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Bedding Materials and Body Size Influence the Stress Responses of Horses Kept in Individual Loose Box Housing

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Simple Summary

Bedding refers to a variety of materials placed on the stall floor to accommodate horses. It is primarily used to absorb urine and other moisture, provide cushioning, and create a dry, comfortable environment. Although the impacts of different bedding materials on equine comfort have been widely studied, the effects of bedding materials on comfort in horses of varying sizes remain insufficiently explored. This study examined how bedding type (straw vs rice husk) and body size (horses taller than the upper bar of the individual box's front gate vs shorter than it) influence horse comfort, as assessed by behavioural scores, recumbent time, and autonomic regulation measured by heart rate and its variability during a 24-hour housing period. Results indicated that airborne particle concentration was significantly higher in stalls with rice husk bedding compared to those with straw. Horses appeared more comfortable when housed on straw bedding. Additionally, horses taller than the upper bar of the box's front gate exhibited lower stress levels than shorter horses. These findings offer insights into stress responses in horses of varying body sizes on different bedding materials. Although this study demonstrated significant effects of bedding material and body size on autonomic regulation, these findings require validation through further long-term research.

Abstract

Bedding serves a vital role in horse stable management. Although earlier research has examined stress responses to bedding materials, the effect of bedding types on these responses in horses of different sizes has not been studied. This study assessed the influence of bedding materials (straw vs rice husk) and body size (horses taller than the upper bar of the front gate [H1] vs shorter ones [H2]) on stress responses. Stress was evaluated using behavioural scores, recumbent time, and autonomic regulation via heart rate (HR) and heart rate variability (HRV). Microenvironmental variables such as relative humidity (RH), air temperature (AT), volatile organic compounds (VOCs), carbon dioxide (CO_2), particle counts (PC; PC1, 2.5 and 5) and fine particulate matter (PM; PM1, 2.5 and 5) were also recorded. No differences were observed in RH, AT, VOCs or CO_2 between stables with different bedding materials. However, PC (1, 2.5 and 5) and PM (1, 2.5 and 5) levels were higher in stables with rice husk than in those with straw. Beat-to-beat (RR) intervals increased, and HR decreased in both H1 and H2 horses on straw during the night ($p < 0.05$). H1 horses generally showed lower HR and higher RR intervals than H2 horses during housing, regardless of the bedding material used ($p < 0.05$–0.0001). Although bedding and body size affected behavioural scores and several HRV metrics, no significant within- or between-group differences were detected. These findings suggest that both bedding material and body size influence stress responses; however, short-term individual box housing may not provide sufficient stressors to produce significant differences.

Keywords: behaviour; body height; horse; heart rate variability; recumbent time; rice husk; stable microclimate; straw; stress; welfare

1. Introduction

In contrast to their wild counterparts, domesticated horses spend most of their time in stables [1–3]. Consequently, ensuring the well-being of horses in stable housing is a significant concern. In addition to stable architecture, bedding type serves a critical role in influencing stereotypic behaviour and, by extension, animal welfare during stabling [4,5]. Bedding is integral to daily stable management and fulfils several functions, such as moisture absorption, creating an appropriate microenvironment for rest, providing thermal insulation and cushioning, and encouraging recumbency in stabled horses [6–9].

A variety of materials have been utilised as bedding in horse stables, including straw, wood shavings, sawdust, compressed wood pellets and rubber mats [10–12]. Among these, straw and wood shavings are the most frequently used in horse stable management [12,13]. Both materials have a dry matter content of 86–89% and a water-binding capacity of 316–321%, which can optimise moisture absorption in the stable and thus reduce the frequency of bedding replacement [12,14]. Straw is often preferred due to its edibility and comfort, which promote recumbency in horses. In contrast, wood shavings offer superior absorbency, and their dust-free properties are advantageous for horses with respiratory conditions [14]. Rice husk, a by-product of rice processing, has also been used as bedding in areas where rice is produced and is considered eco-friendly due to its biodegradability and renewable nature [12,15]. Despite these advantages, high dust levels or low-quality bedding materials can adversely affect indoor air quality. These factors may contribute to an increase in airborne particles, which can cause respiratory problems in stabled horses and stable workers [6,7,10]. Consequently, the selection of bedding materials should prioritise low particle emission, minimise stress and support the welfare of horses in stables.

Horse welfare is commonly assessed using behavioural changes [16,17], hormonal responses [18–20], autonomic regulation as determined by heart rate (HR) and heart rate variability (HRV) [21–23], and combined variables [24–26]. Among these parameters, HRV is widely recognised as a non-invasive indicator of stress levels and a measure of autonomic nervous system (ANS) function in maintaining body homeostasis [27,28]. HRV is defined by rhythmic fluctuations in the intervals between adjacent heartbeats, which are influenced by parasympathetic (vagal) and sympathetic impulses from the ANS. An increase in HRV indicates a shift toward greater vagal tone, which enhances adaptability to physiological challenges, whereas a decrease in HRV reflects reduced adaptability and potential stress [29–31]. Several HRV metrics, such as the square root of the mean squared differences between successive normal beat-to-beat (RR) intervals (RMSSD), the high-frequency (HF) band, and the standard deviation in the Poincaré plot perpendicular to the line of identity (SD1), primarily reflect vagal dominance [28,30,32]. In contrast, RR intervals, the standard deviation of normal-to-normal RR intervals (SDNN), the mean of the standard deviations of normal-to-normal RR intervals in 5-minute segments (SDNNI), the low-frequency (LF) band, and the standard deviation in the Poincaré plot along the line of identity (SD2) are modulated by both sympathetic and vagal components [28,29]. The ratios of the frequency band variables (LF/HF) and non-linear variables (SD2/SD1) indicate sympathovagal balance [29,30,33]. Therefore, HRV metrics are considered reliable indicators of horse welfare and have been employed to assess stress or discomfort in horses during exercise [21,34,35], disease-related discomfort [22,36,37], transportation [38–40] and stable housing [14,41].

Although the stress responses of horses to different bedding materials have been reported, limited information exists regarding how these responses vary across horses of different sizes.

Therefore, this study aimed to examine the effects of bedding materials on stress-related behaviour, recumbent time and autonomic regulation in horses of varying body sizes. It was hypothesised that bedding types would differentially influence stress responses depending on horse size.

2. Materials and Methods

2.1. Animals

Fifteen healthy horses, including six mares and nine geldings aged 12–28 years, from the Horse Lover's Club in Pathum Thani, Thailand (Latitude: 13.9945, Longitude: 100.68081), were included in this study. All animals were housed individually in 4 x 4 m brick stables with plaster insulation. The stables were separated by solid walls up to 170 cm high, with vertical bars above to allow visual contact between neighbouring horses. The front gate of each stable was solid up to 134 cm, with a 50 cm gap at the bottom. As feed, 2–3 kg of commercial pellets were split equally and provided three times daily. Pangola hay and water were freely available in their stables. Horses were usually utilised for school riding for 40–60 minutes, 3–4 days weekly, or for sedentary work. They spent 1–3 hours per day in the paddock on a day off, and spent most of their time in the stables. The horses included in this study were free of injuries and gait abnormalities. Moreover, they had not received any medical or surgical treatment in the 30 days prior to the study. Horses that incurred injuries or illnesses during the study period would have been excluded from the study. No injuries or abnormalities were identified. All 15 horses were included in the study.

2.2. Experimental Design

The study was conducted in October and November 2025 on rest days when exercise and other activities were not permitted. Animals were allocated into two groups according to body weight and height: the first group (H1) consisted of seven horses (average age 19.3 ± 6.6 years, weight 414.4 ± 53.3 kg, height 153.0 ± 8.5 cm) and the second group (H2) included eight horses (average age 21.4 ± 4.5 years, weight 275.5 ± 41.0 kg, height 127.5 ± 7.8 cm). Each group was sequentially housed in their stables with two different bedding materials for each consecutive 24-hour period. Both groups were initially housed with straw bedding and subsequently switched to rice husk bedding on the next experimental date. To minimise confounding factors, stable cleaning was not permitted, and only routine feeding was allowed during the study. Parameters, including airborne particles, noxious gases, stress-related behaviour, recumbent time and autonomic regulation, were monitored during 24-hour study periods for each group. Notably, data from 05:00–07:00 on the following day were excluded from the analysis to avoid confounding by stable cleaning and morning activities. Thus, the data were analysed for 22-hour periods from 07:00 h on the first day to 05:00 h on the following day.

2.3. Data Acquisition

2.3.1. Stable Microenvironment

Two temperature and humidity data loggers (TM-305U; Tenmars Electronics, Taipei, Taiwan) were used to measure relative humidity (RH) and air temperature (AT) within the stables. They were positioned in the stable aisle, approximately 60–70 cm above the concrete floor. The recorded average RH and AT were reported as percentages (%) and degrees Celsius (°C), respectively. Interior air quality was measured in the barn aisle using an air quality monitoring device (Canāree™ Air Quality Monitor, Piera Systems, Ontario, Canada). The device was linked to a laptop running SenseiAQ software (https://github.com/PieraSystems/SenseiAQ; accessed on 24 October 2025) and placed in the middle of the stable aisle to export air quality data. The monitored air quality variables included volatile organic compounds (VOCs), carbon dioxide (CO_2), particle counts (PCs) for particle sizes of 0.5–1 μm (PC1), 1–2.5 μm (PC2.5) and 2.5–5 μm (PC5), and fine particulate matter (PM) for particle sizes of 0.5–1 μm (PM1), 1–2.5 μm (PM2.5) and 2.5–5 μm (PM5). VOCs and CO_2 were expressed as parts per million (ppm), PC as the number per litre (/litre) and PM as micrograms per cubic metre

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(μg/m³). Stable microenvironment data were collected at 60-minute intervals and reported as a 22-hour average.

2.3.2. Stress-Related Behaviour and Recumbent Time

Surveillance cameras (Tapo C210V3, TP-Link Technologies Co., Ltd., Shenzhen, China) were positioned at the front of the stables, approximately 3 m above the floor, to record the stereotypic behaviours and recumbent times of horses housed in individual boxes from 07:00 on the first day to 05:00 on the following day. Three qualified observers evaluated the recorded stereotypic behaviours and assigned stress levels as previously described [42]. Specifically, scores of 1 and 2 were assigned when the horse was calm, unconcerned, relaxed, quiet, listening, accepting, alert or watching. Scores of 3 and 4 were given when the horse was listening, interested, alert, curious, unsettled, or barging. Scores of 5 to 7 were assigned when the horse exhibited a restless or tense body, fidgeting, jumpiness, was easily startled, avoided potential stressors or remained still to focus on the observers. A score of 8 was given if the horse displayed weaving, box walking or repetitive head movements. Scores of 9 and 10 were assigned when the horse appeared agitated, fidgety, anxious, active, aggressive or uncomfortable. Behavioural scores of 1 and 2 indicated no stress; scores of 3 and 4 indicated low stress; scores of 5 to 7 indicated medium stress; and scores of 8 to 10 indicated the highest stress levels. Behavioural scores were averaged across horses and reported every 60 minutes over a consecutive 22-hour period, while the recumbent time was reported as a 22-hour average.

2.3.3. Autonomic Regulation

Autonomic regulation was evaluated by monitoring HR and HRV using a heart rate monitoring (HRM) device (Polar Electro Oy, Kempele, Finland), which reliably produces RR interval data and HRV metrics [27,43–45]. A Polar equine belt, soaked in water and coated with ultrasound gel, was applied to the electrode surface to improve conductivity. An H10 heart rate sensor was attached to the belt and secured around the thorax, with the sensor pocket on the left side and wirelessly connected to a Polar sports watch (Vantage 3) for RR interval data collection. The HRM device remained on each horse throughout the experiment.

The Polar sports watch was connected to FlowSync software (https://flow.polar.com/start; accessed on 28 November 2025) to transfer RR interval data, which were exported as comma-separated value (CSV) files. These files were uploaded to Kubios Premium version 4.1.2.1 (Kubios HRV scientific: https://www.kubios.com/hrv-premium/; accessed on 28 November 2025) for HRV metric computation, and the resulting data were exported as MATLAB MAT files. To address artefacts such as missing, extra, misaligned, or ectopic beats (including premature ventricular contractions or other arrhythmias), an automatic artefact correction feature was applied to ensure accurate RR interval alignment. Automatic noise detection was set to medium to filter out noisy segments that could distort beat analysis. The smoothness prior method was used to remove non-stationarity from the RR interval time series, with a cutoff frequency for trend removal set to 0.035 Hz, as specified in the user guidelines (https://www.kubios.com/downloads/Kubios_HRV_Users_Guide.pdf, accessed 28 November 2025).

HRV metrics were reported in three methodological domains: (1) time domain analysis, including mean HR, RR, SDNN, SDNNI, RMSSD, and stress index; (2) frequency domain methods, comprising the LF band (0.01–0.07 Hz), HF band (0.07–0.6 Hz), LF/HF ratio and respiratory rate estimated from RR intervals (RESP); and (3) non-linear methods, including SD1, SD2, and SD2/SD1 ratio. HRV metrics were collected at 60-minute intervals over a 22-hour period.

2.4. Data Analysis

Data analysis was conducted using GraphPad Prism version 11.0.0 (GraphPad Software Inc., San Diego, CA, USA). Missing data necessitated the use of a linear mixed-effects model (restricted maximum likelihood) to assess the independent effects of bedding materials, body size and time, along with their two- and three-factor interactions, on stress-related behaviour, recumbent time and HRV metrics. Tukey's test was applied for multiple comparisons within and between groups. Since the data were not normally distributed, the Kruskal-Wallis test, followed by Dunn's multiple-comparison test, was used to analyse recumbent time across bedding materials and body sizes. For environmental parameters, the Wilcoxon matched-pairs signed-rank test was used for data with non-normal distributions, and a paired t-test was used for normally distributed data. An unpaired t-test with Welch's correction was used to evaluate differences in age, weight and height between groups. Results are reported as mean ± SD, with significance set at $p < 0.05$.

3. Results

Notably, both horse groups were similar in age ($p = 0.4937$) but differed in body size (weight: $p = 0.0001$; height: $p < 0.0001$). The variables of the stable microenvironment and physiological responses of horses on straw and rice husk beddings are described as follows.

3.1. Noxious Gases and Airborne Particles

The VOC and CO_2 levels did not differ between stables with straw and rice husk beddings. However, the PCs (PC1, PC2.5 and PC5) and PM (PM1, PM2.5 and PM5) appeared to be higher in stables with rice husk bedding than in those with straw bedding (Table 1).

Table 1. Environmental parameters measured during 24-hour housing periods on straw and rice husk beddings.

Variables		**Bedding materials**		***p*-value**
		Straw	**Rice husk**	
Relative humidity (RH) (%)		74.8 ± 10.3	73.2 ± 7.2	$p = 0.1011$
Air temperature (AT) (°C)		29.0 ± 1.5	29.1 ± 1.6	$p = 0.0567$
Volatile organic compounds (VOCs) (ppm)		17.5 ± 19.9	14.4 ± 14.0	$p = 0.3596$
Carbon dioxide (CO_2) (ppm)		1781.0 ± 929.2	1812.0 ± 910.9	$p = 0.6033$
Particle counts (PC) (/litre)	1	635.9 ± 213.4	2421.0 ± 804.3	$p < 0.0001$
	2.5	42.9 ± 10.8	141.4 ± 50.4	$p < 0.0001$
	5	0.9 ± 0.5	4.9 ± 1.8	$p < 0.0001$
Fine particulate matter (PM) (µg/m^3)	1	2.9 ± 0.9	10.5 ± 3.1	$p < 0.0001$
	2.5	3.1 ± 0.9	11.4 ± 3.3	$p < 0.0001$
	5	3.1 ± 1.0	11.2 ± 3.3	$p < 0.0001$

3.2. Stress-Related Behaviour and Recumbent Time

There were effects of independent time and bedding materials on the behavioural scores (time: $F_{(21, 330)} = 3.806$, $p < 0.0001$; bedding materials: $F_{(1, 304)} = 11.09$, $p = 0.0010$). Despite fluctuations, the statistical analysis did not detect within- and between-group differences (Figure 1). Moreover, recumbent time did not differ between horses housed on either bedding material during the 24-hour housing period (straw (H1): 72.1 ± 102.2 min; straw (H2): 82.4 ± 96.3 min; rice husk (H1): 63.3 ± 102.7 min; rice husk (H2): 71.1 ± 78.3 min, $p = 0.5191$) (see Figure S1).

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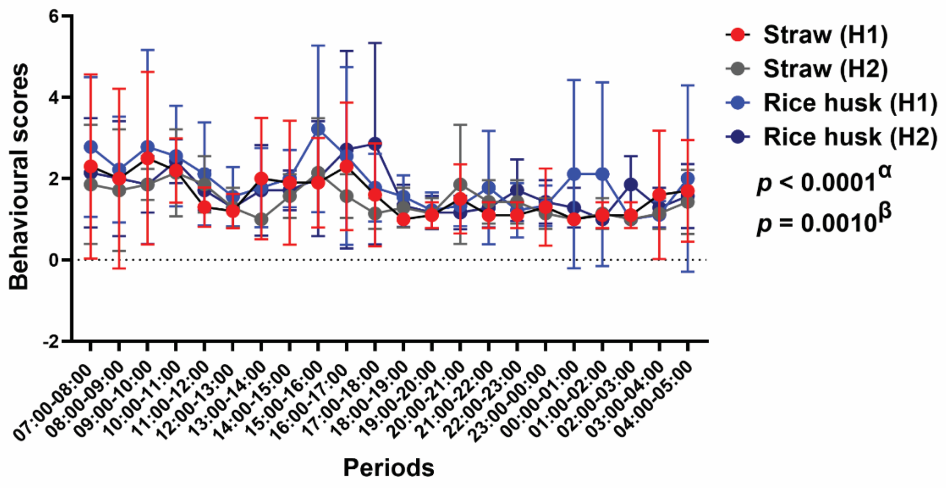

Figure 1. Behavioural scores of horses with different body sizes housed on straw or rice husk over a 24-hour period. α and β indicate the effects of the independent time and bedding materials. **H1**: horses of the first group; **H2**: horses of the second group.

3.3. Heart Rate and Heart Rate Variability

3.3.1. Time Domain Analysis

Independent effects of bedding materials ($F_{(1, 240)} = 4.466$, $p = 0.0356$), body size ($F_{(1, 276)} = 303.5$, $p < 0.0001$) and time ($F_{(21, 276)} = 6.791$, $p < 0.0001$), as well as the interaction between bedding material and body size ($F_{(1, 240)} = 10.53$, $p = 0.0013$), affected RR interval modification. Furthermore, independent body size ($F_{(1, 276)} = 237.1$, $p < 0.0001$) and time ($F_{(21, 276)} = 5.520$, $p < 0.0001$), as well as the interaction between bedding material and body size ($F_{(1, 240)} = 4.405$, $p = 0.0369$), influenced HR changes. The SDNN and stress index shared the same trend because they were modulated as the effects of bedding materials (SDNN: $F_{(1, 242)} = 12.18$, $p = 0.0006$; stress index: $F_{(1, 240)} = 7.785$, $p = 0.0057$), body size (SDNN: $F_{(1, 279)} = 4.080$, $p = 0.0444$; stress index: $F_{(1, 276)} = 31.47$, $p < 0.0001$) and their interaction (SDNN: $F_{(1, 242)} = 10.93$, $p = 0.0011$; stress index: $F_{(1, 240)} = 25.05$, $p < 0.0001$). The SDNNI fluctuated following the effect of body size ($F_{(1, 271)} = 37.31$, $p < 0.0001$) and the interaction between bedding material and body size ($F_{(1, 226)} = 9.243$, $p = 0.0026$). On the contrary, the RMSSD was modified as the effects of bedding materials ($F_{(1, 240)} = 11.85$, $p = 0.0007$) and the interaction between bedding materials and body size ($F_{(1, 240)} = 11.42$, $p = 0.0008$) (Figure 2).

HR decreased between 03.00 and 05.00 in comparison to 07.00–09.00, 12.00–13.00 and 18.00–19.00 in the H2 group on straw bedding ($p < 0.05$–0.01); no within-group differences were observed in the other groups. Likewise, HR in the H2 group on straw was higher than in the H1 group on rice husk at 07.00–09.00 ($p < 0.001$). Moreover, HR in the H2 group was higher than in the H1 group when both were housed on rice husk at 07.00–08.00 and 13.00–14.00 ($p < 0.01$). Simultaneously, HR in the H2 group was higher than in the H1 group when both were housed on straw at 08.00–09.00 ($p < 0.05$) (Figure 2A).

RR intervals increased at 03.00–04.00, compared to the value at 18.00–19.00 in the H1 group housed on the straw ($p < 0.05$). Meanwhile, they increased between 03.00–05.00 when compared to the values at 07.00–08.00, 12.00–13.00 and 18.00–19.00 in the H2 group on straw ($p < 0.05$ for all comparison pairs). No within-group differences were detected in the H1 and H2 groups when they were housed on rice husk. The RR intervals of the H1 group housed on rice husk were greater than those of the H2 group on straw at 07.00–09.00, 13.00–16.00 and 22.00–23.00 ($p < 0.01$–0.0001). Those in the H1 group were higher than in the H2 group when they were housed on rice husk at 07.00–08.00, 13.00–15.00 and 22.00–23.00 ($p < 0.05$–0.001) (Figure 2B).

Despite fluctuations, there were no within- or between-group differences in the time domain results, including SDNN, SDNNI, RMSSD and the stress index (Figures 2C–F).

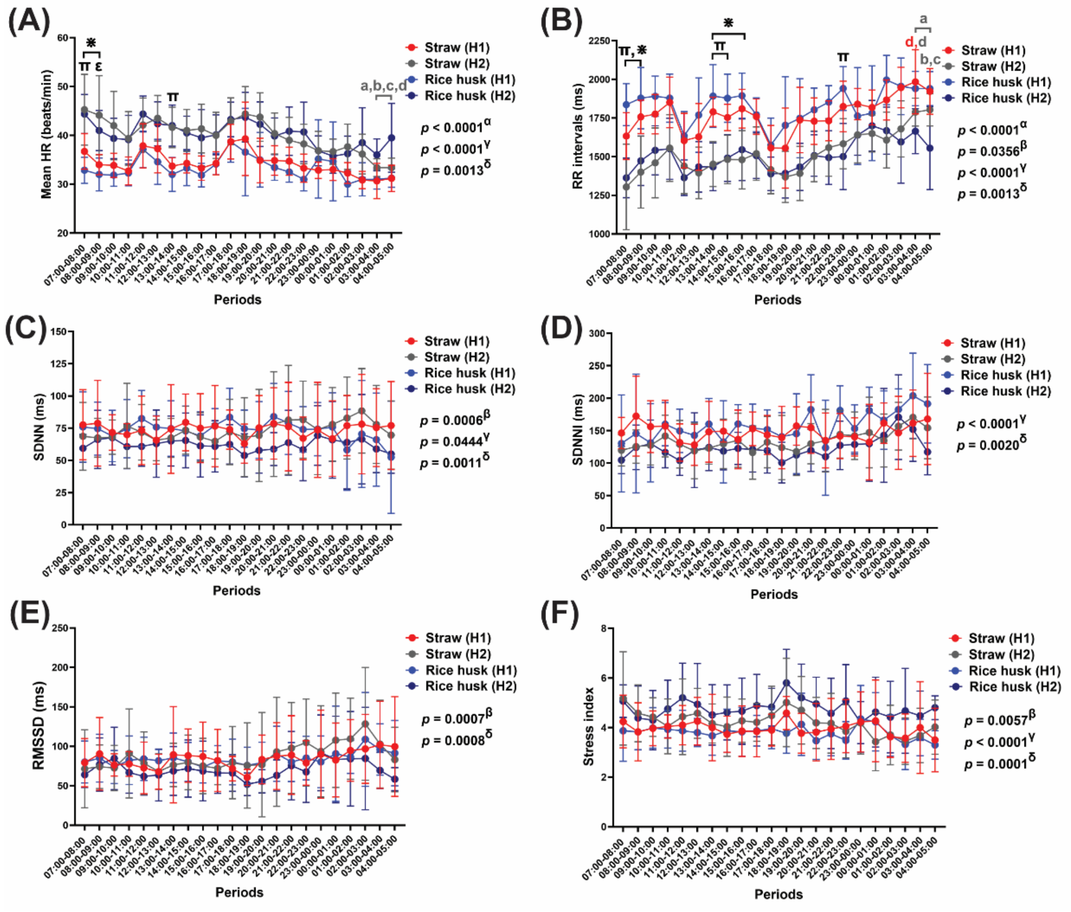

Figure 2. Mean heart rate (HR) (**A**), beat-to-beat (RR) intervals (**B**), standard deviation of normal-to-normal RR intervals (SDNN) (**C**), mean of the standard deviations of normal-to-normal RR intervals in 5 min segments (SDNNI) (**D**), square root of the mean squared differences between successive RR intervals (RMSSD) (**E**) and stress index (**F**) for horses of different sizes housed on straw or rice husk. **α**, **β**, **γ** and **δ** indicate the effects of time, bedding materials, body size, and the interaction between bedding material and body size, respectively. **H1:** horses of the first group, **H2:** horses of the second group. **ε**, ⋇ and **π** indicate significant differences between straw (H1) vs straw (H2), straw (H2) vs rice husk (H1), and rice husk (H1) vs rice husk (H2) at similar time points. **a**, **b**, **c** and **d** indicate within-group differences from values at 07.00–08.00, 08.00–09.00, 12.00–13.00 and 18.00–19.00, respectively. Notably, the colours of the letters align with the line colour of each group.

3.3.2. Frequency Domain Analysis

Bedding materials and body size influenced the LF band (bedding materials: $F_{(1, 240)} = 4.971$, p = 0.0267; body size: $F_{(1, 276)} = 10.21$, p = 0.0016) and the HF band (bedding materials: $F_{(1, 240)} = 5.437$, p = 0.0205; body size: $F_{(1, 276)} = 9.951$, p = 0.0018). By contrast, bedding materials ($F_{(1, 240)} = 4.542$, p = 0.0341), body size ($F_{(1, 276)} = 6.808$, p = 0.0096) and their interaction ($F_{(1, 240)} = 4.329$, p = 0.0385) affected the LF/HF ratio. Moreover, the effects of independent body size ($F_{(1, 276)} = 79.64$, $p < 0.0001$) and the interaction of body size and bedding material ($F_{(1, 240)} = 10.58$, $p = 0.0013$) on RESP expression were observed (Figure 3). As with the time domain results, no within- or between-group differences were observed in the LF band, HF band, LF/HF ratio or RESP (Figures 3A–D).

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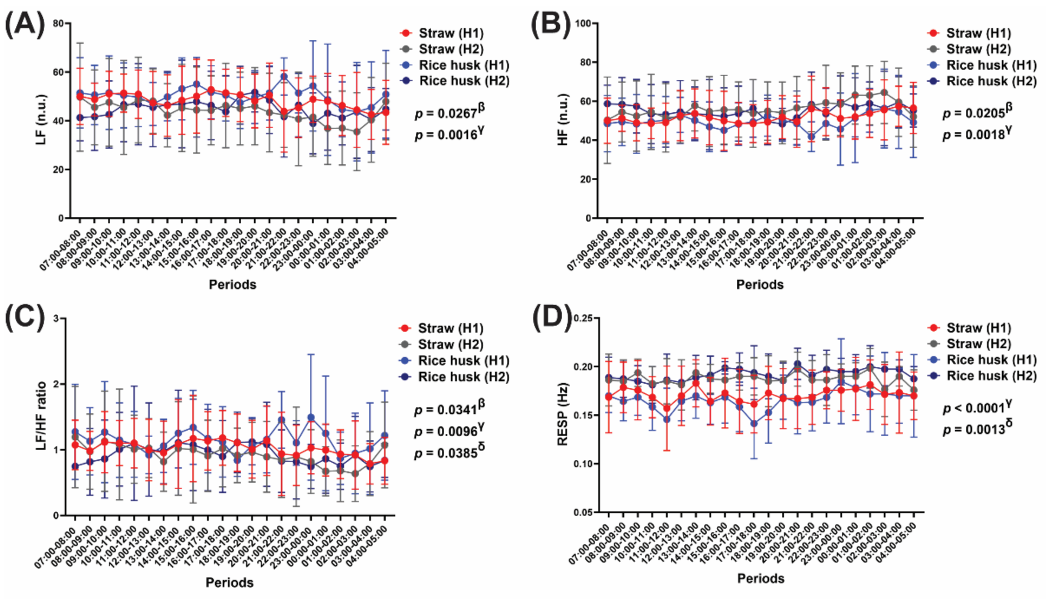

Figure 3. Low-frequency (LF) band (**A**), high-frequency (HF) band (**B**), LF/HF ratio (**C**) and respiratory rate estimated from RR intervals (**D**) in horses of different sizes housed on straw or rice husk bedding. β, γ and δ indicate the effects of bedding material, body size and the interaction between bedding material and body size, respectively. **H1**: horses of the first group; **H2**: horses of the second group.

3.3.2. Non-Linear Analysis

SD1 modulation was affected by bedding materials ($F_{(1, 218)} = 12.71$, $p = 0.0004$) and the interaction between bedding material and body size ($F_{(1, 218)} = 9.904$, $p = 0.0019$). By contrast, bedding material ($F_{(1, 239)} = 7.963$, $p = 0.0052$), body size ($F_{(1, 276)} = 9.076$, $p = 0.0028$), and their interaction ($F_{(1, 239)} = 25.14$, $p < 0.0001$) influenced SD2 fluctuation. The SD2/SD1 ratio was influenced by the main time effect ($F_{(21, 276)} = 2.311$, $p = 0.0012$). Unexpectedly, there were no within- or between-group differences in these non-linear variables (Figures 4A–C).

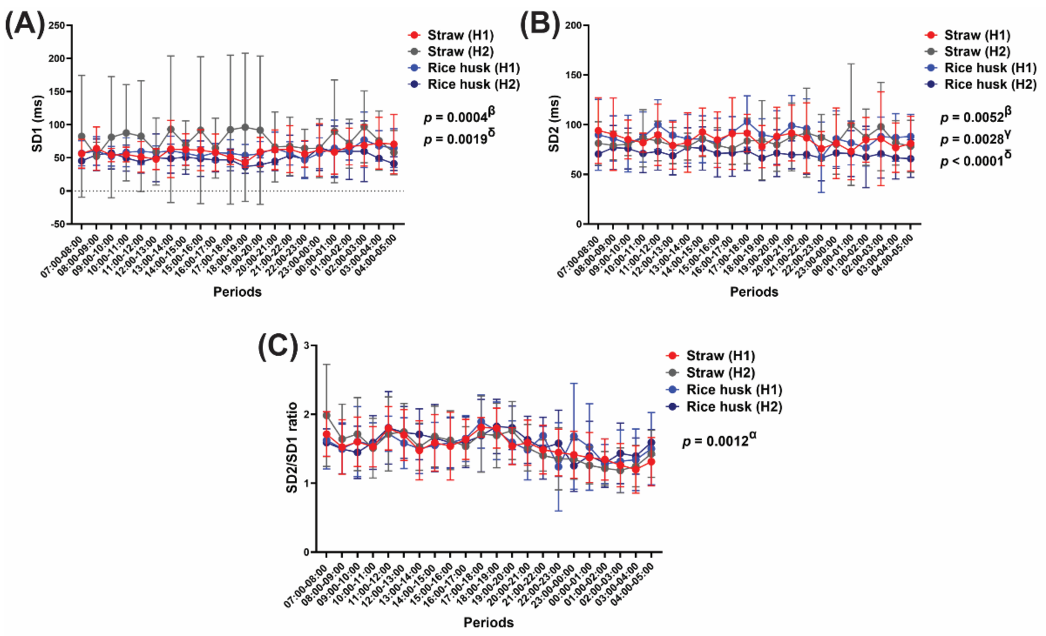

Figure 4. Standard deviation in the Poincaré plot perpendicular to the line of identity (SD1) (**A**), standard deviation in the Poincaré plot along the line of identity (SD2) (**B**) and SD2/SD1 ratio (**C**) in horses of different sizes housed on straw or rice husk. α, β, γ and δ indicate the effects of time, bedding material, body size and the

interaction between bedding material and body size, respectively. **H1**: horses of the first group; **H2**: horses of the second group.

4. Discussion

This study examined how bedding materials affect horses in individual box housing across different body sizes. The key results were as follows: 1) there were no significant differences in VOC and CO_2 emissions between the two bedding types; however, airborne particle concentrations were much higher with rice husk than with straw; 2) behavioural scores, HR and HRV metrics were influenced by both bedding type and horse size; 3) RR intervals increased, and HR decreased only in horses on straw during the night; 4) horses with a height above the upper bar of the front gate of the box tended to show lower HR and higher RR intervals during housing, regardless of bedding type; 5) although bedding type and body size affected behavioural scores and several HRV metrics, no significant within- or between-group differences were observed. These findings suggest that while both bedding materials and body size affect stress responses, short-term individual box housing may not provide sufficient stressors to reveal differences.

In this study, stable microenvironmental factors (e.g. RH, AT, and VOC and CO_2 emissions) were similar between stables using straw and those using rice husk bedding. However, airborne particle concentrations were higher in stables with rice husk compared to those with straw. These results partially align with previous research indicating that bedding material type influences dust and air contamination levels [7,46]. Elevated airborne particulates are associated with respiratory problems and may compromise the health of individual loose boxed horses [5,6,47]. Therefore, the higher concentration of airborne particles observed in rice husk stables could pose a greater risk of respiratory issues. Nevertheless, the concentrations of airborne particles—including PM1, PM2.5 and PM5—ranged from approximately 0.002 to 0.006 mg/m^3 (converted from 1.9–5.8 μg/m^3) in straw-bedded stables and 0.007 to 0.018 mg/m^3 (converted from 7.3–18.4 μg/m^3) in rice husk-bedded stables. These values are substantially lower than those reported in temperate climates, which range from 0.33–1.42 mg/m^3 [48] to 0.68–1.07 mg/m^3 [49]. This discrepancy may be attributed to differences in stable architecture and management practices between temperate and tropical climates. In temperate regions, stables are typically fully enclosed, with scheduled window openings to prevent draughts and maintain an optimal microclimate [50,51]. Notably, such designs may restrict ventilation and allow dust to accumulate. In contrast, stables in tropical climates are often partially covered, with barn gates left open during the day and, in some cases, the absence of permanent external walls [41,52]. These features facilitate greater airflow and reduce dust accumulation during the day. Although dust emissions from rice husk bedding remained below the recommended animal hygiene threshold of 3 mg/m^3 [53], its higher dust output compared to straw and the associated potential risk of respiratory disorders should be considered when selecting rice husk for long-term bedding.

Horses acclimated to the new bedding provided each day and exhibited preferences for specific bedding materials during the initial hours of the study. This observation was supported by variations in HR among groups during the first few hours of the experiment, which partially aligns with previous research reporting increased HR in horses exposed to novel objects [23,54]. The increased RR interval observed overnight in horses from both groups housed on straw bedding suggests increased HRV and a shift toward vagal tone, indicating that straw may be a more favourable bedding option. Likewise, taller horses, defined as those exceeding the front door height of each box, exhibited higher RR intervals compared to shorter horses. Several factors may have contributed to these outcomes. Horses taller than the front gate of their box could extend their heads and necks beyond the door, potentially accessing a more favourable microenvironment outside the box. In contrast, shorter horses remained confined within their boxes, which may have resulted in greater exposure to residual air pollution. Additionally, within the boxes, horses of different heights were likely exposed to varying microenvironmental conditions. RH, which is influenced by differences in moisture content, can vary with height above the floor in individual loose boxes with specific bedding types [15]. Consequently, horses shorter than their box's front gate likely experienced greater discomfort compared to those taller than the front gate. Despite the influence of individual factors and their interactions with autonomic responses, no significant differences were observed within or between groups in several HRV metrics. Thus, it is plausible that the stress levels experienced during the 24-hour housing period were likely insufficient to induce notable changes in autonomic responses. In light of this, further research is warranted to investigate the long-term effects of straw and rice husk beddings on autonomic regulation in horses of varying sizes.

Despite its value, this study presents several limitations. First, measurements of the stable microenvironment were conducted on the stable aisle to ensure safety during device setup. As a result, these measurements may not accurately represent the microenvironment within individual loose boxes. Second, the relatively short study duration may have contributed to the absence of observed differences in behavioural scores and HRV metrics both within and between groups, despite the potential effects of bedding material and body size. Further research with an extended duration is necessary to confirm the effects of bedding materials and body size on these stress responses in horses. Finally, the temporal disconnection between the HR sensor and the sports watch was unavoidable, particularly when horses were recumbent. Notably, this limitation may have influenced the HRV analyses. Therefore, the findings should be interpreted with caution.

5. Conclusions

Bedding material and body size influenced stress responses in horses housed individually in single-box stables. Overall, horses appeared more comfortable when housed on straw bedding. Furthermore, horses whose height exceeded the upper bar of their box's front gate were expected to experience less stress than those whose height fell below it. Nonetheless, these outcomes require validation through additional long-term research. The findings presented here provide insights into the stress responses of horses with varying body sizes utilising different bedding materials, which may inform stable management practices and serve as a foundation for future research on housing management for horses.

Supplementary Materials: Supporting information can be downloaded at: https://www.doi.org/10.6084/m9.figshare.31452076; Figure S1: Recumbent time on different bedding materials in horses of varying body sizes.

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Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
CSV	Comma-separated values
HF	High-frequency band
HR	Heart rate
H1	Horses of the first group
H2	Horses of the second group
HRM	Heart rate monitoring
HRV	Heart rate variability
LF	Low-frequency band
LSD	Least significant difference
RESP	Respiratory rate estimated from RR intervals
RMSSD	Square root of the mean squared differences between successive RR intervals
RR	Beat-to-beat
SDNN	Standard deviation of normal-to-normal RR intervals
SDNNI	Mean of the standard deviations of normal-to-normal RR intervals in 5-min segments
SD1	Standard deviation in the Poincaré plot perpendicular to the line of identity
SD2	Standard deviation in the Poincaré plot along the line of identity

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