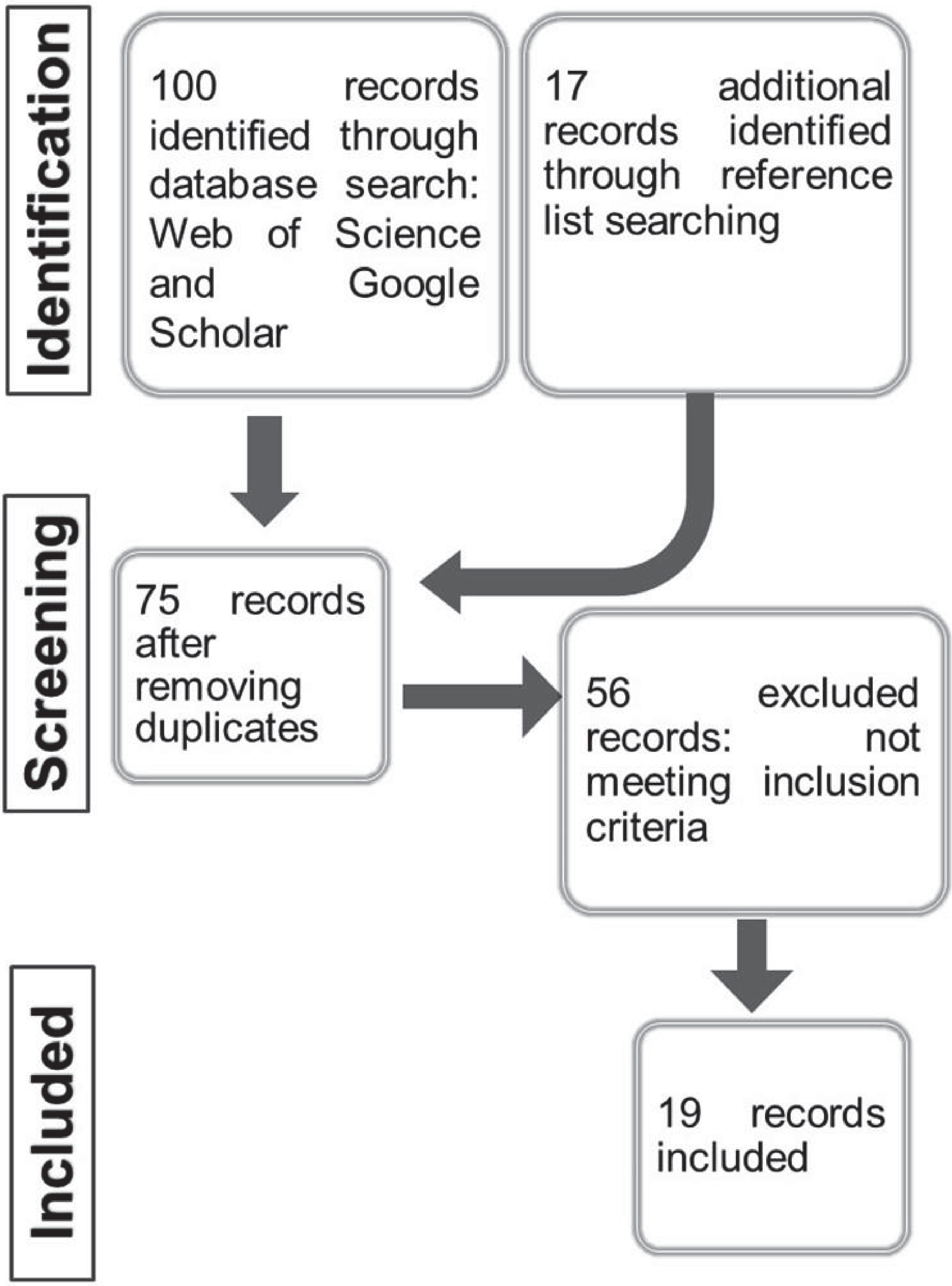
2.2.1 Study Eligibility Criteria

Studies published in indexed double blind peer reviewed journals, as well as monographs, theses, and doctoral dissertations approved by the examining board, were considered eligible if they described the epidemiological characteristics of horses (prevalence of abnormal behaviors, gender, and age) and the methodology used for the behavioral assessments. There were no restrictions regarding the year in which the study was performed or published, the sample size, or the country where it was conducted. Papers written in three languages were accepted: Portuguese, Spanish, and English. The included studies were full text research thesis or articles that addressed issues within the following criteria: (1) presentation of epidemiological information on the population studied; (2) prevalence of abnormal behaviors in equine populations; (3) factors associated with the development or prevalence of stereotypies. Literature reviews, abstracts, and other types of publications were not included in the search strategy.

2.2.2 Search Strategies

The searches were conducted between 2016 and 2020. Web of Science and Google Scholar databases were used to search for cross-sectional and longitudinal studies that reported on the prevalence of abnormal and stereotypic behavior in horses, as shown below (FIGURE 1). An algorithm with keywords (equines, horses, behavior, stereotypies, and behavioral problems) in English, Spanish and Portuguese was used to find eligible publications, and the references section of the included articles were manually inspected to identify additional studies that might fit the requirements. Articles that did not meet the inclusion criteria (either because they did not present most of the necessary information, were poorly executed or not clearly written) were excluded during the process.

FIGURE 1 - FLOW CHART OF THE LITERATURE SEARCH STRATEGY AND THE STUDIES' SELECTION PROCESS.



SOURCE: The Author (2021)

2.2.3. Data Extraction and Analysis

The following data were extracted from each study and inserted into a Microsoft Excel spreadsheet: title, year of publication, the country where the study was conducted, sample size, characteristics of the studied population (gender, age, breed), physical characteristics of the facilities (individual or group housing, access to pasture/paddock, presence of windows), management practices (access to unlimited roughage, daily amount of concentrate feed, imposed exercise, and equestrian sport discipline), prevalence of abnormal behavior, the technique used to evaluate the animals' behavior, outcome assessment, and results.

2.2.4. Data Analysis

Data was tabulated and analyzed through descriptive statistics. Absolute and percentage distributions were determined, and the maximum and minimum values were presented. Subsequently, mean values were calculated to characterize aspects of the prevalence of stereotypies in horses. In addition, the Mann-Whitney U test was used to compare the mean prevalence of abnormal behaviors in the studies with direct observation of the animals and the ones based on questionnaires, using Assistat software (The Assistat Software Version 7.7) (E SILVA, DE AZEVEDO, 2016). The dataset was also analyzed through data mining, with the application of the J48 algorithm, available in the Weka software (WITTEN *et al*., 2016). This algorithm applies a cross validation in ten folds and is an implementation of the C4.5 algorithm, which generates a classification tree that presents a graphical representation of classification rules. The rules are semantic conditionals using logical connectors (IF, AND, OR) to relate classes (THAN) to an event (observation). The database for the analysis included 52 attributes and one classifier (prevalence of abnormal behaviors). This classifier was selected to investigate the main factors that may be related to the high prevalence of abnormal behaviors in horses. The mean prevalence of abnormal behaviors (%) was divided into two categories (Low and High), and three classifying approaches were used to analyze the dataset. In the first approach, prevalence between 1% and 35% of abnormal behaviors in the population was considered low, while prevalence greater than 35% was considered high. In the second approach, the percentages between 1%

and 40% were considered low, and values above that were considered high. Finally, in the third approach, values between 1% and 45% were considered low, and those above 45% were considered to have a high prevalence of abnormal behaviors. All classification models were compared for accuracy, class precision –calculated through a confusion matrix (VALE *et al*., 2010) –, and complexity, based on the number of rules generated and the ability to understand these rules according to expert opinion (CHAPMAN *et al*., 2000). The expert analysts were researchers with a minimum experience of five years in the field of equine science. The knowledge acquired from each tree was described by classification rules (BODY → HEAD). The first root node in the classification tree is the feature with the highest power to classify an object in a predicted class. Each classification rule is located between the root node and the leaf that represents the classification.

2.3 RESULTS

In total, 19 studies (TABLE 1) were analyzed and selected for this systematic literature review and meta-analysis. The 19 selected articles were written in either Portuguese, Spanish, or English, and were carried out in eight different countries: four studies were conducted in Brazil, two in Canada, three in Chile, two in France, one in Malaysia, one in Switzerland, four in the United Kingdom, and two in the United States of America. The oldest study was published in 1998 (LUESCHER; MCKEOWN; DEAN, 1998), and the most recent one in 2020 (RUET *et al.*, 2020). In total, data collected from 18,863 animals were used in the elaboration of these 19 studies. The average number of horses per study was 983, with the smallest study population consisting of 10 animals (SIMÕES, 2019) and the largest consisting of 8,427 animals (MCBRIDE; LONG, 2001). In 89% of the selected articles (17 studies), the horse population was mostly housed in individual stalls, having access to pasture or paddock in only 21% of the studies. Details about the animals' diets are provided only in six studies. The mean amount of concentrate feed offered per day was 4.3 kg, whilst the mean daily roughage intake was 7.6 kg. The studied animals weighed 460 kg on average, had their total diet divided into an average of 2.7 meals per day, and only three studies reported that the horses received roughage *ad libitum*. Based on the articles that provided the animals' gender, it was possible to calculate the prevalence of mares (50.9%), geldings (26.1%) and stallions (23%). In general, the average age of the studied horses was 8.5 years.

However, a wide age variation can be seen in most articles, with some studies including animals aged from one to over 20 years (SACKMAN; HOUPT, 2019).

Thoroughbreds were the most studied horse breed in the selected articles, being present in 11 publications. Warmbloods were present in nine studies, Lusitanian, Arabian, Chilean, and Quarter horses in two each, and *Mangalarga Marchador* in one. In addition, the selected studies included crossbreds in five publications, ponies in two, and draft horses in one. In addition, the selected studies included crossbreds in five publications, ponies in two, and draft horses in one. The animals were used for show jumping and dressage in six of the selected studies, leisure in five, race in four, riding schools in two, mounted police in two, rodeo in two, endurance in one, farm work in one, and other uses in seven publications. In the studies that measured exercise workload, the horses spent about 5.6 hours a week in physical activities.

TABLE 1 – PREVALENCE OF ABNORMAL BEHAVIORS, SAMPLE SIZE OF THE EQUINE POPULATION AND OTHER CHARACTERISTICS OF THE 19 STUDIES INCLUDED IN THE META-ANALYSIS.

Author (Publication year)	**Country**	**Sampling method**	**Animals' Sample size(n)**	**Mean prevalence of abnormal behaviors (%)**
BACHMANN *et al*., 2003	Switzerland	Questionnaires	2341	3.8
CHRISTIE *et al.*, 2006	Canada	Questionnaires	299	12.3
HANIS *et al*., 2020	Malaysia	Direct observation	207	≥54
HOUPT and JOHNSON, 2001	United States	Direct observation	16	12.5
LEME *et al.*, 2014	Brazil	Questionnaires and Direct observation	197	56
LUESCHER *et al*., 1998	Canada	Questionnaires	769	12.3
McBRIDE and LONG, 2001	United Kingdom	Questionnaires	8427	2.5
MILLS *et al*., 2002	United Kingdom	Questionnaires	4061	5.1
MUÑOZ ALONZO *et al*., 2016	Chile	Direct observation	565	6.19
MUÑOZ ALONZO *et al*. 2009	Chile	Direct observation	100	10
REZENDE *et al.,* 2006	Brazil	Direct observation	36	13.4
RUET *et al*., 2019	France	Direct observation	187	≥18.7
RUET *et al*., 2020	France	Direct observation	31	≤38.7
SACKMAN and HOUPT, 2019	United States	Questionnaires	847	39.3
SEABRA, 2017	Brazil	Direct observation	16	94
SIMÕES, 2019	Brazil	Direct observation	10	40
TADICH *et al.,* 2012	Chile	Questionnaires	325	11
VISSER *et al*., 2008	United Kingdom	Direct observation	36	33.3
WATERS *et al.,* 2002	United Kingdom	Questionnaires	225	34.7

Details related to the stalls' architecture are not provided in the vast majority of the selected studies; however, nine of the selected articles mention that some horses had access to a paddock, nine cite the presence of a window between stalls, and two articles mention a view of the horizon. Animals housed in groups, or the presence of toys are also cited in other two studies. The researchers reported that about 26.2% of the studied horses have at least one kind of stereotypy or abnormal behavior. The most common stereotypy assessed in the studies were weaving, identified in 17 publications. The prevalence of Crib-biting was evaluated in 15 studies, box walking in 11, and wind sucking in 10. Another stereotypy addressed in five of the selected studies was nodding, while abnormal movements of the lips and tongue was addressed in four, excessive licking in five, pawing in three, and box-kicking in three. Moreover, the most observed abnormal behaviors were wood chewing, coprophagy, bed-eating and aggressive behavior, being assessed in eight, five, four and three of the selected studies, respectively.

According to the authors, there are several influencing factors on the development and prevalence of stereotypies and other abnormal behaviors in horses. The conditions most frequently cited by the researchers are: providing high levels of concentrate feed (19 studies), reduced feeding time (19 studies), confinement (18 studies), not having access to pasture or paddock (18 studies), limited roughage (18 studies), and social isolation (17 studies). Other factors mentioned in the articles are stall architecture (14 studies), hours of imposed exercise per week (nine studies), weaning technique (five studies), number of daily meals (four studies), number of horses in the yards (two studies), stressful situations (nine studies), frustration (seven studies), boredom (two studies), and pain (one study). Some of the selected studies also suggest that biological factors such as age (11 studies), breed (11 studies), gender (4 studies), and temperament (three studies) may influence the development of abnormal behaviors. There were no statistically significant differences between the mean prevalence of abnormal behaviors in studies with direct observation and the ones that used questionnaires (Mann-Whitney U, $P \geq .10$). After completing the data mining classification task, the three generated models were compared taking into account the model's precision and complexity, as described in TABLE 2. The second model was chosen because it presented greater precision and less complexity, generating a tree easy to understand, without feature selection or class balance.

The classification tree generated by the best model with the data mining technique (FIGURE 2; 86.3% accuracy, and Kappa value of 0.649) showed that animals ingesting bedding material were more likely to be classified in groups with high prevalence of abnormal behaviors. It was also shown that animals that receive limited roughage were more likely to be aggressive and to be classified into groups with high prevalence of abnormal behaviors.

The true-positive values (TABLE 3) represent values correctly classified in their respective class, whereas false-negative values are those incorrectly classified in that class. In total, 51 cases were classified, considering that 34 were correctly classified as low and 10 were correctly classified as high. A decreased precision in the low category was expected due to the imbalance of the classes, meaning that a larger number of cases were categorized in the low prevalence of abnormal behaviors (ZOU *et al.*, 2016).

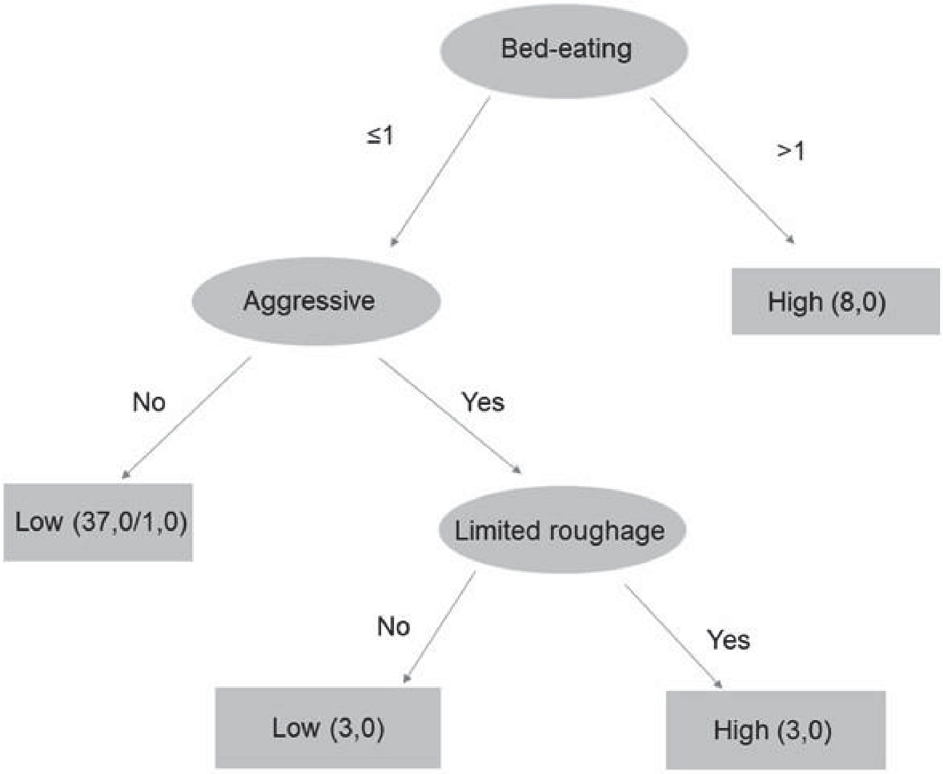
TABLE 2 - CLASSIFICATION APPROACHES USED TO SELECT THE BEST MODEL.

	Classifier: Prevalence of Abnormal Behaviors (%)		
	≤35 Low; >35 High	≤40 Low; >40 High	≤45 Low; >45 High
Model Accuracy	74.50	86.27	74,52
Class precision (Low)	0.77	0.94	0.84
Class precision (High)	0.62	0.67	0.46
Kappa statistic	0.29	0.65	0.31
Root mean squared error	0.48	0.37	0.45
Number of Leaves	4	4	5
Tree size	7	7	9
Model	Simple	Simple	Simple

TABLE 3 – CONFUSION MATRIX WITH THE CLASSIFICATION RESULTS OF THE SELECTED MODEL, SHOWING THE VALUES CORRECTLY OR INCORRECTLY (ERROR) CLASSIFIED BY THE MODEL AND THE RESPECTIVE EVENT TOTALS BY CLASS AND OVERALL.

Classified as	**Low**	**High**
Low	34	5
High	2	10
Total = 51	36	15

FIGURE 2 – DECISION TREE GENERATED FROM THE SECOND MODEL (CLASSIFIER: PREVALENCE OF ABNORMAL BEHAVIORS ≤40% LOW; > 40% HIGH). RULE 1 - IF "BED-EATING" ≤1 AND NO "AGGRESSIVE" = THEN "LOW" CLASS; RULE 2 - IF "BED-EATING" ≤1 AND YES "AGGRESSIVE" = AND NO "LIMITED ROUGHAGE" = THEN "LOW" CLASS; RULE 3 - IF "BED-EATING" ≤1 AND YES "AGGRESSIVE" = AND YES "LIMITED ROUGHAGE" = THEN "HIGH" CLASS; RULE 4 - IF "BED-EATING" > 1 THEN "HIGH" CLASS.



SOURCE: The author (2021)

2.4 DISCUSSION

2.4.1 Study Population

Stereotypies and other abnormal behaviors observed in the selected studies have been affecting the equine industry worldwide for several decades. For instance, the comparison of the cross-sectional study carried out by researchers in 1998 (LUESCHER; MCKEOWN; DEAN, 1998) with recent articles, shows that the management techniques in the horse sector changed very little over the last quarter of century.

The increasing number of publications related to horses indicates a growing concern of the society regarding the health and welfare of this species. However, many strategies developed by researchers to prevent abnormal behaviors are not yet commonly implemented in horse farms and equestrian centers (HANIS *et al.*, 2020;

LEME *et al.*, 2014; SIMÕES, 2019). On the one hand, the progress of technology may have made knowledge more accessible, but on the other, the methods of housing and feeding domestic horses imposed by the needs of the modern society seem to perpetuate management practices that may restrict the species' natural behavior, which stimulates the development of stereotypies and other abnormal behaviors. According to the authors, the population of horses in the selected studies is used mainly for the practice of equestrian sports, work, leisure, and as pets. Conforming to Leme *et al*. (2014), the management practices, diet, and type of housing varies according to the horses' purpose and sport discipline. High performance equines are usually fed with large meals rich in non-fibrous carbohydrates to meet the high nutritional demands resulting from intense physical activity (GEOR; HARRIS, 2007). In addition, the high monetary value associated with these animals, as well as the need to keep them close to urban centers, are common reasons why horse owners decide to keep their animals stabled most of the time (HENDERSON, 2007).

2.4.2. Dietary Problems and the Prevalence of Abnormal Behaviors in Horses

The feeding routine adopted by equestrian centers, where animals are stabled, generally aims to optimize their employees' working scale by dividing the total diet of the horses into two or three daily meals, providing limited amounts of forage. According to the decision tree generated by the most accurate model, the limited supply of roughage was identified as the main factor related to the high prevalence of abnormal behavior in horses.

Firstly, previous studies have already associated diets with inadequate fiber content to the ingestion of bedding substrate (MILLS; ECKLEY; COOPER, 2000; COOPER; MCGREEVY, 2007) and aggressive behavior (BENHAJALI *et al.,* 2009) in this species. Secondly, researchers believe that providing more foraging opportunities and prolonging hay availability are techniques that may increase the frequency of positive social interactions between horses kept in groups, decrease aggression, and improve the animals' welfare (BENHAJALI *et al.*, 2009; BURLA *et al*., 2016). Behaviors like nosing, sifting, and ingesting bed substrate particles are abnormal practices that may be related to limited roughage, with the bedding material being consumed in an attempt to balance diets rich in starch and low in fiber. Another theory suggests that offering high energy meals would decrease the total volume of the diet, not being enough to satisfy the horse's ingestive behavior and causing perseverance of feeding behavior (COOPER; MCGREEVY, 2007). Depending on the material composition of the bedding, this habit could not only be unpleasant but also lead to serious health problems, causing chronic irritation of the respiratory epithelium from dust, noxious gases, and fungal spores (MILLS; ECKLEY; COOPER, 2000). Despite its importance, the prevalence of bed-eating was taken into account in only four studies conducted through direct observations. A high prevalence rate of this abnormal behavior could be evidence of the animals' inadequate nutritional management and should be further evaluated in future studies. Researchers also believe that incorrect feeding management can lead to the development of oral stereotypies in horses (JOHNSON *et al*., 1998; MCBRIDE; HEMMINGS, 2009). Oral stereotypies that are mentioned the most in the selected studies are crib-biting, measured in 15 publications, and wind sucking, measured in 10. The mean prevalence rate of those stereotypies in the studied population was 4.6% and 4%, respectively. However, in a recent observational study conducted with Colombian Creole horses, Mejía *et al.* (2022) found that crib-biting affected 17.65% of the studied population. The authors suggest that restricted feeding and the use of feed concentrates resulted in a higher frequency of oral stereotypies. Roberts and coworkers (ROBERTS *et al*., 2017), also believe that the development of these behaviors may be linked to gastrointestinal discomfort. They suggest that cereal-based concentrate feed reduces mastication and results in decreased saliva production. Saliva is believed to exert a buffering effect, contributing to a decrease in the horses' stomach pH. Thus, meals rich in starch or long periods of fasting would be related to excessive secretion of gastric acid without adequate saliva production, resulting in gastrointestinal discomfort (RUET *et al*., 2019). Recent studies also confirm that the expression of crib-biting involves the interaction between genetic predisposition and environmental conditions, being significantly influenced by the stressful environmental conditions to which the horse is exposed in its life (HEMMANN et *al.,* 2014; MUÑOZ *et al*., 2021). Other oral stereotypies observed in the selected studies were excessive licking and abnormal movements of the lips and tongue. The mean prevalence rate of those stereotypies in the studied population was about 27.3% and 3.9%, respectively. Oral stereotypies are undesirable because they can cause irregular tooth wear, hypertrophy of the neck muscles, and lead to the animals' economic devaluation (NICOLETTI *et al*., 1996).

2.4.3. Other Management Practices that Influence the Development of Abnormal Behaviors

In addition to inadequate diets, Sarrafchi and Blokhuis (2013) believe that the confinement of horses in single stalls, the lack of social interactions, and improperly performed physical exercise are also determining factors for the development and prevalence of locomotor stereotypies. The most observed locomotor stereotypies in all the selected studies were weaving, cited in 17 publications, and box walking, cited in 11 studies. The mean prevalence rate of these stereotypies in the studied population was 5% and 4.5%, respectively. Other locomotor stereotypies observed were pawing (7.7%), nodding (6.5%), and box-kicking (2.6%). These abnormal behaviors are undesirable since they can cause strained ligaments, poor performance, and reduced physical condition of the horse, leading to locomotory problems (MCBRIDE; HEMMINGS, 2009; SARRAFCHI; BLOKHUIS, 2013). In addition, according to five of the selected studies, factors such as the weaning technique and how the animals are housed right after this procedure could also impact the probabilities of a horse developing abnormal behaviors. During a 4-year prospective study on Thoroughbred and part-Thoroughbred horses, researchers observed that abnormal behavior affected 34.7% of the population (WATERS; NICOL; FRENCH, 2002). According to the authors, abrupt weaning, cereal-based meals, and confinement can substantially increase the prevalence of abnormal behavior in young horses. In general, scientists state that chronic stress situations early on in the animal's development may be critical to stereotypy predisposition (MCBRIDE; HEMMINGS, 2009; WATERS; NICOL; FRENCH, 2002). Most of the selected studies (n = 14) also suggest that the presence of windows or openings that allow the horses to observe the external environment and have contact with neighbouring horses can significantly improve their behavioral repertoire. Horses are gregarious animals that have better chances of survival in the wild when organized in social groups. Consequently, their capacity to interact with conspecifics was a crucial characteristic selected during the evolutionary history of the species (MILLS; CLARKE, 2007). Authors of 89% of the selected studies (n = 17) believe that increasing the opportunities for social interaction of stabled horses with conspecifics can decrease boredom and frustration related to the inability to express their natural behavior, which, in turn, can help minimize the prevalence of stereotypies.

A survey was conducted to identify correlations between housing arrangements and prevalence of abnormal repetitive behaviors in populations of sport and racehorses in Hungary. The authors found a positive correlation between prevalence of abnormal repetitive behaviors and use of a stable (0.261), as well as a corral (0.286). The prevalence of abnormal repetitive behaviors correlated negatively with the use of pasture (-0.099) (KÁDÁR *et al.,* 2023). In another study conducted by McAfee *et al* (2002) demonstrates that the provision of mirrors is able to significantly reduce unwanted behaviors of individually housed horses, such as weaving and nodding. Another recent research observes that even a poster of a life-size image of a horse's face can decrease the frequency of some stereotypies when fixed to the inside wall of the stall (MILLS; RIEZEBOS, 2005). Another factor capable of influencing the behavior of horses is the environmental conditions. This aspect was mentioned as being important by only one of the selected studies (REZENDE *et al.,* 2006), however, none of the articles investigated the correlation between climatic conditions and behavioral patterns. These parameters are paramount to maintain the thermal comfort of confined animals, especially when the facilities are located in tropical and subtropical locations, as behavioral changes have been observed in horses exposed to thermal stress. For example, in an experiment carried out in Brazil, the researchers reported that the air temperature was higher in stalls with metal walls, leading to higher thermal discomfort. In addition, the authors reported more reactivity and vocalization, demonstrating the horses' discomfort during the period of thermal stress (DA SILVA *et al.,* 2018). Although there is a great number of research related to behavioral changes motivated by climatic variations in outdoor horses (HOLCOMB; TUCKER; STULL, 2013; MEJDELL; BØE, 2005), there are still not many studies that evaluate the behavior and the prevalence of stereotypies in stabled equines when exposed to different climatic conditions, especially in tropical countries.

2.4.4. Biological Factors and Prevalence of Stereotypies in Horses

Researchers believe that biological factors such as gender (mentioned in 11 of the selected studies), breed (in 11), and age (in four) can influence the probability of a horse developing abnormal behaviors. For example, Luescher *et al* (1998) believe that the probability of developing weaving and crib-biting may be significantly higher in Thoroughbred horses. Arabian horses, on the other hand, are at higher risk of box walking (LUESCHER; MCKEOWN; DEAN, 1998). Rezende *et al* (REZENDE *et al.*, 2006) also observed differences related to the prevalence of abnormal behaviors between different horse breeds. Lusitano horses show a lower prevalence of abnormal behaviors, while crossbred and Brazilian Sport Horses show higher prevalence. Furthermore, Bachmann and coworkers (BACHMANN; AUDIGÉ; STAUFFACHER, 2003) also reported that Thoroughbred and Warmblood breeds are more likely to develop classic stereotypies (weaving, crib-biting, and box walking) and abnormal behaviors, as they are known for having more reactive temperaments. However, Sackman and Houpt (SACKMAN; HOUPT, 2019) did not find any association between reactive temperament and the prevalence of stereotypies in horses. Luescher *et al* (1998) observed that fillies are less likely to develop weaving and crib-biting, while colts are at greater risk. However, in another study, Mills *et al* (2002) observed a higher prevalence of stereotypies in females, geldings, and 2-year-old foals. Other authors state that variations related to the horses' gender can be attributed to different management techniques, as the stallions usually spend more time confined and have less contact with conspecifics (TADICH; WEBER; NICOL, 2013; WICKENS; HELESKI, 2010). Finally, Muñoz Alonzo *et al* (MUÑOZ ALONZO; CRUCES LEAL; BRIONES LUENGO, 2016) did not observe any association between the prevalence of stereotypies and the horses' gender, however, the authors reported that animals at the age of 2 to 3 years old may be more susceptible to the development of box walking. Mills et al (MILLS *et al.*, 2002) believe that young horses (about 2 years old) are more vulnerable to the development of stereotypies when subjected to a chronic stress state, usually generated when the animals are unable to perform species-specific behaviors or during the break in process.

2.4.5 Prevalence of Abnormal Behaviors in the Studied Population

Behavioral studies have shown inconsistent results. For example, among the selected studies we found articles with a mean prevalence of abnormal behaviors as little as 2.4% in horses from various uses and ages in the United Kingdom (MCBRIDE; LONG, 2001), whereas a survey conducted in small equestrian centers in Brazil reported prevalence rates of 95.5% in horses used for rodeo, 74.6% for leisure and 66.7% for foals (LEME *et al.,* 2014). At the same time, another observational study conducted in Brazil reported a prevalence rate of 94% in 2- year-old thoroughbred horses during five months of race training (SEABRA, 2017). Visser and coworkers observed that at the end of a 12-week- period, 67% of the 2-year-old Dutch warmbloods horses housed individually developed a stereotypy (12 out of 18) (VISSER; ELLIS; VAN REENEN, 2008).

The prevalence of abnormal behaviors observed in the selected studies is dependent on multiple environmental and biological factors, however, the methodology may have contributed to the perceived different rates. For example, some studies have only assessed the prevalence of weaving, crib-biting, and box walking, which are classified by the authors as "classic" stereotypies (CHRISTIE *et al*., 2006; MCBRIDE; LONG, 2001; MUÑOZ ALONZO; CRUCES LEAL; BRIONES LUENGO, 2016). Meanwhile, other studies include the observation of other abnormal behaviors that fit into the definition of stereotypies, such as abnormal movements of the lips and tongue, excessive licking, pawing, and box-kicking (HANIS *et al.,* 2020; RUET *et al.,* 2019, 2020; SEABRA, 2017; SIMÕES, 2019). Another factor that may have contributed to the disparity in values is the classification of wood chewing. According to some researchers, this kind of behavior should be considered a stereotypy (RUET *et al.,* 2019; SEABRA, 2017), but other authors prefer to classify the act as an abnormal or redirected behavior that would be mainly related to low fiber diets (HANIS *et al.,* 2020). The mean prevalence of wood chewing was measured in eight studies and affected about 21.2% of the studied population, demonstrating a high prevalence rate in the studies it was registered. An additional aspect that may have contributed to the different prevalence rates of stereotypies in the studies is the data-collection methodology used in the behavioral assessments. Despite the fact that no statistically significant differences were found, studies that directly observed the animals had a mean prevalence rate of 30.4% for stereotypies and other abnormal behaviors. However, studies based on questionnaires answered by the animals' owners had a mean prevalence rate of 19%. The smaller rate of abnormal behaviors in the horse population assessed through questionnaires may have two main reasons. Firstly, abnormal behaviors are undesirable, being commonly associated with low levels of animal welfare and promoting the economic devaluation of affected horses. Therefore, it is possible that equestrian centers with a high prevalence of abnormal behaviors may have chosen not to participate in the research (BACHMANN; AUDIGÉ;

STAUFFACHER, 2003; LUESCHER; MCKEOWN; DEAN, 1998; MILLS *et al.,* 2002). The second factor that may be related to a lower rate of abnormal behaviors is the owners' incapacity of understanding a more complex questionnaire and the difficulty in correctly identifying some stereotypies, leading to errors or inclusion of incorrect information (BACHMANN; AUDIGÉ; STAUFFACHER, 2003; CHRISTIE *et al.*, 2006).

2.5 CONCLUSION

Management practices used in the horse sector have changed very little over the last quarter of century, and the development of strategies is extremely necessary to prevent abnormal behaviors and improve stabled horses' welfare. The different prevalence of abnormal behaviors observed in the selected studies may be associated with the fact that this condition is dependent on multiple environmental (type of housing, diet, social interactions, exercise, climatic conditions) and biological factors (breed, age, gender, temperament). The data mining technique promises to elucidate relevant patterns and has indicated that limited availability of roughage is the main factor related to the development and prevalence of abnormal behaviors in horses. Thus, providing more foraging opportunities and prolonging hay availability may possibly decrease abnormal behaviors and aggression, showing great potential to improve the animals' welfare. Behavioral studies carried out through direct observation presented higher rates of abnormal behaviors. Although there were no statistically significant differences between direct observations and the studies carried out using questionnaires, the first data-collection methodology should be prioritized in future research. Another important fact that needs to be taken into account is that there are still no studies investigating the influence of environmental conditions on the prevalence of abnormal behaviors in horses, and further research is needed to evaluate this hypothesis.

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3. TIME-BUDGET AND WELFARE INDICATORS OF STABLED HORSES IN THREE DIFFERENT STALL ARCHITECTURES: A CROSS-SECTIONAL STUDY

ABSTRACT

Caring about welfare in horses means not only to provide shelter, food, and water, but also an environment in which they are allowed to perform natural behaviors. In equine production, it is common to keep horses in single stalls, however, this housing condition can prevent them from performing natural behaviors due to restricted eating and movement, and social isolation. This condition can lead to a chronic stress state that may trigger the development of abnormal and stereotypic behavior in horses. However, studies show that opportunities for social interactions and the stall architecture are factors that can influence the development and prevalence of stereotypies. Time-budget can be defined as the daily amount of time an animal engages in behavioral activities. Scientists believe that differences in the time-budgets of domesticated horses compared to feral conspecifics in their natural environment can be used to reveal welfare impairment. Other physiological parameters that can be used to measure the stress levels in horses are the cortisol concentrations in the blood, the presence of a cortisol circadian rhythm (CCR), the leucocyte profile, and the neutrophil/lymphocyte ratio. The current study aimed to identify how three different stall architectures can influence the time-budgets, and physiological parameters in full-time stabled horses. Surveillance cameras were installed inside one stall of each three barns. Twenty-nine horses - ten horses from the first and third barns, and nine from the second barn were randomly selected from three different stables located at the same equestrian facility and filmed for 24 hours. The video was analyzed with continuous behavioral sampling and the time spent in stereotypic, abnormal behaviors and other activities was measured second-by-second, inserted into a spreadsheet. Nine horses from the first barn, 8 from the second and 7 from the third were randomly selected for the blood collection performed at 8:00 AM and at 5:00 PM. The samples were used to determine the cortisol levels, CCR, leucocyte profile and neutrophil/lymphocyte ratio. All horses observed presented abnormal and stereotypic behaviors. The restricted availability of food had a direct impact on the horses' time-budget, creating long periods of idleness and fasting. The forage content below ideal values may have led horses to develop abnormal behaviors (bedding-eating, coprophagy and wood-chewing) and to spend more time with those practices compared to eating proper food. Six horses in the studied population also presented alterations in immune cell numbers, and nine horses had cortisol levels above the reference range. These findings indicate that the stabled horses in the current study were in a state of chronic stress and the stalls' architecture and the meteorological conditions did not significantly change the horses' capability to cope with such housing conditions.

Key words: Continuous behavioral sampling; Cortisol circadian rhythm; Equine; Stereotypes; Welfare.

3.1 INTRODUCTION

In recent years, there has been an increasing interest in animals and the conditions under which they are kept and raised. Researchers believe that urbanization, progress of technology, expansion of media reach, and increase in society's education and economic level are the reasons why there is a growing interest in animal welfare in many countries. Caring about welfare in horses means not only to provide shelter, food, and water, but also an environment in which they are allowed to perform natural behaviors (KOKNAROGLU; AKUNAL, 2013).

Scientists define “natural behaviors” as behaviors that are seen in nature, internally motivated, pleasurable, and that can be species-specific or not. Some actions like being able to play and run can be beneficial for different animal species from a welfare perspective. However, other positive behaviors can be highly species-specific, like the rooting in pigs, dustbathing in poultry, and grazing in horses and cattle (BRACKE; HOPSTER, 2006).

Time-budget is a term that can be defined as the daily amount of time an animal engages in behavioral activities. Researchers used direct observations of feral horse populations to construct the natural “time-budget” of this species and calculated the percentage of time horses would spend in different activities. Under natural conditions, horses spend 67 to 75% of their time grazing, 15 to 25% resting or sleeping, and 6 to 10% observing the surroundings, with other activities occupying the remaining time (WARING, 1983). Further performed studies revealed that domestic horses will display time budgets similar to those of feral horses when given the opportunity. Therefore, domesticated horses are often compared to feral individuals from the same species. With that in mind, differences in the time-budgets of domesticated horses compared to feral conspecifics are currently used to reveal welfare impairment (AUER *et al.*, 2021; LESIMPLE, 2020).

In the equine industry, high performance sport horses are commonly housed in single stalls (LESIMPLE *et al.*, 2020). However, this kind of management technique can prevent horses from performing natural behaviors due to restricted feeding and movement, and social isolation, which may lead to a chronic stress state. This condition can cause behavioral, physical, and metabolic disorders that are detrimental to the health of the animal (GONTIJO *et al.,* 2014). The concentration of cortisol in blood is widely used as an indicator of stress, however, the measurements may be affected by time of day, the occurrence of short-term fluctuations, and other environmental factors. Studies show that horses that are well adapted to their environment present a normal oscillation in blood cortisol concentrations, with values that are higher in the morning and decreased throughout the day. Therefore, the absence of a normal cortisol circadian rhythm (CCR) (IRVINE; ALEXANDER, 1994) may be a reliable indicator of chronic stress and poor welfare in horses (LEAL *et al.*, 2011). Immune response changes are another example of induced modifications that may occur in chronically stressed horses. Researchers believe that the use of the leucocyte profile, and especially the neutrophil/lymphocyte ratio may be a reliable indicator to measure stress in vertebrates (Davis *et al.*, 2008). Previous studies have associated a high neutrophil/lymphocyte ratio with horses and mice exposed to chronic stress (HICKMAN, 2017; JUNG *et al.*, 2019; POPESCU; DIUGAN, 2017; SWAN; HICKMAN, 2014).

Another indicator of decreased welfare reported in many studies with different species of animals, including horses, are neurophysiological alterations that may lead to development of stereotypies (CABIB; BONAVENTURA, 1997; MCBRIDE; HEMMINGS, 2005, 2009). Studies suggest that chronic stress in combination with a predisposed genotype contributes to sensitization of the dopaminergic midbrain and striatum, which has been hypothesized as a precursor for stereotypy manifestation in horses (ROBERTS *et al.*, 2017). Stereotypies are invariant and repetitive behavior patterns that seemingly have no function and other abnormal behaviors are practices that do not fit this definition but are not commonly observed in horses living in wild environments (BARTOLOMÉ; COCKRAM, 2016). The prevalence of stereotypies in horses is commonly measured through behavioral assessment, which is a simple method that does not require specific equipment. This technique, alongside the leukogram profile, has been considered appropriate to assess chronic stress in animals (HICKMAN, 2017; JUNG *et al*., 2019; POPESCU; DIUGAN, 2017; SWAN; HICKMAN, 2014). The prevalence of stereotypies in domestic horses has been studied for decades, and researchers believe that several environmental and biological factors may be involved in the development of these behaviors. Studies show that opportunities for social interactions and the stall architecture are factors that can influence the development and prevalence of stereotypies in horses. However, research is still needed to clarify how those aspects can influence the time-budget of stabled horses and their level of welfare. The current study first aimed to identify how three different stall architectures can influence the horses' time-budgets. The second aim was to investigate how those different housing conditions can influence the cortisol circadian rhythm (CCR), leucocyte profile and prevalence of abnormal behaviors in stabled horses.

3.2 MATERIAL AND METHODS

3.2.1 Study population

All experimental procedures were approved by the Ethics Committee on Animal Use of the Federal University of Paraná (UFPR, protocol number 006/2021). The population used for this study consisted of 29 healthy horses (23 geldings and six mares) from different breeds - 21 mixed horses, five Lusitano, and three Brazilian showjumpers. The study was carried out at an Equestrian facility, in Curitiba, Brazil during the fall and winter and seasons (from April 2021 to July 2021). The horses were 13 years old (13 ± 1.1 years), weighed 495.64 ± 15.01 Kg, and had a body condition score of 2.86 ± 0.41 (The 0 to 5 system used by LEIGHTON-HARDMAN, 1980). They had the same management protocol and feeding routine, which remained identical during the whole trial. The daily feeding routine consisted of four meals: 2 Kg of alfalfa hay fed twice a day (4kg in total), and 1.6 Kg of commercial concentrate feed twice a day (3.20 kg in total). The compositions of the hay and the commercial concentrate used for the diet in this trial are listed in TABLE 4. Horses were housed in individual stalls equipped with automatic water, going out only to exercise, with no access to the pasture, paddocks, or any kind of free time outdoors. The horses were user for light exercise, four days a week for different purposes (showjumping, dressage, patrolling and equine assisted therapy), and the same horse could be used in different work modalities. The animals were randomly selected from three different stables located inside the same equestrian facility. All the stalls were bedded with shavings (pine sawdust); wet shavings were removed once a day.

TABLE 4 – COMPOSITION OF THE DIET OFFERED TO ALL HORSES DURING THE TRIAL

	Diet	
	Hay (*Medicago sativa*)	Concentrate
Dry matter (%)	84.61	88.00
Crude Protein (% DM)	18.18	13.00
Ash (% DM)	7.07	10.00
Fat (% DM)	2.01	6.00
Neutral detergent fiber (NDF)	41.51	16.00
Acid detergent fiber (ADF)	28.69	21.60
Calcium (% DM)	1.48	1.50
Phosphorus (% DM)	0.26	0.70
DE Mcal/kg	2.59	2.80

3.2.2 Stall architecture

Twenty-nine horses were randomly selected from three different barns: 10 from the first barn (FIGURE 3), nine from de second barn (FIGURE 4) and 10 from the third barn (FIGURE 5). All barns were built out of masonry with fiber cement roofs. Each barn had different stall architecture and a detailed description can be seen below (TABLE 5).

FIGURE 3 – FIRST BARN

SOURCE: the author

FIGURE 4 – SECOND BARN

SOURCE: Google

FIGURE 5 – THIRD BARN

SOURCE: Google

TABLE 5 - DESCRIPTION OF THE DIFFERENT STALL ARCHITECTURE IN THREE DIFFERENT BARNS

Stall Architecture	First Barn	Second Barn	Third Barn
Dimensions	3.22x3.74 m	2.60x3.50 m	2.30x3.40 m
Door location	Opens to an inner aisle	Opens to the outside patio	Opens to the outside patio
Tactile contact with conspecifics	Yes	No	No
Visual contact with conspecifics	Yes	Yes	Yes
Total number of stalls	24	27	32

3.2.3 Behavioral assessments

Two surveillance cameras were installed in one of the stalls of each barn. One camera was placed over the stall's door and a second camera was placed inside the stall, fixed to the roof beams. Horses of each barn (10 from the first and third brans, and nine from the second barn) were randomly selected in different days, transferred to the monitored stall, and filmed for a period of 24 hours. The video was recorded by and analogical recorder and transferred to a hard drive for posterior analyzes with continuous behavioral sampling. The time spent in stereotypic, abnormal behaviors and other activities was measured second-by-second, inserted into a Microsoft Excel spreadsheet, and used for the determination of the animals' time budgets. Each behavior observed was defined according to the adapted ethogram (TABLE 6) (PESSOA *et al.*, 2016). The footage of 8 horses was analyzed by a trained observer and the rest of the animals were analyzed by the main researcher. The main researcher consisted of an animal scientist with more than seven years of experience with horses and the trained observer was an undergraduate student in agricultural sciences with 3 years of previous experience with horses (amateur rider). The observers in the current study attained ≥90% of agreement for all behaviors.

TABLE 6 – ETHOGRAM OF THE HORSES' BEHAVIORS

Normal Behaviors	
Eating hay	Animal feeding on forage in troughs
Eating concentrate	Animal feeding on commercial grain mix
Drinking	Animal seeking water or drinking water
Observing the surroundings	Animal standing in place, alert, with elevated neck, observing its surroundings
Standing passive	Animal standing in place a little unaware or sleepy, observing the surroundings, with either head or ears turning at short intervals
Other natural behaviors	Not listed above (defecating, lying down, urinating, scratching, walking, rolling over)
Stereotypies (COOPER; MCGREEVY, 2007)	
Crib-biting	involves the grasping of a surface (usually horizontal) in the teeth and the apparent engulfing of air (MCGREEVY; NICOL, 2010)
Excessive licking	The horse licks objects or surfaces with no nutritional purpose
Box-kicking	The horse knocks with the hind limb hoofs against the wall of the stable
Headshaking	The horse moves its head up and down repetitively
Pawing	The horse uses its hoof for digging or scratching a surface
Box-walking	The horse wanders in circles inside the box, walking, trotting, or galloping
Weaving	The horse moves its head from side to side in a rhythmic movement that can involve neck, forelimbs, and even hind limbs (TADICH; WEBER; NICOL, 2013)
Lips and tongue abnormal movements	horse performs repetitive tongue, mouth, or jaw movements without any obvious food substrate in the mouth (COOPER; MCGREEVY, 2007)
Other Abnormal Behaviors	
Bedding-eating	The horse ingests the bedding material of its stable
Wood-chewing	The horse chews and tries to pull out wood pieces in order to eat them
Coprophagy	The horse eats its own feces

3.2.4 Meteorological Recordings

A thermohydrometer was placed inside the experimental stall in each barn to measure the weather conditions, including ambient temperature, relative humidity, and dew point temperature during the recordings' dates. The video and the meteorological data collection were performed in the southern hemisphere during June/July in the first barn, during July in the second barn, and in April/May in the third barn. The average temperature for those months in the study location is displayed in TABLE 7.

TABLE 7 – AVERAGE TEMPERATURE DURING THE STUDY DURATION IN CURITIBA, PR, BRAZIL.

Months	Average Temperature (˚C)	Average Minimum Temperature (˚C)	Average Maximum Temperature (˚C)
April	18.1	15.2	22.3
May	15	12	18.9
June	13.9	10.7	18.2
July	13.4	9.9	18

The environmental data were also collected from the closest meteorological station, about 5000 meters away from the equestrian facility. Then, the data were inserted into an excel spread sheet and used to calculate the average weather conditions during the study. From the environmental data collected inside the experimental stalls, Temperature–Humidity Index (THI) was derived using the following equation (THOM, 1958):

$$THI = T_{db} + 0.36*(T_{dp}) + 41.5$$

Where T_{db} is the dry bulb temperature (˚C) and T_{dp} is the dew point temperature (˚C).

Temperature–Humidity Index (THI) was used as an estimate of the degree of thermal discomfort experienced by horses.

3.2.5 Blood analyses

After finishing the behavioral assessments, twenty-four animals were randomly selected for the blood collection (9 horses from the first barn, 8 from the second and 7 from the third). Venous blood samples were collected from the horses at 8:00 AM and at 5:00 PM (9 hours after the first sample). All blood collection procedures were performed during the horses' day of rest, when animals remain in their stalls for a period of 24 hours. The procedure took place in the horses' stall and was conducted by an experienced veterinarian, which collected the samples quickly to minimize acute stress, and consequently to avoid any changes in the blood cortisol levels. The blood samples were drawn by jugular venipuncture into two vacutainers: the first with ethylenediamine tetra acetic acid (EDTA), and the second with thrombin-based clot activator.

The blood samples were immediately stored at 4°C and transported to the Laboratory of Clinical Pathology of the Federal University of Paraná. The samples collected with EDTA vacutainers were immediately used to identify and quantify the leukogram with differential leukocyte count. The values for the white blood cells (WBC) were determined via the cell counter BC-2800Vet Auto Hematology Analyzer (Mindray). Blood smears were done immediately after blood sampling and stained with a fast Romanowsky-type stain (Newprov ®, Brazil) to determine the differential leukocyte counts using a 100-cell count technique. The neutrophil/lymphocyte ratio was calculated by dividing the absolute number of neutrophils by the absolute number of lymphocytes (ROSSDALE; BURGUEZ; CASH, 1982).

The blood samples collected with thrombin-based clot activator were centrifuged for 1500g for 5 minutes, serum was separated, divided into aliquots, and placed into polypropylene tubes and stored at -80ºC until analysis. Serum cortisol levels were measured by enzyme immunoassay (ELISA, RE52061, Immuno Biological Laboratories, IBL, Hamburg, Germany), a kit developed for the quantitative measurement of cortisol in human serum and plasma. CCR ratios for each horse were calculated by dividing the difference of the two sample values by the higher one. The CCR ratio was considered abnormal when the result was <0.30 (DOUGLAS, 1999; LEAL *et al.*, 2011).

3.2.6 Data Analysis

The horses' time-budgets were calculated by the sum of seconds spent in each behavior observed during the 24 hours period. The seconds were converted into minutes and the comparison of total minutes spent in each behavior in the three different barns were tested via an ANOVA with post hoc Tukey test. The difference in CCR ratio values and the neutrophils/lymphocytes ratio between the three different barns was also assessed by ANOVA analysis. Bartlett's test was used to verify the homogeneity of variances using the statistical program Assistat (E SILVA, DE AZEVEDO, 2016). In addition, the Mann- Whitney U test was used to verify the existence of significant differences between the cortisol levels and the neutrophils/lymphocytes ratio in the samples collected during the morning and afternoon. The accepted level of significance was $P < 0.05$ and data transformations were not required prior to statistical analysis. Temperature and humidity inside the

stalls were compared with the same data collected from the nearest meteorological station using T-Student test ($P < 0.05$). Temperature, humidity, and the Temperature–Humidity Index (THI) could not be compared between barns, because they were collected on different days in each site.
Results are presented as mean values ± standard deviation (SD).

3.3 RESULTS

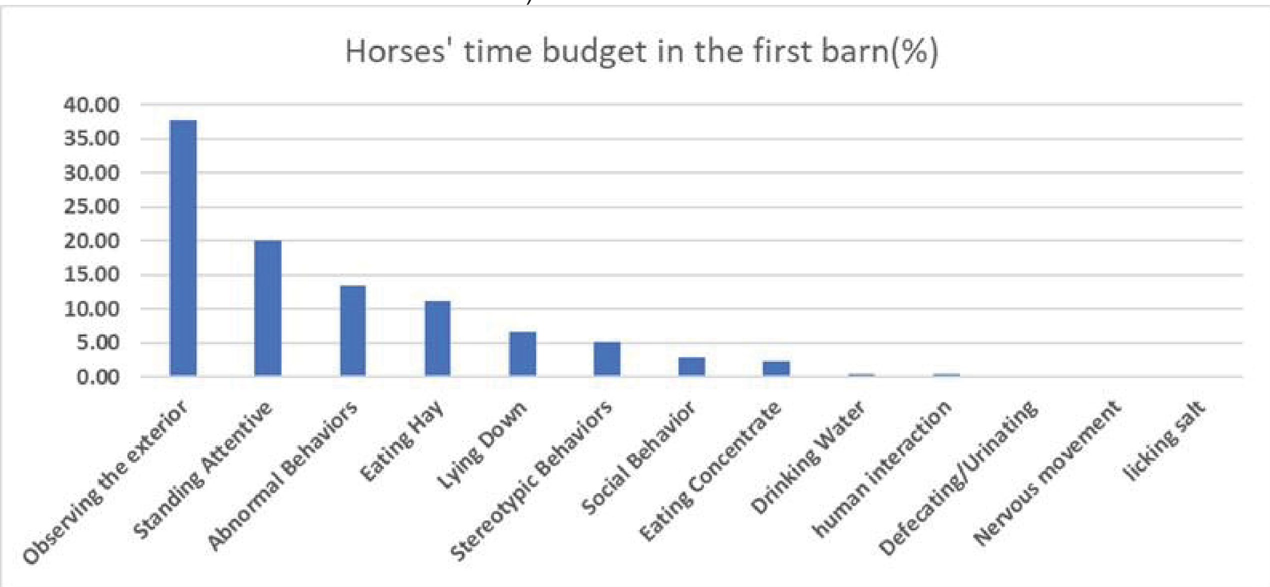
3.3.1 Behavioral assessments

Social interaction was the only behavior that differed significantly between the barns ($P = 0.022$). In the first barn, the horses spent, on average, 2.77% (about 40 ± 18.4 minutes per day) of their time-budgets socializing with their neighboring conspecifics. At the same time, the second and third barns were not significantly different, with horses spending 0 and 0.01% (0 ± 0 and 0.18 ± 0.14 minutes) of their time-budgets with social interactions, respectively. The other behaviors observed were not significantly different between the barns. The percentage of time horses spent in each observed behavior in different barns can be seen below (FIGURE 6-8). In general, horses spent 41.04% of their time-budget observing the exterior of the stall, 18.19% of their time-budget standing attentive inside the stall, 15.57% of their time-budget practicing abnormal behaviors, 11.76% of their time-budget eating hay, 6.58% of their time-budget lying down, 2.55% of their time-budget eating concentrate, and 5.53% of their time-budget practicing stereotypic behaviors. The minutes and the descriptive statistics of these and other observed behaviors can be seen below (TABLE 8).

TABLE 8 – MEAN VALUES IN MINUTES OF OBSERVED BEHAVIORS

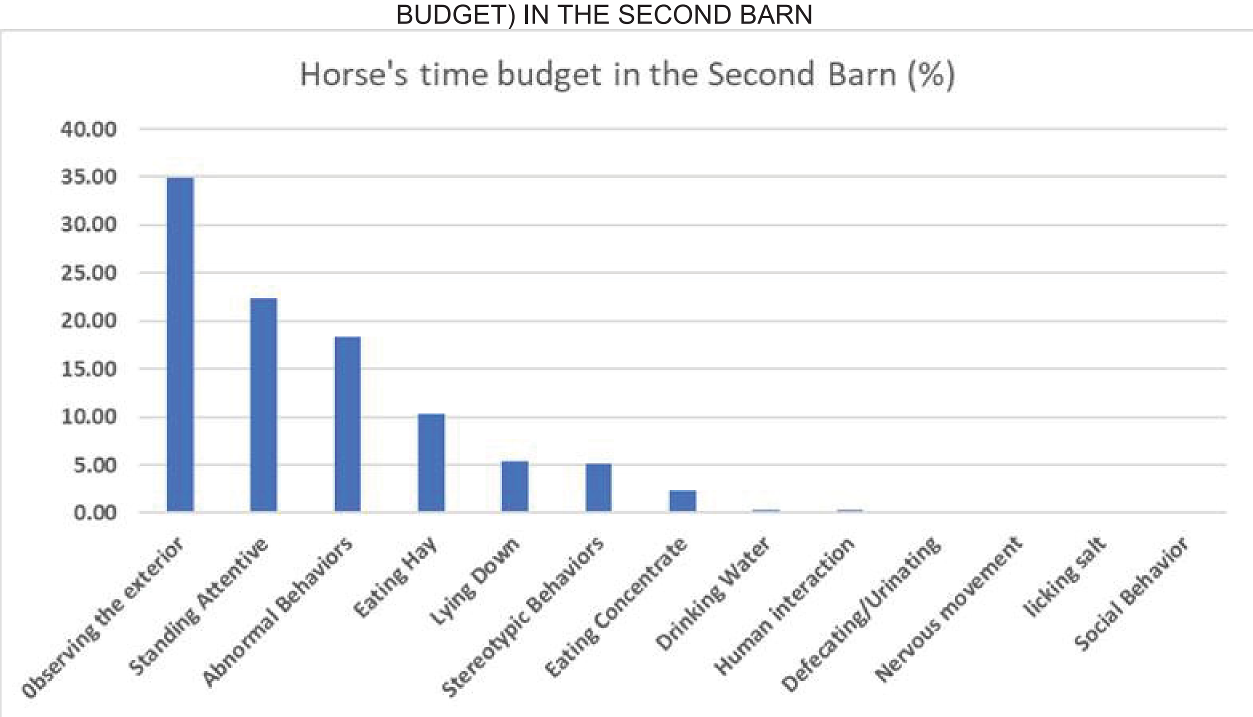
Observed behaviors	Mean ± SD (Minutes)	Median	Mode	Sample Variance	Minimum (Minutes)	Maximum (Minutes)
Stereotypic Behaviors	73.25 ± 56.20	60.83	20.67	3158.29	1.83	201.45
Abnormal Behaviors	224.20 ± 121.49	214.42	214.42	14759.09	64.42	608.75
Observing the exterior	543.5 5± 241.39	595.83	-	58268.53	107.58	930.42
Standing Attentive	251.10 ± 182.15	216.67	170.75	33178.09	0.00	593.17
Lying Down	94.49 ± 59.87	89.83	-	3585.01	0.00	246.83
Eating Concentrate	36.72 ± 14.05	34.75	-	197.32	15.92	70.28
Eating Hay	166.26 ± 59.74	148.58	-	3568.30	75.83	335.83
Drinking Water	7.23 ± 7.18	4.67	1.58	51.55	0.00	26.00
Defecating	1.85 ± 1.40	1.50	1.00	1.95	0.50	6.35
Urinating	1.81 ± 0.97	1.67	3.67	0.94	0.33	3.67
Bedding-eating	175.96 ± 105.66	153.82		11163.62	38.50	436.50
coprophagy	17.97 ± 40.16	1.42	0.00	1612.51	0.00	154.58
Excessive licking	64.28 ± 51.51	57.92	-	2653.48	1.83	195.85
Wood chewing	41.51 ± 100.95	6.00	0.33	10189.92	0.00	503.33
weaving	9.62 ± 34.25	0.00	0.00	1172.92	0.00	155.75
Crib-biting	0.10 ± 0.54	0.00	0.00	0.29	0.00	2.92
box walking	0.67 ± 1.52	0.00	0.00	2.30	0.00	7.17
Headshaking	1.35 ± 2.67	0.08	0.00	7.13	0.00	11.83
pawing	0.58 ± 1.46	0.00	0.00	2.13	0.00	6.08
Nervous movement	1.68 ± 2.50	0.25	0.00	6.26	0.00	9.17
human interaction	5.11 ± 5.12	4.83	0.00	26.23	0.00	23.75
Social Interactions	13.83 ± 38.21	0.00	0.00	1460.10	0.00	184.33
licking salt	1.39 ± 4.50	0.00	0.00	20.28	0.00	23.17
box-kicking	0.11 ± 0.62	0.00	0.00	0.38	0.00	3.33
Lips and tongue abnormal behaviors	3.11 ± 16.66	0.00	0.00	277.66	0.00	89.75

FIGURE 6 – PERCENTAGE OF TIME SPENT ON EACH BEHAVIOR FOR 24 HOURS (TIME-BUDGET) IN THE FIRST BARN



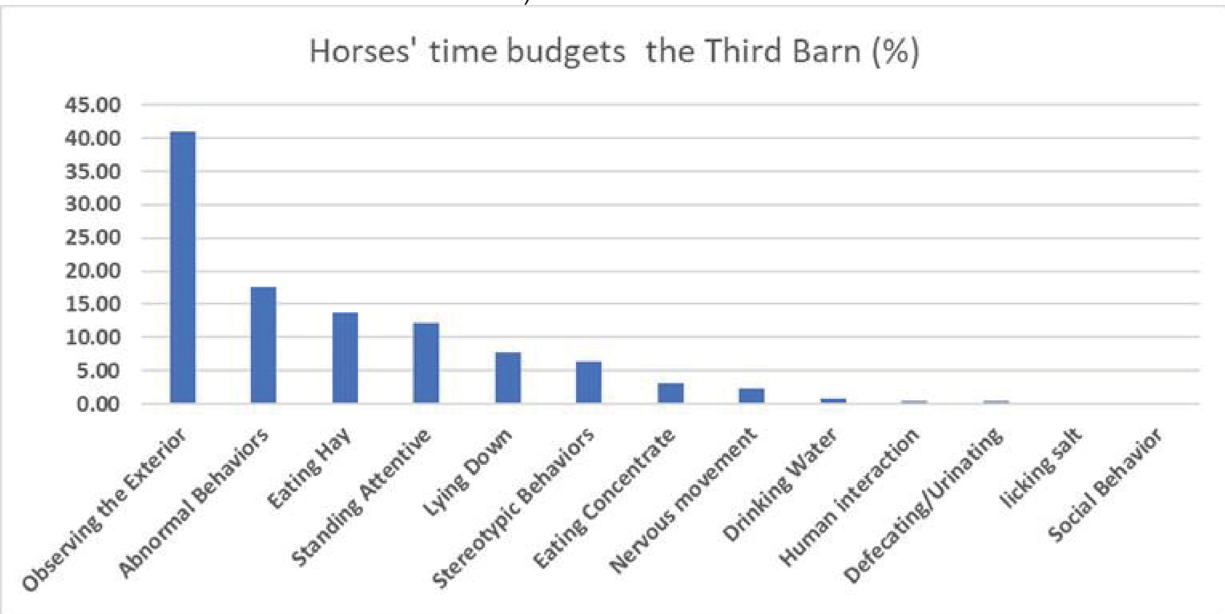
SOURCE: The author

FIGURE 7 - PERCENTAGE OF TIME SPENT ON EACH BEHAVIOR FOR 24 HOURS (TIME-BUDGET) IN THE SECOND BARN



SOURCE: The author.

FIGURE 8 - PERCENTAGE OF TIME SPENT ON EACH BEHAVIOR FOR 24 HOURS (TIME-BUDGET) IN THE THIRD BARN



SOURCE: The author.

In this study, 100% of the horses housed in all three barns performed at least one kind of abnormal and at least one kind of stereotypic behavior. Bed-eating was the most common abnormal behavior featured during the study, affecting 100% of the studied population, followed by wood-chewing (93.10%), and coprophagy (65.52%). Furthermore, 100% of the horses housed in all three barns were observed performing excessive licking, followed by headshaking (51.72%), pawing (41.38%), weaving (24.14%), box-walking (24.14%), lips and tongue abnormal movements (6.90%), box-kicking (3.45%) and crib-biting (3.45%). The time spent practicing abnormal and stereotypic behaviors was not significantly different between the three different barns, but the percentage of horses performing those behaviors in each housing condition can be seen in TABLE 9.

TABLE 9 - PERCENTAGE OF HORSES PERFORMING ABNORMAL AND STEREOTYPIC BEHAVIORS IN THREE DIFFERENT BARNS

Abnormal and Stereotypic Behaviors	First Barn (%)	Second Barn (%)	Third Barn (%)	All Horses (%)
Bed-eating	100.00	100.00	100.00	100.00
Coprophagy	50.00	55.56	90.00	65.52
Wood-chewing	80.00	100.00	100.00	93.10
Excessive licking	100.00	100.00	100.00	100.00
Weaving	0.00	44.44	30.00	24.14
Crib-biting	0.00	0.00	10.00	3.45
Box-walking	30.00	33.33	10.00	24.14
Headshaking	30.00	55.56	70.00	51.72
Pawing	40.00	22.22	60.00	41.38
Box-kicking	0.00	0.00	10.00	3.45
Lips and tongue abnormal movements	20.00	0.00	0.00	6.90

3.3.2 Meteorological Recordings

There was no significative difference between the meteorological data recorded inside the different barns and the data recoded in the closest meteorological station. The meteorological conditions inside each barn could not be compared, because the data were recorded on different days. The calculated Indoor Temperature–Humidity Index (THI) ranged from 51.75 to 75.22 in the total duration of the study. At the same time, temperature and humidity inside the stalls ranged from 8.20 to 28.00 ˚C, and from 47.70 to 98.10% during the current study, respectively. The mean values (± standard deviation) of the temperature and humidity obtained from the thermohydrometers placed inside the experimental stalls (Indoor Temperature) and the closest meteorological station (Outdoor Temperature) throughout the monitoring period are reported in TABLE 10.

TABLE 10 – MEAN VALUES ± STANDARD DEVIATION OF THE TEMPERATURE AND HUMIDITY MEASURED INSIDE THE BRANS AND IN A METEOROLOGICAL STATION THROUGHOUT THE MONITORED PERIOD

	Mean ± SD	Median	Mode	Sample Variance	Minimum	Maximum	Range
Indoor Temperature (°C)							
First barn	15.84 ± 3.79	15.60	12.80	14.39	8.20	23.80	15.6
Second barn	17.61 ± 3.13	17.30	15.20	9.81	13.10	26.10	13.00
Third barn	17.91 ± 3.45	17.60	17.80	11.92	10.40	28.00	17.60
Indoor Humidity (%)							
First barn	79.42 ± 10.83	83.30	83.10	117.39	50.90	91.50	40.60
Second barn	88.86 ± 8.03	90.90	95.50	64.55	61.70	98.10	36.40
Third barn	80.46 ± 9.36	84.40	86.60	87.68	47.70	91.10	43.40
Indoor Temperature–Humidity Index (THI)							
First barn	61.69 ± 4.59	61.77	61.77	21.11	51.75	70.85	19.096
Second barn	64.75 ± 3.75	64.40	65.75	14.10	58.97	74.296	15.33
Third barn	64.58 ± 4.07	64.49	64.95	16.54	54.96	75.22	20.264
Outdoor Temperature (˚C)							
First barn	17.37 ± 5.46	17.90	16.50	29.78	9.30	24.10	14.80
Second barn	16.23 ± 5.09	14.70	24.30	25.94	9.90	24.30	14.40
Third barn	16.56 ± 2.36	16.10	16.10	5.55	13.05	19.35	6.30
Outdoor Humidity (%)							
First barn	72.04 ± 19.77	80.00	85.00	390.74	39.00	98.00	59.00
Second barn	84.50 ± 15.85	91.50	96.00	251.32	52.00	99.00	47.00
Third barn	81.25 ± 12.35	85.50	89.00	152.49	50.00	96.00	46.00

3.3.3 Blood analyses

The absolute number of neutrophils was significantly lower in the blood's samples collected in the second barn (P <0.01). There was no other significant difference between the values of total leukocytes, lymphocytes, neutrophils, neutrophil/lymphocytes ratio and cortisol collected in the different barns and in morning and in afternoon. Mean cortisol plasma concentrations ± standard deviation from samples collected in the morning and afternoon were 78.82 ± 46.43 and 54.70 ± 42.76 ng/mL, respectively. The overall CCR ratio mean was 0.37 ± 0.18. Although there was no significant difference, the horses housed in the third barn had lower CCR ratios and higher incidence of abnormal CCR as compared with all other housings (TABLE 11).

The mean ± SD values and the descriptive statistics for the leucocyte profile, neutrophil/lymphocyte ratio and cortisol levels can be seen in TABLE 12.

TABLE 11 – MEAN CORTISOL CIRCADIAN RHYTHM (CCR) RATIO AND PERCENTAGE OF ANIMALS WITH ALTERED CCR IN EACH BARN

Groups	**N**	**CCR Ratio (ng/mL)**	**Incidence of CCR Ratio <0.30**	
		Mean ± SD	Horses	%
First barn	9	0.42 ± 0.21	2	22.22
Second Barn	8	0.40 ± 0.17	1	12.50
Third Barn	7	0.28 ± 0.15	4	57.14
Total	24	0.37 ± 0.18	7	29.17

TABLE 12 – MEAN VALUES AND DESCRIPTIVE STATISTICS FOR THE LEUCOCYTE PROFILE, NEUTROPHIL/LYMPHOCYTE RATIO AND CORTISOL LEVELS

	First barn						
	Mean ± SD	Median	Mode	Sample Variance	Minimum	Maximum	Range
Total Leukocytes (x 10^3/µL)	6,522.22 ± 1,045.19 ns	6,400.00	6,400.00	1,092,418.30	5,300.00	8,900.00	3,600.00
Neutrophils (x 10^3/µL)	4,046.06 ± 852.73 a	4,112.00	3,248.00	727,145.82	2,703.00	5,184.00	2,481.00
Lymphocytes (x 10^3/µL)	2,536.17 ± 639.24 ns	2,452.00	2,240.00	408,630.85	1,690.00	4,180.00	2,490.00
Neutrophil/ Lymphocytes ratio	1.68 ± 0.55 ns	1.55	2.77	0.31	1.06	2.77	1.71
Cortisol (ng/mL)	62.43 ± 52.62 ns	51.12	-	2,769.19	21.92	213.63	191.71
	Second barn						
	Mean ± SD	Median	Mode	Sample Variance	Minimum	Maximum	Range
Total Leukocytes (x 10^3/µL)	5,956.25 ± 1,472.85 ns	5,850.00	6,000.00	2,169,291.67	4,000.00	9,300.00	5,300.00
Neutrophils (x 10^3/µL)	3,287.06 ± 485.65 b	3,443.50	3,840.00	235,856.86	2,350.00	3,840.00	1,490.00
Lymphocytes (x 10^3/µL)	2,392.19 ± 1,164.36 ns	1,963.50	-	1,355,741.10	1,012.00	5,301.00	4,289.00
Neutrophil/ Lymphocytes ratio	1.61 ± 0.64 ns	1.61	-	0.41	0.68	3.22	2.53
Cortisol (ng/mL)	56.20 ± 35.25 ns	50.65	-	1,242.26	21.24	166.37	145.12
	Third barn						
	Mean ± SD	Median	Mode	Sample Variance	Minimum	Maximum	Range

Total Leukocytes (x 10^3/µL)	6,771.43 ± 746.71 ns	6,950.00	7,400.00	557,582.42	5,200.00	7,600.00	2,400.00
Neutrophils (x 10^3/µL)	3,981.57± 632.65 a	4,029.00	-	400,242.57	2,496.00	4,884.00	2,388.00
Lymphocytes (x 10^3/µL)	2,580.43± 665.53 ns	2,637.00	-	442,926.73	1,500.00	3,550.00	2,050.00
Neutrophil/ Lymphocytes ratio	1.68 ± 0.64 ns	1.61	1.88	0.41	0.94	2.92	1.98
Cortisol (ng/mL)	84.38 ± 45.11 ns	95.15	-	2,034.48	27.39	144.58	117.19

Numbers with different letters are significantly different (Tukey test) at a 5% level. ns not significant (*P* > .05).

3.4 DISCUSSION

3.4.1 Behavioral assessments

The current study observed a very high prevalence of abnormal and stereotypic behaviors (100%) in the studied population. The percentage of horses practicing those behaviors is much higher in the present study than in other papers (BACHMANN; AUDIGÉ; STAUFFACHER, 2003; CHRISTIE *et al.*, 2006; HOUPT *et al.*, 2001; MCBRIDE; LONG, 2001; MILLS *et al.*, 2002; TADICH *et al.*, 2012). According to our previous research, the results found in observational studies can vary greatly depending on the methodology. For example, horse populations studied through direct observation tend to present higher rates of abnormal behaviors when compared to studies based on questionnaires (SEABRA; DITTRICH; VALE, 2021). An additional aspect that may have contributed to the different prevalence rates is that some studies only accessed "classic" stereotypies (weaving, crib-biting, and box walking) (CHRISTIE *et al.*, 2006; MCBRIDE; LONG, 2001; MUÑOZ ALONZO; CRUCES LEAL; BRIONES LUENGO, 2016). In the meanwhile, scientist believe that observational studies will present higher prevalence rates when the researchers decided to also register abnormal behaviors like wood chewing, and bedding-eating, which are known for affecting larger amounts of the stabled population (SEABRA; DITTRICH; VALE, 2021).

According to authors, scan or focal sampling does not provide a continuous assessment of behavior and thus potentially misses or underrepresents important, infrequent behaviors (AUER *et al.*, 2021). Therefore, continuous observation is believed to be the most accurate method for behavioral measurements (MITLÖHNER *et al.*, 2001). The current study registered all behaviors performed by the studied population continuously second-by-second during a period of 24 hours. Therefore, the data-collection methodology used in the current study may have contributed to the higher identification rate of abnormal and stereotypic behaviors in this population.

Feral and wild horses in free-ranging conditions devote most of their time to graze and search for food, spending up to 18 hours a day foraging (75% of their time-budgets) (AUER *et al.*, 2021). However, domestic horses fed with a mixture of concentrate and limited amounts of hay will consume their requirements in a much shorter period of time. The horses that participated in this trial were fed only with 4.00 kg of hay and 3.20 kg of concentrate per day, regardless of their body weight (BW) or level of physical activity. This kind of feeding regime can be seen as inadequate, since scientists recommend a minimum of 1.0 kg long-stem forage per 100 kg BW (i.e., 5.0 kg for a 500-kg horse, as fed basis), and an ideal rate of 1.5 kg per 100 kg BW (i.e., 7.5 kg for a 500-kg horse, as fed basis) (GEOR; HARRIS, 2007). The restricted availability of food had a direct impact on the horses' time-budgets. The studied population spent on average only 2 hours and 47 minutes eating hay and 37 minutes eating concentrate, totalizing only 3 hours and 24 minutes per day feeding (about 14% of their time-budget). The reduction of the time spent foraging cand be seen as a sign of decreased welfare, since it has been associated with health problems in horses, like gastric ulcers and colic, in addition to the emergence of stereotypes and abnormal behaviors. In fact, our recent study suggests that limited availability of roughage may be one of the main factors related to the development and prevalence of abnormal behaviors in horses (SEABRA; DITTRICH; VALE, 2021).

Research show that horses have a behavioral need for chewable fiber and that low forage diets will significantly increase the time that horses will spend performing abnormal behaviors, like sifting through wood shaving bedding for food particles. In a study, the scientists observed that horses eating a pellet diet spent up to 11.5% of their time-budgets sifting through their bedding, while horses eating a hay diet spent only 1.2% of their time practicing that abnormal behavior (ELIA; ERB; HOUPT, 2010). In the current study, all horses were observed bedding-eating and they spent an average time of 2 hours and 56 minutes (12.22% of their time-budgets) practicing that behavior.

Other redirected behaviors that are seen to be directly related to low forage diets and boredom are wood-chewing and coprophagy (HANIS *et al.*, 2020). Wood-chewing was the second most common abnormal behavior observed in the studied population affecting 93.10% of the horses. Other studies with horses reported much lower prevalence rates of wood-chewing (from 8% to 30.3%) (TADICH *et al.,* 2012; WATERS; NICOL; FRENCH, 2002), reinforcing the hypothesis that questionaries, scan, or focal sampling are methodologies that may fail to identify infrequent behavior. The animals in the current trial spent an average of 42 minutes (2.92% of their time-budget) practicing this behavior, which could lead to uneven tooth wear and contribute to acute obstruction of the small intestine (GREEN; TONG, 1988). Authors also believe that wood-chewing may be a behavioral precursor to crib-biting (WICKENS; HELESKI, 2010).

Another abnormal behavior observed in 65.52% of the studied population was coprophagy, and the animals spent an average of 18 minutes eating their own feces. In a recent study, researchers observed that 4 out of 7 horses (57.14% of the studied population) fed limited amounts of roughage practiced coprophagy and would spend 26.6 ± 35.5 minutes on that behavior. However, after introducing slow-feeder hay bags, only one horse (14.30% of the studied population) was observed practicing coprophagy, and the time this horse spent eating feces was reduced to 5.4 ± 14.4 minutes (CORREA *et al.,* 2020). Although some professionals of the equine industry may see this abnormal behavior as harmless, coprophagy should not be taken lightly, since scientists believe that this behavior could increase the transmission of gastrointestinal parasites, and also develop into stereotypic behavior (HANIS *et al.,* 2020)

In the current study, 19 horses (65.52% of the studied population) presented all three measured abnormal behaviors (bedding-eating, wood-chewing, and coprophagy), eight horses (27.59%) presented two different abnormal behaviors, and only two horses (6.90%) presented one single abnormal behavior. In general, horses spent from 1 hour (4.17% of their time-budget) to 10 hours and 9 minutes (42.27%) bedding-eating, chewing wood, and/or eating their own feces. In general, the horses in the current study spent on average 3 hours and 44 minutes (15.57% of their time-budget) practicing abnormal behaviors, therefore, they spent more time in those behaviors than eating proper food (concentrate + hay).

The most common stereotypic behavior observed in the studied population was excessive licking. This behavior affected 100% of the horses, with animals spending on average 1 hour and 12 minutes (4.46% of their time-budget) on this activity.

Excessive licking is considered an oral stereotypy where the horse repetitively licks part of the stable such as an empty feeder or the walls of the stall (FUREIX *et al.,* 2011). Scientists believe that this behavior has not a nutritional purpose, but reflects an inadequate diet, with persistence of the ingestive behavior that could not be satisfied with a low forage meal (SEABRA; DITTRICH; VALE, 2021). A previous study reported that 40.68% of fifty-nine horses observed using a focal sampling method in three different riding schools presented this abnormal behavior (FUREIX *et al.,* 2011). This information suggests that this abnormal behavior can affect large amounts of the stabled population and that the continuous observation methodology used in the current study helped to reliably quantify this behavior.

Headshaking was the second most common stereotypy, affecting 51.72% of the studied population. The horses spent from 0 to 12 minutes practicing headshaking with a general average of 1.35 minutes. Some scientists believe that headshaking can also occur due to an idiopathic neuropathic facial pain syndrome (termed trigeminal-mediated headshaking) (ROSS; MURRAY; ROBERTS, 2018; STANGE; KRIETER; CZYCHOLL, 2022). A study that investigated the owner-reported prevalence of headshaking in the general horse population in the UK reported that only 4.6% of the investigated horse population showed signs of headshaking along the year preceding the study (ROSS; MURRAY; ROBERTS, 2018). This information indicates that this may be an infrequent behavior, which can be difficult to identify without the continuous observation methodology.

The third most common behavior was pawing, affecting 41.38% of the studied population. This behavior also had a high prevalence rate, however it could be considered an infrequent behavior, since the animals spent only one minute on average pawing. The next most prevalent behavior was weaving, affecting 24.14% of the studied population. Weaving is classified as locomotor stereotypy and was observed in 0% of horses housed in the first barn, 44.44% of horses in the second barn and 30.00% of horses in the third barn. The first bar had bigger stalls and was the only construction that allowed the animals to have tactile contact with conspecifics. This finding corroborates with other published research where authors state that weaving can be linked to the confinement in stalls, restricted movements, and social isolation (MILLS; NANKERVIS, 1999). In the current study, weavers spent up to 3 hours per day practicing this behavior and the general prevalence rate is similar to what was found in some previous studies. For example, scientists found that 22% of purebred

Arabian mares at a stallion breeding facility were waivers (BENHAJALI *et al.,* 2014), and an observational study conducted with twenty police horses had a prevalence rate up to 29% (ZULUAGA *et al.,* 2018). This stereotypy is undesirable because it can cause weight loss, uneven muscle development (MCBRIDE; LONG, 2001), excessive hoof or shoe wear, ligament strain, may exacerbate sub-clinical orthopedic conditions (WINSKILL *et al.,* 1995), decrease conception rate (BENHAJALI *et al.,* 2014), and decrease the commercial value of the affected horses. Because of those negative consequences, scientists have been investigating techniques that could be used to decrease the prevalence of this stereotypy. In a study, researchers were able to significantly reduce the incidence of weaving and head nodding in all horses by placing a 1m x 1.5 m acrylic mirror inside the horses' stalls (MCAFEE; MILLS; COOPER, 2002). Another study, scientists found similar results: weaving was significantly decrease by placing a poster image consisted of a life-size image of a horse's face inside the stalls (MILLS; RIEZEBOS, 2005). However, the best results were found by (COOPER; MCDONALD; MILLS, 2000) in a study that was able to entirely cease weaving by allowing horses to have tactile contact with a neighbor and observe the surroundings by leaving half doors open at front and back.

Box-walking was also observed in 24.14% of the studied population, but it could also be considered an infrequent behavior, since the affected horses spent only 40 seconds on average performing it. Lips and tongue abnormal movements were observed only in 6.90% of the studied population, however, the animals spent from 0 minutes to 1 hour and 30 minutes on this behavior (up to 6.23% of the time-budget). Other observed stereotypies were crib-biting and box-kicking, both affecting only 3.45% of the studied population.

In general, two horses were affected by five different stereotypies (6.90% of the studied population), two horses were affected by four (6.90%), eleven horses were affected by three (37.93%), nine horses were affected by two (31.03%) and five horses were affected by only one stereotypy (17.24%). The studied population spent most of their time observing the exterior (an average of 9 hours and 37.74% of their time-budget) and standing attentive inside their stalls (an average of 4 hours and 12 minutes and 17.44% of their time-budget). Considering the total time spent in those two behaviors, it is possible to say that the observed horses spent 13 hours per day (55.18% of their time-budget) just standing.

The deficit in environmental stimulation, inadequate stall size (in the third barn) (MINERO *et al.,* 2015), inadequate diet, and the incapacity of performing behavioral needs, as social interactions, and foraging behavior (COOPER; MCGREEVY, 2007) can lead to very high prevalence of abnormal and stereotypic behavior.

3.4.2 Meteorological Recordings

The average temperatures inside the first barn during the video recordings ranged between 8.20 and 23.80°C, in the second barn they ranged between 13.10 and 26.10°C and in the third barn they ranged between 10 and 28.00°C. There was no statistical difference (P>0.05) between the indoor temperatures and the ones recorded at the closest meteorological station. The temperatures inside the different barns could not be compared since the data collection was conducted on different days. The average THI index calculated for the first, second and third barns was 61.69 ± 4.59, 64.75 ± 3.75 and 64.58 ± 4.07 respectively. These conditions could still be considered normal for livestock animals (TABLE 13) (LCI, 1970), however, there was only a few days with THI values above 74 during the study period, which would be considered an alert. The third barn had the highest THI, however, since there was no statistical difference on prevalence of abnormal and stereotypic behaviors between different barns, there is no evidence that the meteorological conditions can affect the manifestation of those behaviors.

TABLE 13 - HEAT STRESS RISK CLASSES ACCORDING TO THE LIVESTOCK WEATHER SAFETY INDEX

THI value	**Heat stress class**
THI<74	Normal
74<THI<79	Alert
79<THI<84	Danger
THI≥84	Emergency

THI=Temperature Humidity Class

3.4.3 Blood analyses

The normal range of the neutrophil/lymphocyte ratio for healthy horses is 0.8-2.8 (MORRIS, LARGE, 1990). The blood samples were collected on the horses' day of rest and the average values are within the normal range. This result indicates that horses used in this study were healthy and were not experiencing stress at the time of collection, based on this specific immunological measurement.

There were no significant differences (*P*>0.05) between the blood samples collected in the morning and in the afternoon in all parameters analyzed. The reference range for total leukocyte values is 5.5-14.3 (10^3/µL) (GRONDIN; DEWITT, 2000) and the average values observed in the current study are within the normal range. However, six horses presented results below the reference range during the morning and three horses during the afternoon, indicating that their immunity was decreased. The reference range for neutrophil counts are 2.3-8.6 (10^3/µL) (GRONDIN; DEWITT, 2000) and the average and individual values observed in the current study are within the normal range. Although all the values were still within the normal range, the animals housed in the second barn had significantly lower neutrophil values (*P* <0.01). The reference range for lymphocyte counts are 1.5-7.7 (10^3/ µL) (GRONDIN; DEWITT, 2000) and the average values observed in the current study are within the normal range. However, two horses presented results below the reference range during the morning and one horse during the afternoon.

In a study, researchers relocated twelve German Warmblood geldings that were kept on pasture to single-housing conditions in a stable. They performed blood collections one day and eight days after the change in housing conditions. The samples were used to determine the total leukocyte counts, leukocyte distribution and neutrophil-to-lymphocyte ratio. The authors concluded that the relocation of the horses to single housing led to changes in the numbers of all investigated immune cell types. The number of eosinophils, monocytes and T cells (type of lymphocyte) declined, whereas the number of neutrophils increased resulting in an increased neutrophil-to-lymphocyte ratio. Those alterations persisted, pointing to a longer-lasting effect on the immune system of the horses (SCHMUCKER *et al.,* 2022).

In another study, authors evaluated how 24 horses (mostly French Saddlebreds) used in courses for riding instructors and housed in single stalls reacted to daily paddock release. They found that up to 92% of horses kept stabled (N = 22/24) presented abnormally low concentrations of leucocytes, which is similar to the results found in the current study where 29% of the analyzed animals had the same condition (LESIMPLE *et al.,* 2020). Both authors believe that housing horses in individual stalls can lead them to a chronic stress state that can cause alterations in immune cell numbers. This condition can lead to chronic immune dysregulation, and it might increase the horses' susceptibility diseases (LESIMPLE *et al.*, 2020; PADALINO *et al.,* 2017; SCHMUCKER *et al.,* 2022).

Although not significant differences were found between groups (*P*>0.05), the mean value calculated for barn three is above basal cortisol concentrations described in the literature for fit adult horses (35 – 70 ng/ml) (WATSON, 1998). Besides, all groups have individuals with cortisol levels above the reference range. The first barn had four horses above the maximum values in the morning and one horse was still above 70 ng/ml in the afternoon. The second barn had one horse with cortisol levels above the reference range in the morning and afternoon. The third barn had four horses above the maximum in the morning and afternoon blood collections. In total, nine out of twenty-four horses presented cortisol values above the reference range (37.50% of the studied population). Similar result was found by researchers that accessed the welfare state of urban working horses and Chilean rodeo horses. The authors observed that both groups had higher basal cortisol concentrations than those described in the literature, and they believe that the high basal cortisol levels can be often associated to chronic intermittent stress situations where the horse cannot predict the stressor stimulus (GONZÁLEZ *et al.,* 2019).

There was no significant difference in the CCR ratio between the different barns. The CCR ratio mean in the current study was 0.37 ± 0.18, higher than the average of 0.26 ± 0.16 found by researchers that evaluated the CCR ratio of police horses subjected to different housing and work conditions (LEAL *et al.,* 2011). Abnormal CCR ratio was observed in 22.22% of the horses in the first barn, 12.50% in the second barn, and 57.14% in the third barn. In total, 29.17% of all horses in the current study had abnormal CCR ratio. According to Leal *et al.* (2011), disturbances of the circadian rhythm in cortisol are compatible with chronic stress situations, which corroborates with other findings in the current study.

3.5 CONCLUSION

All horses observed in this study presented abnormal and stereotypic behaviors. The inadequate diet had a direct impact on the horses' time-budget, creating long periods of idleness and fasting. The forage content below ideal values may have led horses to develop abnormal behaviors (bedding-eating, coprophagy and wood-chewing) and to spend more time with those practices than eating proper food. Some horses in the studied population also presented alterations in immune cell numbers, and cortisol levels above the reference range. These findings indicate that the stabled horses in the current study were in a state of chronic stress and the stalls' architecture and the meteorological conditions did not significantly change the horses' capability to cope with such housing conditions. In future studies, the impact of different feeding and housing conditions on horses' time-budgets and physiological parameters should be investigated.

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4. EFFECTS OF DIFFERENT HAY FEEDERS, AVAILABILITY OF ROUGHAGE ON ABNORMAL BEHAVIORS AND TIME-BUDGET OF HORSES KEPT IN DRY LOTS

ABSTRACT

Automatic hay boxes feeders are devices that can be used to schedule feeding times throughout the day, facilitating the management routine in equine facilities. However, there is little to no research on how this feeding technique can affect horse behavior. A recent study indicated that limited availability of roughage may be the main factor related to the development and prevalence of abnormal behaviors in horses. In this light, offering roughage *ad libitum* could be the best option regarding horse welfare. Nevertheless, this practice has been associated with incapacity to control body weight, increases in body condition score (BCS), and a great waste of hay. Devices such as hay-nets/bags and slow-feeders have been developed and aimed to decrease food waste, increase the time horses spend feeding on roughage, and with that, reduce "undesirable" behaviors, such as abnormal and stereotypic behaviors. The objective of this study was to compare the effects of feeding free choice hay, a slow feeder and a box feeder on the equine behavior by 24-hour continuous sampling. The study was designed as a 3 x 3 Latin Square with 15 healthy school horses belonging to the Colorado State University. Three groups of five horses were randomly assigned to one of the treatments for 15 days, and then switched over to the next treatment, until all horses are submitted to all three treatments. A camera was installed on each pen at a height of 3 m. The animals' behavior was recorded during the last 24 hours of the last day of each treatment period and the video was analyzed with continuous behavioral sampling. The time spent in stereotypic, abnormal behaviors and other activities was measured second-by-second, inserted into a spreadsheet, and used for the determination of the animals' time-budgets. Horses in the free choice treatment used 64.5% more hay than the daily recommended intake of 2% of the body weight (BW), whereas horses in the box and slow feeders had the lower hay utilization rate and lower weight gain and both treatments can be considered equivalent in food efficiency and intake control. Horses in both free choice and slow feeder treatments were able to spend more than 50% of the time-budget foraging, generating a time distribution similar to horses in their natural environment. However, the box feeder treatment decreased the time that horses spent eating to an average of 25.81% of their time-budget, and significantly increased the time horses spent in other activities (standing attentive, sniffing the ground, and practicing coprophagy). The level of aggression between conspecifics became significantly higher as the area per feeder became smaller and the food source became less accessible, with horses in the box feeder treatment showing higher levels of aggression. Therefore, more research is needed in order to develop an automatic feeder that will provide optimal welfare levels by allowing horses to fulfill their behavioral needs.

Key words: Aggression; Automatic feeder; Equine; Free choice hay; Limited forage; Slow feeder.

4.1 INTRODUCTION

Free-ranging horses can spend around 16 hours per day grazing (MAYES; DUNCAN, 1986) and offering restricted access to food can change the natural behavior of this species, causing disorders that may be detrimental to the health of the animal (GONTIJO *et al.,* 2014). Stereotypies are invariant and repetitive behavior patterns that seemingly have no function and other abnormal behaviors are practices that do not fit this definition but are not commonly observed in horses living in wild environments (MASON, 2006).

The prevalence of stereotypies and other abnormal behaviors in domesticated horses have been studied for decades, and researchers believe that several environmental and biological factors may be involved in the development of these behaviors. Abnormal behaviors and stereotypies are commonly recognized as indicators of poor welfare (RUET *et al.*, 2019) and may directly impair athletic performance, resulting in great economic losses to horse owners.

Researchers theorize that the prevalence of those behaviors can be attributed to the inability to express highly motivated behaviors, such as foraging activity (MCBRIDE; HEMMINGS, 2009). Firstly, previous studies have already associated diets with inadequate forage content to the ingestion of bedding substrate (COOPER; MCGREEVY, 2007; MILLS; ECKLEY; COOPER, 2000) and aggressive behavior (BENHAJALI *et al.,* 2009) in this species. Secondly, researchers believe that providing more foraging opportunities and prolonging hay availability are techniques that may increase the frequency of positive social interactions between horses kept in groups, decrease aggression, and improve the animals' welfare (BENHAJALI *et al.,* 2009; BURLA *et al.,* 2016). The feeding routine adopted by equestrian facilities, generally aims to minimize food waste, and optimize their employees' working scale by providing limited amounts of forage (SEABRA; DITTRICH; VALE, 2021). Automatic hay boxes are devices that can be used to schedule feeding times throughout the day, facilitating the management routine in equine facilities. However, there is little to no research on how this feeding technique can affect horse behavior. A recent study indicated that limited availability of roughage may be the main factor related to the development and prevalence of abnormal behaviors in horses (SEABRA; DITTRICH; VALE, 2021). In this light, offering roughage *ad libitum* could be the best option regarding horse welfare. Nevertheless, this practice has been associated with incapacity to control body weight, increases in body condition score (BCS), and a great waste of hay (MARTINSON *et al.,* 2012). Devices such as hay-nets/bags and slow-feeders have been developed and aimed to decrease food waste, increase the time horses spend feeding on roughage, and with that, reduce “undesirable” behaviors, such as abnormal and stereotypic behaviors (CORREA *et al.,* 2020).

In a recent systematic review and meta-analysis (SEABRA; DITTRICH; VALE, 2021) summarizing the main risk factors associated with the development of abnormal behaviors in horses, they concluded that the management practices used in the horse industry have changed very little over the last quarter of a century, and the development of strategies to prevent abnormal behaviors and improve horses’ welfare is needed. Limited availability of roughage is the main factor related to the development and prevalence of abnormal behaviors in horses. Although there were no statistically significant differences between direct observations and the studies carried out using questionnaires, first data-collection methodology should be prioritized in future research. Other researchers (MITLÖHNER *et al.,* 2001) also state that continuous observations are the most accurate method for behavioral measurements. Moreover, another study (CORREA *et al.*, 2020) evaluated horses before and after 10 days of forage feeding a slow-feeder hay bag after being fed hay twice a day. At the end of this period, the authors observed an increased ingestion time, reduction and/or elimination of stereotyped behaviors, and regularization of cortisol circadian rhythm (CCR), demonstrating that the use of a slow-feeder hay bag can consistently improve the welfare of horses. Therefore, the objective of this study is to compare the effects of feeding free choice hay (FC), a slow feeder (SF) and a box feeder (BF) on the equine behavior by 24-hour continuous sampling.

4.2 MATERIAL AND METHODS

4.2.1 Study population

All experimental procedures were approved by the Animal Care and Use Committee of Colorado State University (CSU, protocol number 2873). The population used for this study consisted of 15 healthy thoroughbred mix horses (2 geldings and 13 mares) belonging to the Polo Club at Colorado State University (Fort Collins, Colorado – US). At the beginning of the trial, the horses were 14.86 ± 4.15 years old (from eight to 20 years old) and weighed 489.58 ± 40.10 kg. They all followed the same light training protocol and had similar exercise routines, being exercised aerobically for one hour, three times a week. The horses were assigned to three groups of five animals at least six months before the study started, according to their already established hierarchy. The three groups were kept in identical dry lots measuring 20x30 meters with shelters and iron pipe fencing at the Equine Teaching and Research Center (ETRC). Horses were fed with free choice hay for 10 days before the study and had unlimited access to mineral blocks and water during the total duration of the experiment.

4.2.2 Study design

The study was designed as a 3 x 3 Latin Square with fifteen horses in total. The animals were divided into 3 groups of 5 horses according to their already established hierarchy. The groups remained the same for at least 6 months before the start, and during the total duration of the study. One day before being randomly assigned to a treatment, the horses had the body weight (BW), and BCS determined with a scale from 1 (poor) to 9 (extremely fat) (CARROLL, HUNTINGTON, 1988). Horses were assigned to one of the 3 treatments for 15 days, and then switched over to the next treatment, until all horses were submitted to all treatments (45 days in total).

4.2.3 Diet composition and estimated hay consumption

Hay was weighed before being placed in the feeders and leftovers were weighed before a new bale was put in (scale specs). Total hay consumption for each treatment was calculated by the initial weight of the bale, minus the leftover hay for the treatment period. The horses were weighed, and body condition score was assessed (BCS) by two experienced independent evaluators before the experiment and after every treatment (every 15 days). Horses had access to mineral blocks and salt blocks. All animals were fed the same hay (*orchard brome grass*) and each horse also received 100 grams per day of a gastric support supplement (Purina® Outlast™). The compositions of the hay and the gastric support supplement used for the diet in this trial are listed in TABLE 14.

TABLE 14 – COMPOSITION OF THE DIET OFFERED TO ALL HORSES DURING THE TRIAL

	Diet	
	Hay (*Orchard Brome grass*)	Supplement
Dry matter (%)	91.42	91
Crude Protein (% DM)	18.11	13.00
Ash (% DM)	11.23	17.00
Fat (% DM)	2.81	1.10
Neutral detergent fiber (NDF)	48.96	35.00
Acid detergent fiber (ADF)	30.17	21.00
Calcium (% DM)	0.82	5.00
Phosphorus (% DM)	0.30	0.45
Starch (% DM)	1.12	9.00
DE Mcal/kg	2.21	2.32

DM= Dry matter; DE= Digestible Energy

4.2.4 Treatments

The treatments consisted of three different hay feeders and availability of roughage: (BF) an automatic hay box (Hay Box Dual Pro Long) (FIGURE 9 and FIGURE 10) offering hay *ad libitum* for 60 minutes 6 times a day through large square bales placed inside the box feeder; (FC) a traditional feeder offering unlimited access to hay 24/7 (FIGURE 11), and (SF) unlimited access to hay through a slow feeder (Hayhut feeder with netted square hay bales, Hay Chix) (FIGURE 12 and FIGURE 13). The specifications of each feeder are displayed in TABLE 15.

FIGURE 9 – CLOSED BOX FEEDER

SOURCE: The author.

FIGURE 10 – OPEN BOX FEEDER

SOURCE: CSU Facebook (2022)

FIGURE 11 – TRADICIONAL FEEDER (FREE CHOICE)

SOURCE: The author

FIGURE 12 – HAYHUT FEEDER

SOURCE: The author

FIGURE 13 – SLOW FEEDER (HAY CHIX, LARGE BALE NET, 1 ¼'' NET)

SOURCE: The author

TABLE 15 – FEEDERS' SPECIFICATIONS

Feeder	Depth (meters)	Width (meters)	Height (meters)	Openings (meters)	Space per horse (meters)	Access to hay
Box Feeder	2.97	1.30	1.52	Along the length only	1.19	*ad libitum* for 60 minutes 6 times a day
Free Choice Feeder	4.27	1.22	2.13	Along the length and on the sides	2.20	*ad libitum*
Slow Feeder	2.13	1.83	2.18	Along the length and on the sides	1.58	*ad libitum* but through a 1 ¼'' net

4.2.5 Behavioral assessments

A camera (AXIS P3719-PLE Network Camera, Mexico) was installed on the fence of each pen at a height of 3 meters (FIGURE 11). The animals' behavior was recorded during the last 24 hours of the last day of each treatment period with a digital recorder (AXIS S3008 Recorder 8TB, Poland). During recording days, all horses were kept inside the pens for the 24 hours of recording. The images were stored in an external hard drive for further analysis with continuous behavioral sampling using the software AXIS Camera Station Client. The time spent in stereotypic, abnormal behaviors and other activities was measured second-by-second, inserted into a spreadsheet, and used for the determination of the animals' time budgets. Each behavior observed was defined according to the adapted ethogram (TABLE 16) (PESSOA *et al.,* 2016). The footage was analyzed by an animal scientist specialized in equine behavior with more than seven years of experience with horses.

FIGURE 14 – INSTALLED CAMERA

SOURCE: The author

TABLE 16 – ETHOGRAM OF HORSES' BEHAVIORS

Normal Behaviors	
Eating hay	Animal feeding on forage in troughs
Drinking	Animal seeking water or drinking water
Observing the surroundings	Next to the fence observing outside the pen
Standing alert	Animal standing in place, alert, with elevated neck, observing its surroundings
Other natural behaviors	Not listed above (defecating, lying down, urinating, walking, rolling over)
Stereotypies (SARRAFCHI; BLOKHUIS, 2013)	
Excessive licking	The horse licks objects or surfaces with no nutritional purpose
Kicking	The horse knocks with the hind limb hoofs against surfaces (feeder, fence or similar)
Nodding	The horse moves its head up and down repetitively

Pawing	The horse uses its hoof for digging or scratching a surface
Box-walking	The horse wanders in circles inside the box or pen, walking, trotting, or galloping
Weaving	The horse sway laterally, moving the head, neck, forequarters and sometimes hindquarters
Crib-biting	horse fixes its incisors on a hard and horizontal surface, and then, it tenses the neck muscles and forces air into the cranial portion of the esophagus, making a characteristic noise
Windsucking	The horse opens the mouth, contracting the pharyngeal muscles, flexing the neck muscles, and swallowing air, usually making a characteristic grunting noise
Other Abnormal Behaviors	
Coprophagy	The horse eats its own feces
Wood or Metal-chewing	The horse chews on wood or metal objects
Affiliative interactions (PIERARD; MCGREEVY; GEERS, 2019)	
Allogroom	Two horses standing next to each other, rubbing the other's hair
Approach	Horse moving toward another, stopping within touching distance and standing there for at least 5 seconds, without any signs of aggression
Agonistic interactions (PIERARD; MCGREEVY; GEERS, 2019)	
Displace	Horse moving toward another horse and taking the exact same place after the other horse moved away
Avoid	Horse moving and increasing distance with other horse that is moving closer without tacking the same place
Head threat	Rapid extension of the head toward another horse, with ears pinned and mouth closed
Threat strike	Rapid extension of one or both front limbs toward another horse but without contact
Threat kick	Horse turning its hindquarters toward another horse and walking backwards
Threat bite	Extension of the neck toward another horse, opening and closing mouth but no contact with other horse
Chase	Running after another horse for at least 3 strides, often trying to bite or strike the other horse, while the latter is running away
Strike	Rapid extension of one or both front limbs, hitting another horse
Kick	Rapid extension of one or both hind limbs toward another horse
Bite	Extension of the neck toward another horse, opening mouth and closing it in contact with other horse

4.2.6 Meteorological Recordings

The video recording and the meteorological data collection were performed during the Spring season (April/May). Environmental data was collected from the closest meteorological station, about 2736 meters away from the dry lot pens. The weather conditions, including ambient temperature, relative humidity, dew point temperature, solar radiation, wind speed, wind direction, and gust were registered during the recordings' dates. Then, the data were inserted into an excel spread sheet and used to calculate the average weather conditions during the video recording dates.

From the environmental data collected from the meteorological station, Temperature–Humidity Index (THI) was derived using the following equation (THOM, 1958):

$$THI = T_{db} + 0.36*(T_{dp}) + 41.5$$

Where T_{db} is the dry bulb temperature (˚C) and T_{dp} is the dew point temperature (˚C).

Temperature–Humidity Index (THI) was used as an estimate of the degree of thermal discomfort experienced by horses.

4.2.7 Data Analysis

All data were tabulated and analyzed through descriptive statistics. Absolute and percentage distributions of all observed behaviors were determined, and the maximum and minimum values were presented. For the statistical analysis, treatments were considered independent variables (Box Feeder (BF), Free Choice (FC), Slow Feeder (SF), and horse within treatment was considered the subject. The seconds were converted into minutes for all observed behaviors in the time-budget. The dependent variables were the time spent for each behavior in minutes and plasma cortisol. Body weight and the body condition score (BCS) were also dependent variables. The horses' time-budgets were calculated by the sum of seconds spent on each behavior observed during the 24 hours period. The effect of the tree treatments on each behavior was analyzed by ANOVA with repeated measures in a mixed model and applied to the 15 horses with SAS (9.2) software (Cary, NC). The difference in CCR ratio values, the body weight and the body condition score (BCS) between the 3 different treatments was also assessed by ANOVA analysis. Least-square means analysis was used to determine significance with a threshold of $P \leq 0.05$. results are presented as means and SD. Bartlett's test was used to verify the homogeneity of variances using the statistical program Assistat (E SILVA, DE AZEVEDO, 2016). Data transformations were not required prior to statistical analysis.

4.3 RESULTS

4.3.1 Estimated hay consumption

The treatments affected the daily rate of hay use (*P*<0.0001). Horses used more hay (*P*<0.0001) in the free choice treatment when compared to the box feeder, and to the slow feeder, however, the hay consumed between the box feeder and slow feeder treatments was not different (P=0.092). In the free choice treatment, each horse used an average of 16.58 ± 0.45 Kg (DM) of hay daily, in the meanwhile each individual used an average of 10.39 ± 0.45 Kg and 9.30 ± 0.45 kg of hay daily in the slow feeder and box feeder treatments, respectively. Horses in the free choice treatment used 64.5% more hay than the daily recommended intake of 2% of the body weight (NRC, 2007). The *P* values and the mean ± standard deviation values for hay use in different treatments are displayed in TABLE 17.

TABLE 17 – DISCRETION OF ESTIMATED AND REAL DAILY INTAKE AND USE OF HAY IN DIFFERENT TREATMENTS.

	Free choice	**Slow Feeder**	**Box Feeder**	**ANOVA P value**
Estimated daily intake as (% of BW)	2.00	2.00	2.00	-
Real daily use of hay (% of BW)	3.29 ± 0.10[a]	2.08 ± 0.10[b]	1.95 ± 0.10[b]	<0.0001
Estimated daily hay intake per animal (Kg of DM)	10.12 ± 0.85	10.04 ± 0.94	9.58 ± 0.86	-
Real daily hay use per animal (Kg of DM)	16.58 ± 0.45[a]	10.39 ± 0.45[b]	9.30 ± 0.45[b]	<0.0001

4.3.2 Body condition score and body weight

The body condition score (BCS) (P=0.821) and the horses’ body weight (P=0.2026) did not differ between the treatments. However, the treatments affected the horses’ weight change (P=0.0017). The weight of the animals increased an average of 23.51 kg at the end of the free choice treatment and this weight gain was higher than in the slow (P=0.0011), and box feeder (P=0.0042). However, the box and slow feeders did not differ in this variable (P=0.91). The P values and the mean ±

standard deviation values for BW change and BCS in different treatments are displayed in TABLE 18.

TABLE 18 – HORSES' BODY CONDITION SCORE, BODY WEGHT, AND BODY WEIGHT CHANGE IN DIFFERENT TREATMENTS

	Free choice	**Slow Feeder**	**Box Feeder**	**ANOVA *P* value**
Horses' body weight	406.00 ± 11.42	502.12 ± 11.42	478.81 ± 11.42	0.2026
Horses' weight change (Kg)	23.51 ± 4.62^{a}	0.37 ± 4.6204^{b}	1.21 ± 5.66^{b}	0.0017
Body Condition Score	5.27 ± 0.16	5.13 ± 0.16	5.15 ± 1.20	0.8210

4.3.3 Behavioral assessments

Nine hundred and sixty hours of video recordings were analyzed with continuous behavioral sampling by one observer, generating a total of 57,600 observations. The box feeder treatment had only two repetitions because of malfunction. During one of the recording days, the box feeder stopped working and did not open during the entire 24 hours session. Therefore, the video recordings of that day had to be discarded and excluded from the analysis.

The treatments affected the time horses spent eating (P< 0.0001). The box feeder decreased (P< 0.0001) the time horses spent eating when compared to the free choice and slow feeder treatments, however the free choice and the slow feeder did not differ in this variable (P=0.22). The horses spent an average of 791.16 ± 26.37 min eating hay (54.94% of their time-budget) in the slow feeder treatment, 744.00 ± 26.38 minutes (51.70% of their time-budget) in the free choice treatment, and it was 371.61 ± 32.31 minutes (25.81% of their time-budget) in the box feeder treatment.

The time horses spent drinking water was affected by the treatments (P=0.0017). It was higher in the free choice treatment when compared with the slow feeder (P=0.0218) and box feeder treatments (P=0.0005). But the box and slow feeder were not different (P=0.0983). The horses spent an average of 16.98 ± 1.83 minutes drinking water (1.18 % of their time-budget) in the free choice treatment, and this time was lower in the slow feeder (10.78 ± 1.83 minutes; 0.75% of their time-budget) and box feeder treatment (5.88 ± 2.24 minutes; 0.41% of their time-budget).

The time horses spent standing attentive was influenced by the treatments (*P*<0.0001). The box feeder treatment increased the time horses spent standing attentive (*P*< 0.0001) when compared to the free choice and slow feeder treatments, however the free choice and the slow feeder did not differ in this variable (*P*=0.16). The horses spent an average of 753.85 ± 29.57 minutes standing attentive (52.35% of their time-budget) in the box feeder treatment, and the time spent in this behavior was 469.03 ± 24.15 minutes (32.57% of their time-budget) in the free choice treatment, and 420.47 ± 24.15 minutes (29.20% of their time-budget) in the slow feeder treatment.

The time horses spent lying down was affected by the treatments (*P*= 0.0252). Horses spent less time lying down in the box feeder treatment when compared to the slow feeder (P=0.0317) and free choice (P=0.0089), however, there was no difference between the free choice and slow feeder treatments (P=0.56). On average, horses spent 36.13 ± 18.60 minutes lying down (2.51% of their time-budget) in the box feeder treatment, and this time was 89.75 ± 15.19 in the slow feeder treatment (6.23% of their time-budget), and 102.75 ± 15.19 minutes (7.12% of their time-budget) in the free choice treatment.

Another behavior that was affected by the treatments was walking (*P* = 0.0109). The time Horses spent walking was lower in the free choice treatment when compared to the box feeder (P=0.0029), however, the free choice treatment was similar to the slow feeder (P=0.11), and the slow feeder was similar to the box feeder (P=0.096). The horses in the box feeder treatment spent 57.13 ± 4.84 minutes (3.97% of their time-budget) walking, horses in the slow feeder spent 46.44 ± 3.96 minutes (3.23% of their time-budget), and horse in the free choice treatment spent 37.18 ± 3.96 minutes (2.58% of their time-budget) walking.

Coprophagy was also affected by treatments (*P*<0.0001). Horses in the box feeder spent more time practicing coprophagy (*P*<0.0001) when compared to the slow feeder and free choice treatments, however, there was no difference between the free choice and slow feeder treatments (*P*=0.16). The horses in the box feeder spent an average of 96.06 ± 11.74 minutes practicing coprophagy (6.67% of their time-budget). At the same time, the horses spent an average of 19.40 ± 9.58 minutes practicing coprophagy (1.35% of their time-budget) in the slow feeder treatment, and no horses were observed practicing this behavior in the free choice treatment.

Allogroom was another behavior that was significantly affected by the treatments (*P* = 0.0382). The horses in the free choice treatment were the only ones that presented that behavior, and this treatment differed from the box (*P*=0.0392) and slow feeders (*P*=0.0220). There was no difference between the box feeder and slow feeder treatments (*P*=1.0). The horses in the free choice treatment spent an average of 0.59 ± 0.17 minutes practicing this behavior (0.04% of their time-budget).

The treatments influenced the time horses spent kicking each other (*P*= 0.0022). The time horses spent kicking was higher in the box feeder treatment when compared to the slow feeder (*P*=0.0076) and free choice (*P*=0.0006). But there was no difference between the free choice and slow feeder treatments (*P*=0.32). The horses spent an average of 0.22 ± 0.04 minutes kicking their conspecifics (0.015 of their time-budget) in the box feeder treatment, and the time spent in this behavior was lower in the free choice treatment (0.01 ± 0.04 minutes; 0.001% of their time-budget), and 0.06 ± 0.04 minutes (0.004% of their time-budget) during the slow feeder treatment.

The treatments also influenced the time horses spend biting each other (*P*= 0.0430). The horses in the free choice treatment spent less time biting each other when compared to the box feeder (*P*=0.0127), however, the box feeder and slow feeder were similar (*P*=0.13), and there was no difference between the free choice and slow feeder (*P*=0.24). The horses in the free choice treatment spent 0.04 ± 0.03 minutes biting each other, while the horses in the slow feeder spent 0.09 ± 0.03, and the horses in the box feeder spent 0.18 ± 0.04 minutes.

Other behaviors did not significantly differ between the treatments (*P*>0.05). Significance levels for all behaviors are displayed in TABLE 19. The mean ± standard deviation values and the descriptive statistics for minutes spent in each observed behavior can be seen in TABLE 20. A graphic representation with the average percentage of time spent on each behavior in different treatments can be seen in FIGURE 15.

TABLE 19 – EFECTS OF DIFFERENT FEEDERS AND FOOD AVAILABILITY ON HORSES BEHAVIOR

Observed behaviors	**Box Feeder (Minutes)**	**Free Choice (Minutes)**	**Slow Feeder (Minutes)**	**ANOVAS *P* value**
Eating Hay	371.61 ± 32.31[a]	744 ± 26.38[b]	791.16 ± 26.37[b]	*P*< 0.0001
Eating Supplement	9.32 ± 2.24	7.68 ± 1.83	6.89 ± 1.83	*P* = 0.70
Drinking Water	5.88 ± 2.24[b]	16.98 ± 1.83[a]	10.78 ± 1.83[b]	*P*= 0.0017
Licking salt	12.69 ± 2.11	10.98 ± 1.72	6.18 ± 1.72	*P*= 0.0800
Standing Attentive	753.85 ± 29.57[a]	469.03 ± 24.15[b]	420.47 ± 24.15[b]	*P*< 0.0001
Observing the exterior	23.26 ± 7.39	24.63 ± 6.04	19.38 ± 6.04	*P*= 0.8194
Lying Down	36.13 ± 18.60[b]	102.75 ± 15.19[a]	89.75 ± 15.19[a]	*P*= 0.0252
Walking	57.13 ± 4.84[a]	37.18 ± 3.96[b]	46.44 ±3.96[ab]	*P*= 0.0109
Trotting	0.63 ± 0.23	0.36 ± 0.19	0.32 ± 0.19	*P*= 0.63
Galloping	0.15 ± 0.06	0.06 ± 0.05	0.08 ± 0.05	*P*= 0.4900
Defecating	1.19 ± 0.19	1.42 ± 0.15	1.39 ± 0.15	*P*= 0.6057
Urinating	1.76 ± 0.37	2.56 ±0.30	2.38 ± 0.30	*P*= 0.2466
Rolling over	0.11 ± 0.09	0.23 ± 0.07	0.06 ± 0.07	*P*= 0.2397
Sniffing the ground	47.28 ± 4.50[a]	5.28 ± 3.68[b]	2.02 ± 3.68 [b]	*P*< 0.001
Coprophagy	96.06 ± 11.74[a]	0 ± 9.58[b]	19.40 ± 9.58[b]	*P*<0.0001
Pawing	0 ± 0.03	0.04 ± 0.02	0.00 ± 0.02	*P*= 0.4458
Nodding	0.21 ± 0.10	0 ± 0.08	0.01 ± 0.08	*P*= 0.2464
Human interaction	4. 07 ± 4.23	5.38 ± 3.46	8.84 ± 3.46	*P*= 0.6451
Wood or Metal chewing	0.58 ± 0.27	0 ± 0.22	0 ± 0.22	*P*= 0.1781
Allogroom	0 ± 0.2133[b]	0.59 ± 0.17[a]	0 ± 0.17 [b]	*P*= 0.0382
Approach	0.44 ± 0.32	0.50 ± 0.26	0.59 ± 0.26	*P*= 0.9290
Displace	4.73 ± 1.38	3.55 ± 1.13	5.29 ± 1.13	*P*= 0.5446
Avoid	9.63 ± 1.42	6.29 ± 1.42	7.37 ± 1.16	*P*= 0.1990
Head threat	0.12 ± 0.23	6.29 ± 1.42	7.37 ± 1.16	*P*= 0.1990
Threat strike	0.75 ± 0.25	0.12 ± 0.20	0.21 ± 0.20	*P*= 0.1311
Threat kick	0.01 ± 0.00	0 ± 0.00	0.00 ± 0.00	*P*= 0.2275
Threat bite	0.13 ± 0.07	0.02 ± 0.06	0.13 ± 0.06	*P*= 0.2868
Chase	0.33 ± 0.19	0.19 ± 0.15	0.02 ± 0.15	*P*= 0.4166
Strike	0.03 ± 0.02	0.02 ± 0.02	0.01 ± 0.02	*P*= 0.6700
Kick	0.22 ± 0.04[a]	0.01 ± 0.04[b]	0.06 ± 0.04[b]	*P*= 0.0022
Bite	0.18 ± 0.04[a]	0.04 ± 0.03[b]	0.09 ± 0.03[ab]	*P*= 0.0430

TABLE 20 - DESCRIPTIVE DATA FOR MINUTES SPEND IN EACH OBSERVED BEHAVIOR IN DIFFETENT TREATMENTS. (MEAN ± STANDARD DEVIATION)

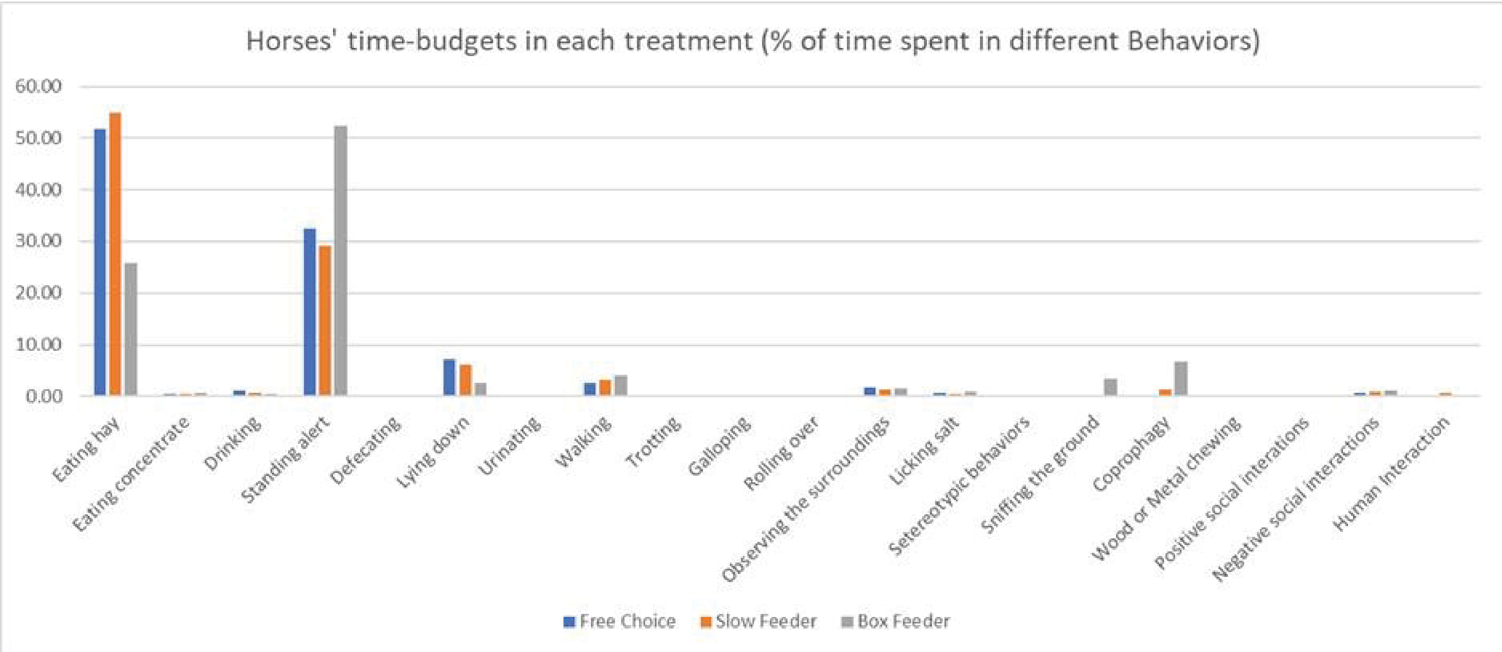
			Free choice		
Observed behaviors	Mean (Minutes)	Median	Sample Variance	Minimum (Minutes)	Maximum (Minutes)
Eating Hay	744.45 ± 85.08	720.17	7239.25	605.58	891.17
Eating Supplement	7.69 ± 6.21	7.42	38.56	0.75	18.58
Drinking Water	16.98 ± 8.13	16.83	66.02	5.75	32.50
Licking salt	10.98 ± 7.32	11.08	53.56	0.00	27.92

Standing Attentive	469.03 ± 89.53	453.83	8015.61	269.42	600.45
Observing the exterior	24.63 ± 25.05	20.00	627.35	1.17	75.42
Lying Down	102.47 ± 61.87	95.50	3827.77	33.17	243.08
Walking	37.18 ± 9.80	36.25	96.05	23.25	59.33
Trotting	0.36 ± 0.68	0.00	0.46	0.00	2.17
Galloping	0.06 ± 0.12	0.00	0.01	0.00	0.33
Defecating	1.42 ± 0.56	1.33	0.31	0.42	2.75
Urinating	2.56 ± 1.17	2.67	1.36	0.50	4.08
Rolling over	0.23 ± 0.39	0.00	0.15	0.00	1.42
Sniffing the ground	5.28 ± 4.48	4.75	20.10	0.00	14.78
Coprophagy	0	0	0	0	0
Pawing	0.04 ± 0.15	0.00	0.02	0.00	0.58
Nodding	0	0	0	0	0
human interaction	5.38± 6.67	3.17	44.49	0.00	23.50
Lips and tongue abnormal behaviors	0.0 2± 0.09	0.00	0.01	0.00	0.33
Wood or Metal chewing	0	0	0	0	0
Allogroom	0.59 ±1.10	0.00	1.20	0.00	3.58
Approach	0.50 ±1.08	0.17	1.17	0.00	4.08
Displace	3.55 ±3.62	2.25	13.10	0.00	11.42
Avoid	6.29 ±4.30	6.50	18.49	0.00	15.25
Head threat	0.12 ±0.23	0.00	0.05	0.00	0.67
Threat strike	0.00 ±0.00	0.00	0.00	0.00	0.00
Threat kick	0.04 ±0.08	0.00	0.01	0.00	0.25
Threat bite	0.02 ±0.04	0.00	0.00	0.00	0.12
Chase	0.19 ±0.64	0.00	0.41	0.00	2.50
Strike	0.02 ±0.09	0.00	0.01	0.00	0.33
Kick	0.01 ±0.03	0.00	0.00	0.00	0.08
Bite	0.04 ±0.06	0.00	0.00	0.00	0.17
			Box feeder		
Observed behaviors	Mean (Minutes)	Median	Sample Variance	Minimum (Minutes)	Maximum (Minutes)
Eating Hay	371.61 ± 54.99	355.08	3024.26	296.67	448.83
Eating Supplement	9.32 ± 6.15	8.88	37.78	2.42	19.58
Drinking Water	5.88 ± 2.17	6.13	4.73	2.58	10.42
Licking salt	12.69 ± 6.80	12.67	46.29	4.67	23.17
Standing Attentive	753.85 ± 80.60	745.83	6495.57	625.75	904.67
Observing the exterior	23.26 ± 19.90	16.88	395.93	1.83	71.83
Lying Down	36.13 ± 46.89	23.04	2198.31	0.00	127.25
Walking	57.13±21.57	55.08	465.24	29.00	98.83
Trotting	0.63±0.77	0.33	0.59	0.00	2.17
Galloping	0.15±0.24	0.00	0.06	0.00	0.75
Defecating	1.19 ± 0.73	1.21	0.54	0.42	2.92
Urinating	1.76 ± 0.68	1.54	0.46	0.67	2.75
Rolling over	0.11± 0.23	0.00	0.05	0.00	0.58
Sniffing the ground	47.93 ± 28.19	39.83	794.71	8.92	92.17
Coprophagy	96.06 ± 58.80	96.58	3457.73	9.58	184.58
Pawing	0	0	0	0	0
Nodding	0.21 ± 0.66	0.00	0.43	0.00	2.08
human interaction	4.07±6.36	1.08	40.42	0.00	19.17

Lips and tongue abnormal behaviors	0	0	0	0	0
Wood or Metal chewing	0.58 ± 1.70	0.00	2.90	0.00	5.42
Allogroom	0	0	0	0	0
Approach	0.44 ± 0.63	0.17	0.40	0.00	1.83
Displace	4.73 ± 4.21	4.38	17.73	0.08	13.25
Avoid	9.63 ± 5.43	10.17	29.48	0.00	16.83
Head threat	0.75 ± 1.52	0.08	2.32	0.00	4.92
Threat strike	0.01 ± 0.03	0.00	0.00	0.00	0.08
Threat kick	0.43 ± 0.76	0.17	0.57	0.00	2.33
Threat bite	0.13 ± 0.13	0.08	0.02	0.33	0.00
Chase	0.33 ± 0.88	0.00	0.78	2.83	0.00
Strike	0.03 ± 0.06	0.17	0.00	0.00	0.00
Kick	0.22 ± 0.25	0.08	0.06	0.00	0.67
Bite	0.18 ± 0.22	0.13	0.05	0.00	0.75
			Slow Feeder		
Observed behaviors	Mean (Minutes)	Median	Sample Variance	Minimum (Minutes)	Maximum (Minutes)
Eating Hay	791.16 ± 35.02	135.65	18400.89	634.3	1160.67
Eating Supplement	6.89±2.16	8.35	69.73	0.00	26.92
Drinking Water	10.78 ± 2.06	7.99	63.78	2.08	32.25
Licking salt	6.78±1.52	5.90	34.85	0.08	17.92
Standing Attentive	420.47 ± 26.98	104.5	10920.32	140.00	544.25
Observing the exterior	19.38 ± 6.12	23.72	562.63	0.33	78.75
Lying Down	89.75 ± 16.15	62.53	3910.15	5.75	265.17
Walking	46.44 ± 3.87	15.00	225.04	21.75	69.50
Trotting	0.39 ± 0.20	0.78	0.61	0.00	2.50
Galloping	0.08 ± 0.06	0.21	0.05	0.00	0.83
Defecating	1.39 ± 0.13	0.52	0.27	0.42	2.58
Urinating	2.38 ± 0.37	1.42	2.03	0.33	5.67
Rolling over	0.06 ± 0.04	0.16	0.03	0.00	0.58
Sniffing the ground	2.02±0.55	2.12	4.48	0.00	6.83
Coprophagy	19.40 ± 9.73	37.68	1420.03	0.00	108.50
Pawing	0	0	0	0	0
Nodding	0.01 ± 0.01	0.04	0.00	0.00	0.17
human interaction	8.83 ± 5.18	20.07	402.64	0.00	79.58
Lips and tongue abnormal behaviors	0	0	0	0	0
Wood or Metal chewing	0	0	0	0	0
Allogroom	0	0	0	0	0
Approach	0.59 ± 0.29	1.13	1.27	0.00	3.75
Displace	5.29 ± 1.32	5.11	26.15	0.08	18.83
Avoid	7.37 ± 1.02	3.97	15.73	0.00	11.33
Head threat	0.21 ± 0.07	0.29	0.08	0.00	1.08
Threat strike	0	0	0	0	0
Threat kick	0.07 ± 0.02	0.09	0.01	0.00	0.33
Threat bite	0.13 ± 0.09	0.34	0.11	0.00	1.00
Chase	0.02 ± 0.01	0.03	0.00	0.08	0.00
Strike	0.01 ± 0.01	0.02	0.00	0.08	0.00

Kick	0.06 ± 0.02	0.25	0.09	0.00	0.01
Bite	0.09 ± 0.02	0.08	0.01	0.00	0.25

FIGURE 15 – GRAPHIC REPRESENTATION WITH THE AVERAGE PERCENTAGE OF TIME SPENT ON EACH BEHAVIOR IN DIFFERENT TREATMENTS



SOURCE: The author

4.3.4 Meteorological Recordings

The ambient temperature ranged from -3.40 to 29 °C during the video recordings in the free choice treatment, from 5.50 to 18.80 °C during the recordings of slow feeder treatment, and from 5.90 to 26.50 °C in the video recordings of box feeder treatment. At the same time, the relative humidity (RH) ranged from 11.10 to 92.90% during the video recordings in the free choice treatment, from 9.40 to 73.30% during the recordings of slow feeder treatment, and from 12.50 to 90.30% in the video recordings of box feeder treatment.

The calculated Indoor Temperature–Humidity Index (THI) ranged from 35.44 to 70.88 in the total duration of the video recordings. More details regarding the meteorological conditions during the video recordings days can be seen in TABLE 21.

The mean minimum, maximum and average temperature during all study duration was 2.55 ± 5.04, 19.76 ± 7.12, and 11.48 ± 5.41 °C, respectively. And the mean minimum, maximum and average relative humidity (RH) during all study duration was 18.93 ± 10.94, 76.58 ± 16.04, 47.76 ± 11.38%. More details regarding the meteorological conditions during the first, second and third rounds (15-day period) of the study are displayed in TABLE 22.

TABLE 21 – ENVIRONAMETAL DATA FOR EACH GROUP OF FIVE HORSES DURING THE DIFFERENT TREATMENTS (SLOW FEEDER, FREE CHOICE AND BOX FEEDER)

				Free choice			
Group 1		Mean	Median	Sample Variance	Range	Minimum	Maximum
	Air Temperature °C	5.03 ± 4.88	4.30	23.77	15.10	-3.40	11.70
	RH (%)	41.49 ± 14.29	38.40	204.18	51.40	25.80	77.20
	Dewpoint (°C)	-7.76 ± 1.86	-7.85	3.46	8.70	-10.90	-2.20
	Solar Radiation (W/m2)	186.26 ± 243.23	27.35	59162.01	748.70	0.00	748.70
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Wind (m/s)	3.65 ± 2.21	2.45	4.87	7.40	1.10	8.50
	Wind Direction (°N)	219.79 ± 111.20	222.50	12366.42	341.00	9.00	350.00
	Gust (m/s)	6.71 ± 3.79	5.85	14.33	11.40	2.40	13.80
	Gust Direction (°N)	220.50 ± 125.45	248.00	15737.51	359.00	0.00	359.00
	THI	43.74 ± 5.15	42.34	26.54	15.60	35.44	51.04
Group 2	Air Temperature °C	18.69 ± 6.53	18.45	42.58	18.30	10.70	29.00
	RH (%)	52.15 ± 29.49	48.80	869.78	78.10	11.20	89.30
	Dewpoint (°C)	5.35 ± 5.24	8.70	27.47	15.20	-4.60	10.60
	Solar Radiation (W/m2)	266.89 ± 346.10	52.50	119786.58	920.00	0.00	920.00
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Wind (m/s)	2.88 ± 1.85	2.20	3.42	6.70	0.80	7.50
	Wind Direction (°N)	180.86 ± 118.79	141.50	14110.64	355.00	3.00	358.00
	Gust (m/s)	5.61 ± 2.97	4.60	8.81	10.90	2.30	13.20
	Gust Direction (°N)	181.95 ± 118.98	122.50	14155.82	340.00	18.00	358.00
	THI	62.12 ± 5.14	62.74	26.39	15.55	55.33	70.88

Group 3	Air Temperature °C	10.30 ± 3.85	10.70	14.84	14.30	5.20	19.50
	RH (%)	70.65 ± 16.54	67.95	273.42	63.90	29.00	92.90
	Dewpoint (°C)	4.69 ± 1.15	4.70	1.32	5.60	1.00	6.60
	Solar Radiation (W/m2)	256.75 ± 258.01	189.80	66569.41	789.80	0.00	789.80
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Wind (m/s)	2.00 ± 0.80	2.00	0.65	2.90	0.40	3.30
	Wind Direction (°N)	192.58 ± 92.92	167.00	8634.91	344.00	15.00	359.00
	Gust (m/s)	3.70 ± 1.45	3.60	2.11	4.90	1.40	6.30
	Gust Direction (°N)	191.54 ± 91.19	167.00	8316.02	330.00	21.00	351.00
	THI	53.49 ± 3.89	53.86	15.14	13.47	47.89	61.36
			Slow feeder				
Group 1	Air Temperature °C	13.93 ± 3.43	13.40	11.74	12.00	6.80	18.80
	RH (%)	26.76 ± 13.54	24.50	183.41	63.90	9.40	73.30
	Dewpoint (°C)	-6.34 ± 5.00	-5.80	24.97	24.00	-15.00	9.00
	Solar Radiation (W/m2)	301.59 ± 334.73	157.05	112043.74	921.90	0.10	922.00
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Wind (m/s)	5.58 ± 3.27	6.45	10.66	9.70	1.30	11.00
	Wind Direction (°N)	217.27 ± 79.45	242.00	6312.66	267.00	64.00	331.00
	Gust (m/s)	11.35 ± 5.46	12.80	29.76	17.40	3.30	20.70
	Gust Direction (°N)	225.25 ± 70.43	262.50	4960.80	237.00	70.00	307.00
	THI	53.15 ± 3.49	53.14	12.20	14.09	46.25	60.34
Group 2	Air Temperature °C	8.43 ± 2.16	8.30	4.68	5.80	5.50	11.30
	RH (%)	36.06 ± 6.67	36.05	44.44	22.00	26.30	48.30
	Dewpoint (°C)	-6.02 ± 1.45	-5.90	2.11	6.40	-8.70	-2.30
	Solar Radiation (W/m2)	193.02 ± 218.30	57.20	47654.62	557.60	0.00	557.60
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00

	Wind (m/s)	7.31 ± 2.41	6.60	5.79	8.10	3.50	11.60
	Wind Direction (°N)	290.15 ± 64.89	304.50	4210.83	348.00	4.00	352.00
	Gust (m/s)	13.69 ± 4.58	12.40	20.99	13.90	8.00	21.90
	Gust Direction (°N)	302.40 ± 21.44	299.50	459.48	88.00	259.00	347.00
	THI	47.76 ± 2.23	48.77	4.97	6.39	44.54	50.93
Group 3	Air Temperature °C	8.48 ± 2.16	8.80	4.68	5.80	5.50	11.30
	RH (%)	36.11 ± 6.63	36.05	44.01	22.00	26.30	48.30
	Dewpoint (°C)	-5.95 ± 1.38	-5.90	1.91	5.90	-8.20	-2.30
	Solar Radiation (W/m2)	192.45 ± 218.17	57.20	47598.43	557.60	0.00	557.60
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Wind (m/s)	7.28 ± 2.42	6.55	5.84	8.10	3.50	11.60
	Wind Direction (°N)	288.71 ± 65.19	303.00	4249.66	348.00	4.00	352.00
	Gust (m/s)	13.78 ± 4.57	12.80	20.89	13.90	8.00	21.90
	Gust Direction (°N)	301.88 ± 22.01	299.50	484.45	88.00	259.00	347.00
	THI	47.84 ± 2.21	48.79	4.88	6.39	44.54	50.93
				Box feeder			
Group 1	Air Temperature °C	9.74 ± 4.48	7.35	20.10	11.90	5.90	17.80
	RH (%)	68.75 ± 28.73	87.20	825.68	71.60	18.70	90.30
	Dewpoint (°C)	2.28 ± 4.31	4.40	18.57	14.70	-7.10	7.60
	Solar Radiation (W/m2)	232.32 ± 310.81	40.35	96605.09	792.70	0.00	792.70
	Precipitation (mm)	0.28 ± 0.45	0.00	0.20	1.30	0.00	1.30
	Wind (m/s)	3.81 ± 3.35	2.30	11.19	10.50	0.70	11.20
	Wind Direction (°N)	249.47 ± 68.21	275.50	4651.97	212.00	112.00	324.00
	Gust (m/s)	6.99 ± 5.52	4.20	30.51	16.80	1.80	18.60
	Gust Direction (°N)	255.99 ± 74.32	287.50	5523.36	253.00	107.00	360.00
	THI	52.06 ± 3.11	50.57	9.67	8.87	48.98	57.86

Group 3	Air Temperature °C	20.14 ± 4.27	17.45	18.25	10.90	15.60	26.50
	RH (%)	29.94 ± 10.28	31.85	105.64	42.40	12.50	54.90
	Dewpoint (°C)	1.00 ± 3.80	1.05	14.44	13.90	-6.60	7.30
	Solar Radiation (W/m2)	144.60 ± 207.08	43.10	42884.02	728.90	0.00	728.90
	Precipitation (mm)	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
	Wind (m/s)	4.51 ± 2.03	4.35	4.12	7.90	0.50	8.40
	Wind Direction (°N)	240.54 ± 87.16	245.00	7596.69	329.00	24.00	353.00
	Gust (m/s)	9.37 ± 3.59	9.30	12.87	13.30	2.10	15.40
	Gust Direction (°N)	233.13 ± 72.37	241.50	5237.50	251.00	105.00	356.00
	THI	62.00 ± 4.37	60.48	19.09	13.24	57.29	70.53

TABLE 22 – METEOROLOGICAL CONDITIONS DURING THE FIRST, SECOND AND THIRD STUDY ROUNDS (15-DAY PERIODS)

First Round					
	Mean ± SD	Sample Variance	Range	Minimum	Maximum
Average Temperature °C	11.86 ± 4.41	19.45	14.30	3.90	18.20
Maximum Temperature °C	19.86 ± 6.58	43.28	24.30	5.70	30.00
Minimum Temperature °C	3.88 ± 4.00	16.03	14.80	-4.70	10.10
RH Maximum (%)	80.02 ± 14.59	212.82	46.80	51.10	97.90
RH Minimum (%)	24.15 ± 18.77	352.13	65.50	7.70	73.20
RH Average (%)	52.08 ± 14.37	206.64	49.35	35.25	84.60
Solar Radiation (W/m2)	238.71 ± 72.27	5222.89	247.40	65.80	313.20
Precipitation (mm)	2.14 ± 4.23	17.87	15.50	0.00	15.50
Wind Run (m/s)	295.11 ± 141.82	20112.98	573.20	156.60	729.80
Second round					
Average Temperature °C	8.21 ± 4.73	22.37	18.90	-2.10	16.80
Maximum Temperature °C	16.53 ± 6.56	42.98	24.70	4.40	29.10
Minimum Temperature °C	-1.19 ± 4.32	18.67	16.40	-10.70	5.70
RH Maximum (%)	70.91 ± 16.82	283.05	52.10	39.20	91.30
RH Minimum (%)	16.03 ± 5.58	31.17	17.30	6.80	24.10
RH Average (%)	43.47 ± 8.84	78.18	31.40	26.05	57.45
Solar Radiation (W/m2)	246.21 ± 35.37	1251.29	114.10	177.90	292.00

Precipitation (mm)	0.10 ± 0.36	0.13	1.50	0.00	1.50
Wind Run (m/s)	405.40 ± 182.79	33410.50	531.60	182.10	713.70
		Third round			
Average Temperature °C	14.23 ±5.27	27.76	17.10	3.30	20.40
Maximum Temperature °C	22.28 ±7.43	55.21	26.10	5.60	31.70
Minimum Temperature °C	5.52 ±3.78	14.30	13.30	-1.70	11.60
RH Maximum (%)	79.08 ±15.49	240.09	53.60	42.60	96.20
RH Minimum (%)	21.66 ±12.99	168.75	41.80	9.70	51.50
RH Average (%)	50.37 ±12.79	163.63	47.70	26.15	73.85
Solar Radiation (W/m2)	236.17 ±73.46	5396.86	257.60	68.90	326.50
Precipitation (mm)	0.97 ±1.70	2.88	4.80	0.00	4.80
Wind Run (m/s)	259.83 ±67.34	4534.06	280.30	166.60	446.90

4.4 DISCUSSION

4.4.1 Estimated hay consumption

Automatic feeding is gaining more importance in the livestock industry because it can reduce the workload of feeding crews as well as make it easier to control feed intake, reduce waste and improve feed efficiency (ARTHUR; ARCHER; HERD, 2004; SCHNEIDER *et al.,* 2020a). However, during the current study, the box feeder presented malfunction on the recording day of group 3, leaving horses without food for 24 hours. After detecting the malfunction, the automatic feeder was fixed and worked normally for all study duration. The findings of the current study corroborate with the information regarding food efficiency and intake control, since horses in the box feeder treatment had the lower hay utilization rate during the study (SCHNEIDER *et al.,* 2020b). They consumed an average of 1.95% of the BW in hay (DM) per day, very similar to the daily recommended requirements of 2.00% for horses in light exercise (NRC, 2007). The animals on the box feeder treatment gained 1.21 Kg at the end of the treatment, however, the slow feeder also had similar performance and horses gained similar amount of weight. In fact, horses in the slow feeder treatment had a little higher hay utilization rate (2.08%) but had lower weight gain rates at the end of the treatment (0.37 Kg). On the other hand, the free choice treatment had the highest rate of hay utilization, with each horse utilizing an average of 3.29% of their BW in hay per day, 64.0% above the daily recommendation of 2.00% for horses in light exercise (NRC, 2007). However, we theorize that some part of the hay in the free choice treatment was not consumed by the animals, but instead, it was dragged to the ground and used as bedding or even carried away by the wind, leading to great food waste, and contributing to the high rate of hay utilization observed in this treatment. Horses in the free choice treatment also gained more weight than the other treatments, putting up an average of 23.51kg at the end of the treatment. Horses in the free choice treatment also spent more time drinking water when compared to the other treatments. Horses in the free choice treatment spent 16.98 minutes (1.18% of the time-budget) drinking water and this value decreased to 10.78 minutes (0.75% of the time-budget) in the slow feeder, and to 5.88 minutes (0.41% of the time budget) in the box feeder treatment. Scientists believe that the water intake is highly correlated to the dry matter intake of the diet (FONNESBECK, 1968), and that would explain why horses in the free choice treatment spent more time drinking than the animals in other treatments. In ponies fed free choice hay, time spent drinking water was 21 to 27 minutes per day (SUFIT; HOUPT; SWEETING, 1985). In limit fed ponies, however, time spent drinking was 5-9 minutes (HOUPT *et al.,* 1988). In another study (NYMAN *et al.,* 2010), Standardbred geldings watered by buckets, float vales, or pressure valves, spent 3-15 minutes per day drinking.

Positive energy balance in horses can lead to fat deposition and the excess of adipose tissue can result in metabolic disturbances. Some pathological processes that have been associated to the excess of adipose tissue in horses include inflammation, oxidative stress, over stimulation of the hypothalamic-pituitary-adrenal axis, disturbances of cortisol and lipid metabolism, vascular dysfunction (RENDLE *et al.,* 2018), decreased insulin sensitivity (HOFFMAN *et al.,* 2003), and a major risk factor for laminitis (TREIBER; KRONFELD; GEOR, 2006). A survey conducted in the UK revealed that 54% of pleasure riding horses were overweight or obese (STEPHENSON; GREEN; FREEMAN, 2011). This number represents a substantial proportion of the equine population and according to the findings in the current study, it would not be recommended to keep horses affected by this condition in a free choice regimen. Although BCS did not change during the study, longer times would certainly affect the fat deposition, increasing BCS. Considering the displayed data, the slow feeder and box feeder would be better opinions to control weight gain and avoid food waste in horses.

4.4.2 Behavioral assessments

The results found in the current study suggest that the box feeder treatment had a negative influence on equine behavior. Scientists that conducted studies on horses living in their natural environment observed that this species spent 51-63% of their time-budget feeding (DUNCAN, 1980; LAMOOT and HOFFMAN, 2004). The horses in the free choice and slow feeder treatments spent 51.70 and 54.94 % of their time foraging, which confirms other authors findings that domestic horses will display time-budgets similar to those of feral horses in their natural environment when given the opportunity (AUER *et al.,* 2021; LESIMPLE, 2020). As expected, the box feeder treatment provided restricted access to roughage, reducing the time that horses spent eating to an average of 25.81% of their time-budget.

According to researchers, decreasing the feeding time can lead horses to long periods of fasting and boredom (HANIS *et al.,* 2020). This tendency was also observed in the current study, since the horses in the box feeder spent more time standing attentive (52.35% of the time-budget) when compared to the free choice and slow feeder treatments, (32.57 and 29.20% of the time-budget, respectively). In feral horses (DUNCAN, 1980), time spent standing varied between 11-23% of the day. Thus, the incapacity of fulfilling the behavioral need of foraging can leave horses with a high feeding motivation, which may contribute to the development of abnormal behaviors, such as coprophagy (HOTHERSALL; NICOL, 2009). This information also corroborates with the findings of the current study, since horses in the free choice treatment were not observed practicing coprophagy, whereas horses in the box feeder treatment spent an average of 6.67% of their time-budget eating feces. An interesting finding of the current study is that even with unlimited access to hay through a net, horses in the slow feeder treatment also developed coprophagy. Although the value was not significantly different from the free choice treatment, horses in the slow feeder treatment were also observed spending an average of 1.35% of their time-budget eating feces. A factor that could have influenced the development of this abnormal behavior in the slow feeder treatment is the group hierarchy. The time horses spent eating in the slow feeder had great variation with values ranging from 634.3 minutes (44.05% of the time-budget) to 1160.67 minutes (80.60% of the time-budget), a difference of 45.35%. We theorize that because of the small area of the hut feeder available for five horses to access the hay, the dominant horses spent more time eating while the submissive horses had fewer foraging opportunities, which may have led them to develop this abnormal behavior. Future studies will be necessary to test this hypothesis, and if confirmed, it could mean that only one unity of the slow feeder used in this study is not enough to provide equal foraging opportunities for five horses. We theorize that two units of the described slow feeder would be able to provide more access to hay for submissive horses and consequently, decrease aggression, provide more homogeneous feeding time between individuals, and eliminate coprophagy.

The horse is a highly social species that form social groups when living in natural conditions and specialists believe that group housing is the best option regarding welfare (LESIMPLE *et al.,* 2020). However, many owners keep horses in individual stalls for fear of aggressive interactions and injury risks (FLAUGER; KRUEGER, 2013). Scientists believe that higher aggression levels in horses is linked to limited resources, whether due to lack of physical space (FLAUGER; KRUEGER, 2013) of restricted food (BURLA *et al.,* 2016), which corroborates with the current findings. In the current study, the free choice treatment presented lower levels of aggression and was the only group where the behavior allogroom, an affiliative social interaction, was observed. On the other hand, the horses in the slow feeder and box feeder treatment had similar numbers of bites and the box feeder had the highest number of kicks, which is concerning, since this kind of negative social interaction can increase the risk of injury.

Similar results were found in a recent study conducted to evaluate the effect of round bale hay nets on horse aggression. The horses were given a round bale within a ring feeder and the treatments consisted of: (1) without a hay net, (2) surrounded with a hay net with small diameter openings (4.445 cm diameter), or (3) surrounded with a hay net with large diameter openings (7.62 cm diameter). The authors observed that horses fed using round bale hay nets with small openings (4.445 cm diameter) were observed to have increased agonistic threats and avoidance behavior. They theorized that the hay nets with smaller openings may increase horse frustration, and therefore, aggression between horses (SUNDMAN *et al.,* 2022). Those findings corroborate with the data from the current study where the horses in the slow feeder treatment showed an increase in the biting behavior when compared to the free choice treatment and had similar values of this variable to the box feeder treatment.

A great variation of the feeding time was also observed in the box feeder treatment, where the time that horses spent eating ranged from 296.67 minutes (20.60% of the time-budget) to 448.83 minutes (31.17% of the time-budget), a variation of 33.91%. An increase in negative social interactions (bite and kick) was observed in this treatment, corroborating with previous findings described in the scientific literature. For example, a study conducted with 188 mares and 202 geldings kept in group housing systems found that the aggressive behavior between conspecifics decreased substantially during feeding with an increasing duration of hay availability (BURLA *et al.*, 2016).

Another factor that could have contributed to the aggression between horses in the current study is the size of the enclosure and the size of the feeders. For example, in a previous study, researchers found a significant correlation between the groups' enclosure sizes and the horses' aggressive behavior. The authors observed that aggressiveness among horses approaches zero when the enclosure is sized 331 m^2 per horse or more. The dry lots of the current study were 20x30 (600m^2), which provides 120 m^2 of area per horse, 63.75% less than the recommendation (FLAUGER; KRUEGER, 2013). Therefore, a certain level of aggression between conspecifics was expected, but it became significantly worse as the area per feeder became smaller and the food source became less accessible.

Researchers believe that the amount of space between horses that are feeding can influence agonistic behavior (BURLA *et al*., 2016; SUNDMAN *et al*., 2022). In a study, scientists observed above-average agonistic behavior when horses were required to stand in close proximity and entailed feeding with heads close together (BURLA *et al.,* 2016). According to the scientific literature, horses prefer to maintain an individual distance of 1.0–1.5 meters while feeding (McGREEVY, 2004). The box feeder used in the current study offered 1.19 meters per horse, the slow feeder offered 1.58 meters per horse, and the free choice feeder offered 2.20 meters per horse. Although all feeders used in the current study offered more space per horse than the minimum recommendation, only the free choice feeder had lower levels of agonistic behaviors. Therefore, using distant feeding places seems like a better option that would allow horses to maintain their perceived individual distances, and help to reduce the occurrence of agonistic behavior. According to authors, if horses are required to eat in close proximity, they should be provided with individually feeding places, which limit or prevent physical contact (BURLA *et al.,* 2016).

As expected, horses in the box feeder also spent more time sniffing the ground than the other treatments (47.93 minutes or 3.33% of the time-budget). At the same time, the horses in the free choice treatment spent an average of 5.28 minutes (0.37%

of the time-budget), and the horses in the slow feeder treatment spent an average of 2.02 minutes (0.14% of the time-budget) sniffing the ground. Nosing and sifting the ground for feed or forage closely resembles the natural foraging patterns of feeding horses (COOPER; MCGREEVY, 2007). Researchers believe that this behavior may represent attempts to find spilt food or perseverance of feeding behavior that was not satisfied by the previous meal (COOPER; MCGREEVY, 2007). This theory corroborates with the finding of the current study since the box feeder treatment, which provided limited access to roughage, had the highest value for this variable. It indicates that horses in this treatment were not able to satisfy their ingestive behavior with the limited period of hay availability.

Horses in the box feeder and slow feeder treatment also spent more time walking than in the free choice treatment. Those two treatments had similar values with horses in the box feeder spending 57.13 minutes (3.97% of the time-budget) and horses in the slow feeder spending 46.44 minutes (3.23% of the time-budget) walking. On the other hand, horses in the free choice treatment spent only 37.18 minutes walking (2.58% of the time budget). Comparable results were also found in a previous study where the scientists aimed to evaluate if different feeding systems would affect the locomotor activity. They observed that horses fed in feeding systems with higher levels of agonistic behavior would show more locomotor activity and this data corroborates with the findings in the current study (BURLA *et al.,* 2016).

Another behavior that was affected by the treatment was the time horses spent lying down. Horses in the box feeder treatment spent less time lying down than the animals in the other treatments. The horses in the free choice and slow feeder treatment did not differ in this variable. Researchers studying the lying behavior of fifty-six horses in a modern group housing system observed that the daily total lying time per horse varied from 26.7 to 74.3 minutes depending on the bedding material (rubber mats, sand bedding) and on the horse rank. They found that horses which were lower in rank showed fewer and shorter lying bouts (BAUMGARTNER *et al.,* 2015). In the current study, horses in the free choice treatment spent 102.47 minutes (7.12% of their time-budget) lying down, the horses in the slow feeder treatment spent 89.75 minutes (6.23% of their time-budget), and in the box feeder this time decreased to 36.13 minutes (2.50% of their time-budget). Since the sleeping area provided for all groups were the same, we theorize that groups with higher levels of agonistic behavior also presented shorter periods of time lying down.

Horses in the current study presented extremely low incidence of stereotypy behavior and the difference between treatments were not significant. The animals in the free choice and slow feeder treatments spent 0% of their time-budgets in this behavior and the horses in the box feeder spent 0.01% of their time-budget in this kind of behavior. Nodding was observed in the box feeder and slow feeder treatments (0.21 and 0.01 minutes on average, respectively). Pawing and abnormal movements of lips and tongue we observed in the free choice treatment (0.04 and 0.02 minutes on average, respectively). Researchers believe that a genetic pre-disposition, chronic stress, and the incapacity of performing natural behaviors due to restricted feeding and movement, and social isolation are the main causes of the development of stereotypies in horses (SEABRA; DITTRICH; VALE, 2021). The extremely low incidence of stereotypic behaviors in the current study indicates that the horses used in this trial presented good levels of welfare or that fifteen days of treatment were not enough to cause the development of stereotypies in the studied population.

4.4.3 Meteorological Recordings

The calculated Indoor Temperature–Humidity Index (THI) values in our study ranged from 35.44 to 70.88. The maximum THI was still within the normal range and did not reach values that would characterize heat stress situations (THOM, 1958). According to previous research, values up to 74 are classified as ‘normal’, values from 75 to 79 as ‘alert’, values from 79 to 84 as ‘danger’ and a value of 84 or above as ‘emergency’ (THOM, 1958). To the best of our knowledge, the THI has not yet been used in studies on horses kept in cold regions. Therefore, it was not possible to place the minimum value encountered in our study in context.

According to authors, the thermoneutral zone of the mature horse housed outdoors during winter ranges from approximately - l5°C to 10°C (MCBRIDE; CHRISTOPHERSON; SAUER, 1985). During the current study, the mean minimal temperature was 2.55 ± 5.04, above the lower critical temperature, indicating that the horses were not suffering from cold stress. The mean average temperature was 11.48 ± 5.41 °C and the mean maximum temperature during the study duration was 19.76 ± 7.12. According to McBride and coworkers, these values are above the higher critical temperature (MCBRIDE; CHRISTOPHERSON; SAUER, 1985). However, other researchers state that the thermoneutral zone of horses should not be considered a fixed value, because it will depend on animal factors such as age, breed, physiological status, acclimatization, hair coat quality, feeding, and body condition (AUTIO, 2008; MEJDELL; BØE; JØRGENSEN, 2020). For example, Morgan and collaborators estimated the general thermoneutral zone for horses to range from 5 to 25°C (MORGAN; EHRLEMARK; SÄLLVIK, 1997). The calculated Indoor Temperature–Humidity Index (THI) values in our study were also within the normal range, indicating that the animals were not under heat stress conditions.

4.5 CONCLUSION

Horses in the box and slow feeders had the lower hay utilization rate and lower weight gain in the current study and both treatments can be considered equivalent in food efficiency and intake control. Horses in the free choice treatment had the highest rates of hay utilization and weight gain, and this regimen should not be recommended to overweight or obese horses. Horses in both free choice and slow feeder treatments were able to spend more than 50% of the time-budget foraging, generating a time distribution similar to feral horses in their natural environment. However, the results found in the current study suggest that the box feeder treatment had a negative influence on equine behavior, decreasing the time that horses spent eating to an average of 25.81% of their time-budget. Consequently, horses in the box feeder treatment spent significantly more time standing attentive, sniffing the ground, and practicing coprophagy than horses in the other treatments. A great variation of the feeding time was observed in the box feeder and slow feeder treatments, with horses in the box feeder treatment showing higher levels of aggression. The level of aggression between conspecifics became significantly higher as the area per feeder became smaller and the food source became less accessible. Although all feeders used in the current study offered more space per horse than the minimum recommendation, only the free choice feeder had lower levels of agonistic behaviors. We theorize that the dominant horses spent more time eating while the submissive horses had fewer foraging opportunities. Future studies will be necessary to test this hypothesis, and if confirmed, it could mean that only one unity of the slow feeder and box feeder used in this study is not enough to provide equal foraging opportunities for five horses and two unities of the described feeders would be needed to provide equal access to hay, decrease aggression, and eliminate coprophagy. Finally, automatic feeders are a good option to maximize feed efficiency while minimizing the workload of feeding crews, however, it should be designed by animal scientists taking into consideration the feeding behavior and welfare of the target species. Therefore, more research is needed in order to develop an automatic feeder that will provide optimal welfare levels by allowing horses to fulfill their behavioral needs. The THI calculated based on environmental conditions and the mean minimal air temperatures recorded during the footage recordings shows that horses were not under heat or cold stress during the behavioral assessments.

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5. FINAL CONSIDERATIONS

This dissertation shows that many strategies developed by researchers to prevent abnormal behaviors are not yet commonly implemented in horse farms and equestrian centers, especially in developing countries. The fulltime stabled horses observed in the second chapter of this dissertation presented very high prevalence of abnormal and stereotypic behaviors, along with other physiological alterations consistent with a chronic stress state. The knowledge on horse welfare has become more accessible for elite horse owners, but many equestrian facilities that use horses as workforce insist on perpetuating management practices that restricts the species' natural behavior, leading to poor welfare conditions.

The restricted availability of forage observed in the third chapter and in the box feeder treatment in the fourth chapter had a negative impact on the horses' time-budget, decreasing the feeding time and increasing the time horses spend in abnormal behaviors and idleness. On the other hand, horses in both free choice and slow feeder treatments in the third chapter were able to spend more than 50% of the time-budget foraging, generating a time distribution similar to feral horses in their natural environment. Horses in the free choice treatment had the highest rates of hay utilization and weight gain, and this regimen should not be recommended to overweight or obese horses. Therefore, we can conclude that the slow feeder is a good opinion to improve health and welfare in horses, since it provides more natural distribution of time, along with food efficiency and intake control.

Finally, actions to ensure good levels of welfare to working horses should be prioritized. Researchers working with animal welfare in developing countries should receive more support from the equine industry and from international nonprofit organizations. Thus, more research is needed to validate affordable methods to measure stress levels and to develop accessible techniques to improve the welfare conditions for working horses.

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ANNEX 1 – CERTIFICATE OF THE ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ, PROTOCOL NUMBER 006/202021

UNIVERSIDADE FEDERAL DO PARANÁ SETOR DE CIÊNCIAS AGRÁRIAS
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o protocolo número 006/2021, referente ao projeto de pesquisa **"Práticas de manejo e outros fatores associados à prevalência de distúrbios comportamentais em equinos e uso de técnicas de enriquecimento ambiental para melhoria do bem-estar de cavalos estabulados",** sob a responsabilidade de **João Ricardo Dittrich** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de Outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) DO SETOR DE CIÊNCIAS AGRÁRIAS DA UNIVERSIDADE FEDERAL DO PARANÁ - BRASIL, com grau 1 de invasividade, em 06/04/2021.

Finalidade	Pesquisa
Vigência da autorização	Junho/2021 até Dezembro/2022
Espécie/Linhagem	*Equus caballus* (equino)
Número de animais	100
Peso/Idade	3-25 anos/450kg
Sexo	Machos e fêmeas
Origem	Regimento da Polícia Montada de Curitiba, Paraná, Brasil.

*A autorização para início da pesquisa se torna válida a partir da data de emissão deste certificado.

CERTIFICATE

We certify that the protocol number 006/2021, regarding the research project **"Husbandry practices and other factors associated with the prevalence of behavioral disorders in horses and use of environmental enrichment techniques to improve the welfare of stabled horses"** under **João Ricardo Dittrich** – which includes the production, maintenance and/or utilization of animals from Chordata phylum, Vertebrata subphylum (except Humans), for scientific or teaching purposes – is in accordance with the precepts of Law nº 11.794, of 8 October 2008, of Decree nº 6.899, of 15 July 2009, and with the edited rules from Conselho Nacional de Controle da Experimentação Animal (CONCEA), and it was approved by the ANIMAL USE ETHICS COMMITTEE OF THE AGRICULTURAL SCIENCES CAMPUS OF THE UNIVERSIDADE FEDERAL DO PARANÁ (Federal University of Paraná, Brazil), with degree 1 of invasiveness, on 2021, April 6th.

Purpose	Research
Validity	June/2021 until December/2022
Specie/Line	*Equus caballus* (equine)
Number of animals	100
Weight/Age	From 3 up to 25 years old/992lb
Sex	Male/Female
Origin	The Mounted Police Regiment of Curitiba, Parana, Brazil.

*The authorization to start the research becomes valid from the date of issue of this certificate.

Curitiba, 06 de abril de 2021

Maity Zopollatro

Coordenadora pro-tempore CEUA/AG/UFPR

Comissão de Ética no Uso de Animais do Setor de Ciências Agrárias - UFPR

ANNEX 2 – CERTIFICATE OF INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) AT COLORADO STATE UNIVERSITY - PROTOCOL NUMBER 2873

PROTOCOLS kuali

#2873 - Effects of different hay feeders, availability of roughage on abnormal behaviors and cortisol circadian rhythm in horses kept in dry lots

Protocol Information

Review Type	Status	Approval Date	Continuing Review Date
Designated Member Review	Approved	Oct 28, 2021	Oct 27, 2022

Expiration Date	Initial Approval Date	Initial Review Type
Oct 27, 2024	Oct 28, 2021	Designated Member Review

Feedback

Approval Comment

None