Competition horses housed in single stalls (2nd communication): Effects of free exercise on the behaviour in the stable, the behaviour during training and the degree of stress

Hanna Werhahn*, M. Sc.; Engel F. Hessel, PD Dr.; Herman F. A. Van den Weghe, Prof. Dr. Ir.

Department of Animal Sciences, Division: Process Engineering, Georg-August University of Goettingen, Universitaetsstr. 7, D-49377 Vechta, Germany

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* Corresponding author. Universitaetsstr. 7, D-49377 Vechta, Germany. Tel.: 0049-551-3922176. Fax: 0049-4441-15448. E-mail address: hwerhah@gwdg.de (H. Werhahn).

ABSTRACT

Although housing horses in single stalls limits their natural behaviour to a great extent, this housing system is widespread in Germany, especially for competition horses. To improve the welfare of this system, free exercise on pastures or paddocks is deemed suitable, but it is also feared because of injuries and decreased willingness/motivation to perform. In the present study, three treatments were investigated with regard to their effect on the behaviour of six competition horses in the stable and during training, and on their degree of stress: daily training without free exercise (NT), solitary turnout for two hours after training (ST) and two-hour turnout in groups of two after training (GT). The horses' behaviour in the stable was continuously analysed via video recordings (2 pm to 6 am) on three days at the end of each treatment. The degree of stress was evaluated daily by heart rate variability (HRV) at rest. The behaviour during training was evaluated by a questionnaire answered by the riders and the distance covered during training was measured by GPS. When no turnout was allowed (NT), the horses showed less lying in the stable compared to the treatments with turnout. HRV measurements resulted in great individual differences but generally there was a higher degree of stress shown with the treatment NT according to the parameters SDNN, RMSSD and LF/HF. The willingness to perform was evaluated as being slightly better in the treatments with turnout than in the treatment without turnout.

Keywords: horse, turnout, single stall, behaviour, HRV.

1. Introduction

Free-ranging horses spend up to 16 hours a day foraging, which generally happens with a slow and steady walk [1, 2]. They also spend their whole lives in (family) bands with a constant social hierarchy [3, 4]. Although housing horses in single stalls limits their natural behaviour to a great extent (especially exercise and social behaviour), this housing system is widespread in Germany, especially for competition horses. Free exercise on pastures or dry lots can improve the degree of animal welfare in the system [5], but allowing free exercise is not taken for granted by many horse keepers [6]. Particularly competition horses can be worth a lot of money, so the most frequent reason for not allowing free exercise is the risk of injury [7, 8]. Some riders also fear that free exercise might decrease performance in sport. Nevertheless, preventing horses' natural requirement for exercise most likely poses a stressful situation for them.

The conventional way to get information on animal welfare is the analysis of animal behaviour. In the absence of illness, behaviour patterns (regarding locomotion, social interactions, ingestion, resting, etc.) are supposed to be performed as closely as possible to an animal's behaviour under natural conditions [9]. Deviations from natural behaviour or from the normal behaviour of individuals due to changes in the environment are able to reveal valuable indications of animal welfare [10]. In the last fifteen years, measuring heart rate variability (HRV) has become an accepted method for evaluating the degree of stress in organisms caused by disease or physical or mental strain [11]. Developed in the field of human medicine, the technique has also been used to evaluate stress in animals in the recent years, amongst others in horses [12, 13, 14]. The analysis of HRV is based on the irregular time intervals between consecutive heart beats regulated by the autonomic nervous system (ANS). The primary pulse generator for heart beats, the sinoatrial node, is under control of the parasympathetic (vagal; high frequency impulse) and sympathetic (low frequency impulse) nervous systems. At rest, both branches of the ANS are active, with a dominance of vagal regulation. Irregular time intervals between consecutive heart beats (great HRV) characterise the physiological and psychological flexibility of an organism and is, therefore, an indicator of its ability to respond to stress. Due to the great importance of stress in animal welfare, HRV is an adequate tool to evaluate the effect of defined situations on an animal's well-being [11].

The aim of the present study was a systematic investigation of the effects of the prevention of free exercise and social interactions on the behaviour in the stable, during turnout and training, and on the degree of stress in competition horses under conditions of practice. Therefore, multiple parameters of behaviour and stress measured in six horses housed in single stalls were analysed in three treatments varying in the configuration of free exercise. Changes between the treatments *within* the horses were in the focus of the study instead of differences *between* the horses. To compare different horses to each other is of little information, because of very individual experiences in upbringing, housing, treatment, training etc. until becoming a competition horse. As they are widely used in Germany, the following management practices were selected: daily training without additional free exercise (no turnout = NT), free exercise in groups of two horses after training (group turnout = GT) and free exercise separately after training (solitary turnout = ST). The present manuscript focuses on the degree of stress and behaviour in the stable and during training. The effect of the treatments is evaluated with regard to animal welfare to provide indications on how the single-stall housing system may be improved.

2. Material and methods

2.1. Location of the study

The research was carried out in a training and competition yard in Noerten-Hardenberg (County of Goettingen, Lower Saxony, Germany) in the period from 07th June until 18th July, 2010. The stable contained 16 single stalls (3.00 m x 3.50 m) in two rows with an aisle (width: 4.00 m) in the middle (Fig. 1). The stalls were 0.20 m deeper than the aisle and were separated by 1.30 m hardwood walls with 1.00 m vertical lattice bars on top (distance between bars: 6.00 cm). The fronts had sliding doors (width: 1.50 m) consisting of the same material. The lattice bars of the doors had windows (width: 0.70 m) allowing the horses to put their heads into the aisle. These windows were open all the time during the investigation. The brick walls of the stable formed the back of the stalls. The stable ceiling height was 3.00 m. Along the southern side of the stable, 17 windows (0.70 m x 1.20 m) at a height of 1.50 m and with a distance of 1.20 m provided light and ventilation (windows were left slightly open). Two doors (3.00 m x 3.00 m) at the ends of the aisle were open during the day and closed at night between 10 pm and 6 am. Each stall was equipped with a feeding trough for concentrates and an automatic drinking bowl. Six stalls situated next to each other were used for the investigation (Fig. 1). All the stalls in the stable had horses in them and the horse next to Horse 6 did not change during the investigation.

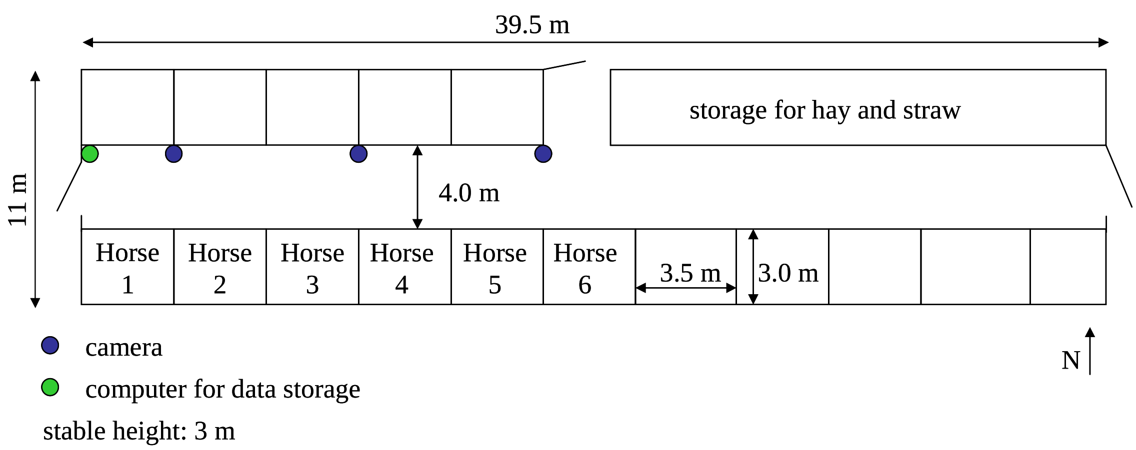

Fig. 1. Outline of the stable including the positions of the experimental stalls (Horses 1-6), cameras and computer.

The pasture was located about 300 m southwest of the stable. Its dimensions were 60 m x 90 m. For the investigation, the area was divided into three paddocks of 30 m x 60 m using electrical fences. This size gave the horses enough space for free exercise in walk, trot and canter (German guidelines advise at least 150 m^2 for two horses [15]).

2.2. Animals

Six German Warmblood Horses (height between 1.65 m and 1.75 m; weight between 600 kg and 650 kg) were used for the investigation. All six horses were schooled in dressage and show jumping, and were deployed in competitions in one of these disciplines at pre-novice to advanced class. Horse 1 (H1) was a four-year-old Hanoverian mare. Horse 2 (H2) was a six-year-old Hanoverian gelding. Horse 3 (H3) was a seven-year-old Holstein gelding. Horse 4 (H4) was a ten-year-old Hanoverian gelding. Horse 5 (H5) was a four-year-old Hanoverian gelding. Horse 6 (H6) was a six-year-old Hanoverian gelding. These horses were selected because they were of similar age, available for the whole study (not for sale within the time of the study), trained by one rider, trained at the same time of day during the whole study and their owners agreed that they passed all three treatments.

All six horses were moved into their experimental stalls two weeks before the investigation was started. They were accommodated in six stalls next to each other and the experimental group partners were kept in neighbouring stalls. They were used to having free exercise three to four times a week on pasture or paddock (both on their own and with their experimental group partner for one to two hours). The riders were asked to retain their way of training during the investigation according to their normal routine. As the way of training was not standardised duration varied (between 21 and 65 minutes).

2.3. Bedding materials and feed

The experimental stalls were strewn with wheat straw. New straw (approximately 10 kg per stall) was given every morning after feeding and the stalls were mucked out only once every four weeks.

Oats and muesli (Torneo Muesli, onOvo GmbH, Hann. Muenden, Germany) were fed three times a day (06:00 am, 12:00 am, 04:00 pm). At 12:00 am, 50 g of mineral feed (Torneo Mineral, onOvo GmbH, Hann. Muenden, Germany) was added to the concentrates. H1 received 1.3 kg muesli three times a day. All other horses received 1 kg of oats at 6:00 am and 4:00 pm and 0.5 kg of oats and 0.7 kg of muesli at 12:00 am. Hay was given in the morning and in the afternoon before the concentrates. H3 received 4 kg of hay at any one time and while all other horses received 5 kg. The amount of feed, feeding quality and type remained constant over the course of the experiments. Water was available in the stable at all times for each individual horse.

2.4. Measurement techniques

Behaviour in the stable

Three video cameras (Panasonic CCTV WV-BP 310, Panasonic Corporation, Kadoma, Osaka, Japan) were fixed underneath the ceiling opposite to the experimental stalls (Fig. 1), so each camera recorded the behaviour of two horses. To allow recording at night, infrared light sources were fixed underneath the ceiling within each stall. The videos were recorded in digital format using a Noldus MPEG Recorder 2.1 (Noldus Information Technology, Wageningen, The Netherlands). A computer to record data (Dell Precision T3400 Workstation, Dell Inc., Round Rock, Texas, USA) and a monitor screen to control recordings were located in a metal box within the stable (Fig. 1). The recordings were stored daily on external hard disks. Subsequently, the data was analysed using the Observer XT 9.0 (Noldus Information Technology, Wageningen, The Netherlands).

Heart rate variability

Six Polar Equine RS800CX Science devices (Polar Electro Oy, Kempele, Finland) were used to measure the HRV of the horses at rest. The devices consist of a measuring girth containing two electrodes, a sender fixed on the girth, and a receiver. The receiver was located in a pocket fixed on an ordinary blanket girth. Each horse was equipped with a measuring girth and a blanket girth carrying the receiver daily from about 9:00 pm until 8:00 am. For better conduction of the electric potential of the heart action from the body surface, the horse's coat underneath the electrodes was wetted using ultrasound gel. The girths were padded in the area of the withers to avoid physical irritation. After measurement, the data were read out and stored using the software program Polar ProTrainer Equine Edition (Polar Electro Oy, Kempele, Finland).

Distance Covered during training

Four global positioning system (GPS) devices — Garmin Forerunner 205 (Garmin, Olathe, Kansas, USA) — were used to record the distance covered and the duration of training. The devices were fixed around the riders' wrists. The measurements were started when the horse and rider left the stable for training and stopped when they returned to the stable. After training, the data was read out and stored using the software program Garmin Training Center (Garmin, Olathe, Kansas, USA).

Behaviour during training

The riders registered the horses' behaviour during training with the aid of a questionnaire. The horses' behaviour during the working phase of training (answer possibilities: particularly quiet, rather quiet, normal, rather agitated, particularly agitated), its concentration (answer possibilities: particularly good, rather good, normal, rather bad, particularly bad), contumacy (bucking, rearing, working against the rider etc.; answer possibilities: particularly little, rather little, normal, rather intense, particularly intense) and the horse's urge to move (answer possibilities: particularly low, rather low, normal, rather strong, particularly strong) had to be described. The riders were asked to compare each horse's behaviour during training in each of the respective treatments to the horse's previous behaviour during training. For organizational reasons it was not possible to blind the riders to the treatments. Training was carried out by three experienced riders (one rider trained H1, H2, H3 and H5; one rider trained H4; one rider trained H6), who also presented the horses at competitions. To retain the experimental rhythm of the day, the riders sometimes had to swap horses (e.g. if a rider had no time to ride in the morning one of the other riders trained the horse). To avoid differences in evaluation within the horses caused by different riders, only evaluations of the "main" rider of each horse were considered in the final analysis.

Temperature and relative humidity

During the experiments, two Tinytag Plus 2 loggers (Gemini Data Loggers Ltd., Chichester, UK) recorded air temperature and relative humidity hourly outside and within the stable.

2.5. Experimental design

The study was carried out under conditions of practice, because results of absolutely standardized conditions in relation to this topic cannot be transferred to practice and so are of little information. The whole investigation took six weeks and was divided into three two-week-periods. The experimental horses were also arranged into three groups (Group 1 = H1 and H2; Group 2 = H3 and H4; Group 3 = H5 and H6). During the investigation, all six horses passed through each of the three treatments; each treatment lasted for two weeks. In the first treatment, free exercise was allowed after training for two hours in groups of two horses (group turnout = GT). In the second treatment, solitary turnout (one horse per pasture) was allowed for two hours after training (solitary turnout = ST), while in the third treatment, no free exercise was allowed in addition to training (no turnout = NT). Group 1 started with ST then was subjected to GT and then NT. Group 2 was given NT at first then ST and then GT. Group 3 started with GT then was subjected to NT and then ST (Table 1).

Table 1: Experimental design.

Period of time	Group 1 (Horses 1+2)	Group 2 (Horses 3+4)	Group 3 (Horses 5+6)
Period 1 (weeks 1+2)	ST	NT	GT
Period 2 (weeks 3+4)	GT	ST	NT
Period 3 (weeks 5+6)	NT	GT	ST

NT = no turnout. ST = solitary turnout. GT = group turnout.

Training was carried out according to the horses' individual training schedule between 8:00 and 11:00 am with the aim to achieve better performance at competition. Training was not standardised, because this would have been against practice, put the horses behind their individual training pensum and so caused a financial loss for the owners if offered for sale. After training, between 11:00 am and 2:00 pm, free exercise on pasture was allowed according to the respective treatment. In the treatment with turnout, the midday feeding was given after turnout. Depending on the particular time of training, the horses spent 30 to 120 minutes within their stalls between training and turnout.

2.6. Data collection

The data was collected daily from Monday to Friday. No data was collected on Saturdays and Sundays, because most of the experimental horses were taking part in competitions on one of the days within the region around the stable (so they did not stay over night). On days of competition, the free exercise was given according to the treatment the horses were being subjected to at the time. The exact time of day for training and turnout sometimes differed from the experimental times on Mondays to Fridays. The day after competition was always excluded of the analysis.

Behaviour in the stable

Video recordings of the horses' behaviour in the stalls were analysed on three consecutive days of each period of the investigation. Because the horses were supposed to be acclimatized to the treatment, the days at the end of the second week were chosen for behaviour observation. The observations were carried out continuously by one person (who also carried out the experiment) between 2.00 pm and 6.00 am the next morning (16 h), while all the horses were in their stalls. *Frequency*, *mean duration* per appearance and *total duration* within the observation time were documented for each recorded type of behaviour.

The observed behaviours were grouped into five categories: "eating" (hay and concentrates), "standing alert" (watching the surroundings attentively or nervously), "occupation" (investigating/eating bedding material, drinking, investigating stall equipment like trough, grits, drinking bowl, etc.), "dozing" (stand quietly, one hind leg relaxed) and "lying" (sternal and lateral recumbency). Additionally, the *frequency* of "locomotion" (more than three steps without interruption) was recorded.

Heart rate variability

To estimate HRV, the inter-beat-interval data of four (the least common denominator of lying periods per night) five-minute intervals from different periods of recumbency between 0 am and 5 am were selected and analysed for each horse and each night. The five-minute intervals were chosen in the middle of the periods of recumbency (mean duration: 29.07 min.) when the horse was lying quietly.

To ensure a correct interpretation of the measurements, it is advised to analyse more than one parameter [16]. The following HRV variables were quantified in the present study: two time domain parameters, SDNN (standard deviation of inter-beat-intervals in ms; indicates general variability) and RMSSD (square root of the mean of the sum of the squares of differences between successive inter-beat-intervals in ms; indicates vagal activity), and one frequency domain parameter LF/HF (ratio between low frequency

(LF, ms²) and high frequency (HF, ms²) content of the IBI signal; indicates sympathovagal balance of regulation). The normal values for LF/HF range between 1.5 and 2.0. The parameters SDNN and RMSSD vary widely so normal values have not been defined [16].

The software program Kubios HRV (Biosignal Analysis and Medical Imaging Group, Kuopio, Finland) was used to calculate the chosen parameters. Artefact correction was adjusted at 0.5 sec (i.e. intervals deviating more than 50% from the previous interval are deleted) and interpolation rate at 2 Hz. The frequency band ranges were set from 0.01 to 0.07 Hz for LF and from 0.07 to 0.6 Hz for HF according to the recommendations of Kuwahara et al. [17] for HRV analysis in horses. The mean values of the aforementioned parameters were calculated per horse and night and these were used for statistical analysis.

Behaviour and distance covered during training

To create more concrete statements about the horses' willingness to perform out of the differentiated answers given in the questionnaire, the riders' answers about the horses' behaviour during the working phase, concentration and contumacy were summarized into the feature "willingness to perform". In addition to the questionnaire, the distance covered during training and the total duration of training was documented by the GPS devices. This was conduced to get an indication on the amount of exercise provided by training.

Temperature and relative humidity

The air temperature and relative humidity values were averaged according to the focus of the analysis. For the video observations, the values measured within the stable were averaged over the observation time (2:00 pm to 6:00 am). For the HRV data, the temperature and relative humidity measured within the stable were averaged over night (9:00 pm to 6:00 am). For the GPS data and behaviour during training, the values measured outside the stable were averaged over the period of training (8:00 to 11:00 am).

2.6. Statistical analysis

The statistical evaluation of the data was carried out with the software program SAS 9.1 (SAS Inst. Inc., Cary NC, USA).

The video observations resulted in values for the parameters *total duration*, *frequency* and *mean duration per appearance* for the behaviours "eating", "occupation", "standing alert", "dozing" and "lying". As "locomotion" only occurred briefly, just *frequency* was recorded. If the data sets were not available in Gaussian distributions, they were transformed by taking the logarithm. The analysis of variance was computed by GLM procedure (general linear model) considering the random effect of the horse, the fixed effect of the treatment and the interaction between horse and treatment (for all behaviours except for "locomotion"). The fixed effect of the period had no significant influence on the variables and so was not included in the model. For the behaviour “locomotion”, the random effect of the horse and the fixed effects of the period and of the treatment within the period were considered. The interaction between horse and treatment had no significant influence on the variable and so was not included in the model. For all the behaviours, temperature and relative humidity were taken into account as co-variables. In total, the video observations created 54 data sets (three days in three treatments for six horses).

The HRV was measured 180 times (6 horses on 30 days) in total and resulted in 131 analysable data sets (19 measurements in ST, 13 in GT and 17 in NT could not be analysed because of technical problems). To create a Gaussian distribution, the logarithm was taken of the SDNN data. For the parameters RMSSD and LF/HF, the logarithm was taken twice to generate Gaussian distributions. The GLM procedure was used utilising the same model described above for the video analysis (model for all the behaviours except for “locomotion”). The coefficient of correlation (Pearson product-moment correlation coefficient, r; procedure CORR PEARSON) was computed between SDNN, RMSSD, LF/HF, temperature and relative humidity.

The parameters “distance covered”, “average speed” and “duration of training” were supposed to be measured 180 times (6 horses on 30 days). Because of the reasons mentioned in section 2.4 and technical problems, only 118 measurements could be analysed (ST = 38, GT = 41, NT = 39; 4-9 measurements per horse and treatment). The logarithm was taken of all three data sets to create Gaussian distributions. Again, the GLM was computed utilising the model described for the video analysis above (model for all the behaviours except for “locomotion”). The coefficient of correlation (Pearson product-moment correlation coefficient, r; procedure CORR PEARSON) between distance, duration and average speed was computed.

Questioning the riders about the horses’ willingness to perform was supposed to be carried out 180 times (6 horses on 30 days). Because of the reasons mentioned in section 2.4., only 123 questionnaires could be analysed (ST = 39, GT = 42, NT = 42; 5-9 evaluations per horse and treatment). Because of the structure of the data (only five answer possibilities), only the frequency of answers were evaluated. The coefficient of correlation (Spearman's rank correlation coefficient, r; procedure CORR SPEARMAN) between the duration of training, distance covered, willingness to perform, urge to move, temperature and relative humidity was computed.

The significance level in all data sets was $P \leq 0.05$ and P-values between 0.05 and 0.1 were seen as tendencies (t-test). If not explicitly indicated, the first three days of the treatments were not taken into account. This was done to reduce the effect of the previous treatment on the present treatment.

3. Results

During the investigation, the air temperature within the stable (averaged between 2 pm and 6 am) varied between 18 and 28°C and the relative humidity varied between 53 and

67% (Period 1: 18-22°C and 53-63%. Period 2: 24-28°C and 57-63%. Period 3: 22-28°C and 54-67%). Outside the stable, the temperature (averaged between 8 and 11 pm) varied between 12 and 29°C and the relative humidity varied between 51 and 88% (Period 1: 15-22°C and 51-88%. Period 2: 12-27°C and 58-80%. Period 3: 17-29°C and 54-85%).

3.1. Behaviour in the stable

The individual horse had a significant influence ($P < 0.05$) on the parameters *total duration*, *frequency* and *mean duration* per appearance in all behaviours except for the *frequency* of "standing alert".

The *total duration* of the horses' behaviour in the stable during the daily observation time (2 pm to 6 am) is presented in Figure 2. The treatments resulted in significant differences in the time budgets. If turnout was allowed in groups (GT), the *total duration* of "occupation" was significantly shorter than with NT ($P = 0.0037$). In the treatment ST, the *total duration* of this behaviour was between the other treatments without any significant difference. The behaviour "lying" was performed significantly longer in the treatment GT compared to NT ($P = 0.0165$). The *total duration* of "lying" in ST was again between the other treatments without any significant difference. No significant differences could be found between the treatments in *total duration* of the behaviours "standing alert", "dozing" and "eating". The interaction between horse and treatment had a significant influence on "lying" and "standing alert" ($P \leq 0.05$). The *total duration* of the behaviours was not significantly influenced by either the temperature or relative humidity.

"Occupation" was observed significantly more *frequently* in NT than in GT ($P = 0.0211$) and "lying" was observed significantly more *frequently* in GT than in ST ($P = 0.0239$; Table 2). The *frequency* of the behaviour "lying" was significantly influenced by temperature ($P = 0.0340$): the higher the temperature, the less frequently "lying" was performed. In "locomotion", the period had a significant influence on the *frequency* of appearance. It was performed most frequently during Period 3 (58.42 times). In Period 1, "locomotion" was performed significantly more frequently in NT (45.58 times) than in GT (25.57 times; $P = 0.0195$), while the treatments did not differ significantly during the other periods. All the other behaviours did not show any significant differences in *frequency*. Figure 3 presents the development of the *frequency* of "locomotion". Between 2 and 8 pm, the *frequency* in GT varied to a considerably greater degree and was mostly lower than in ST and NT. From 8 pm onwards, "locomotion" decreased and hardly differed between the treatments. Between 5 and 6 am, "locomotion" increased again in all three treatments.

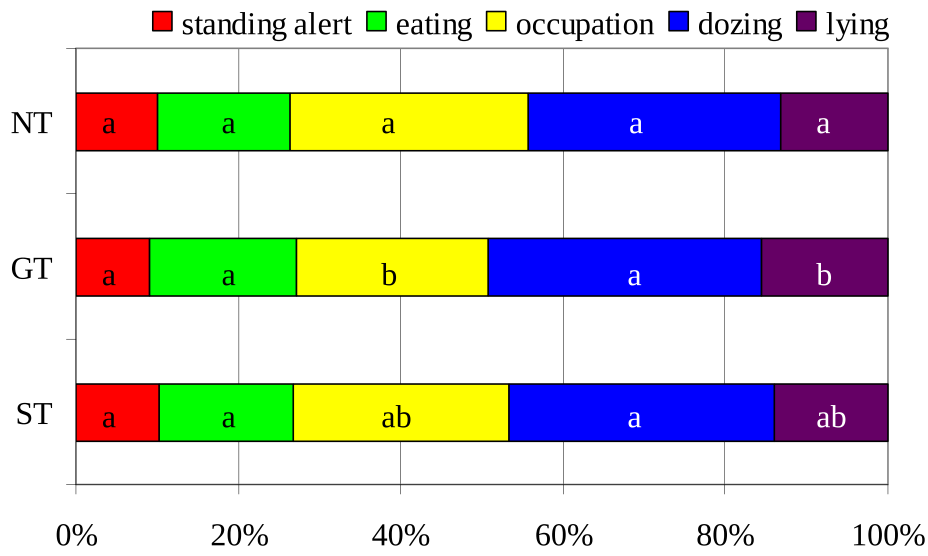

Fig. 2. Percentage of the behaviour patterns within the stall of the total observation time (16 hours: 2 pm to 6 am) subdivided according to treatment [NT = no turnout; ST = solitary turnout; GT = group turnout; n = 54 (18 observations per treatment); a, b = least squares means within a behaviour with different letters are significantly different (*P < 0.05*)].

Table 2: *Frequency* and *mean duration* per appearance (minutes) of the behaviours "occupation" and "lying" in the stable subdivided according to treatment.

Behaviour	Treatment	n	Frequency		Mean Duration	
			LSM	SE	LSM	SE
occupation	ST	18	22.45[ab]	1.23	11.82[a]	0.58
	GT	18	20.69[a]	1.20	11.26[a]	0.55
	NT	18	24,59[b]	1.23	11.95[a]	0.57
lying	ST	18	4.69[a]	0.24	30.46[a]	1.43
	GT	18	5.48[b]	0.23	28.95[a]	1.37
	NT	18	4.94[ab]	0.23	27.78[a]	1.41

NT = no turnout. ST = solitary turnout. GT = group turnout. n = number of values.
LSM = least squares means. SE = standard error. a, b = least squares means within a behaviour with different letters are significantly different (P < 0.05).

The behaviours' *mean duration* per appearance did not differ significantly between the treatments (Table 2). "Lying" was significantly influenced by the relative humidity: the higher the relative humidity, the longer was the *mean duration* of "lying" (*P* = 0.0273).

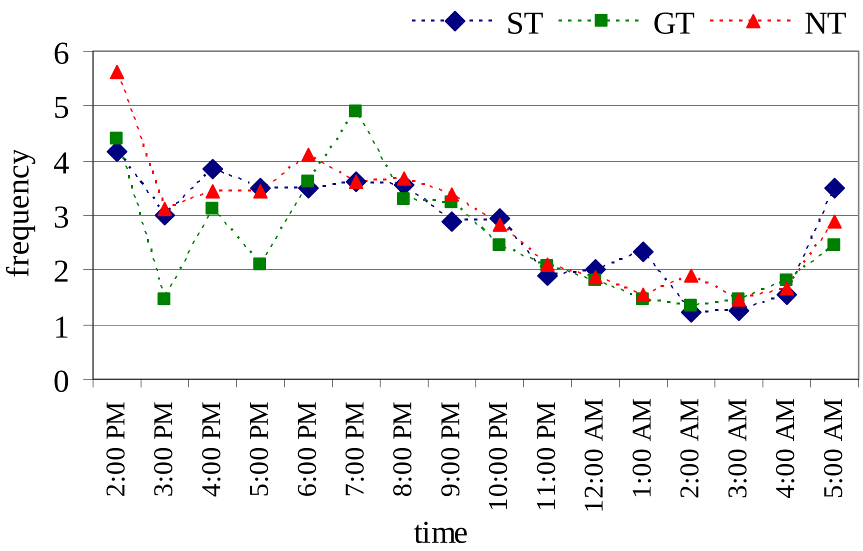

Fig. 3. Mean frequency of "locomotion" in the course of the observation time subdivided according to treatment. [NT = no turnout; ST = solitary turnout; GT = group turnout; n = 54 (18 observation periods per treatment)]

3.2. Heart rate variability (HRV)

The HRV analysis revealed differences in the degree of stress between the treatments (Figure 4). The treatments with turnout did not differ significantly in any of the three parameters (SDNN, RMSSD and LF/HF), and the values describing the greatest degree of stress were found when no turnout (NT) was allowed. The standard deviation of all inter-beat-intervals (SDNN) revealed a significantly lower value (indicating lower HRV and hence a greater degree of stress) in NT compared to turnout in groups (GT; P = 0.0466). The vagal regulatory activity (RMSSD) in the treatment NT tended to be lower (indicating a lower HRV) than in both treatments with turnout (ST: P = 0.0707; GT: P = 0.0632). The values of LF/HF ranged from 2.21 (GT) to 2.56 (NT) and did not show any significant differences. SDNN was significantly higher when the temperature was higher (P = 0.0254), while the LF/HF was significantly higher when the relative humidity was higher (P = 0.0202).

The individual horse had a significant influence on all parameters. H6 showed the lowest value for SDNN when solitary turnout (ST) was allowed (GT: P = 0.0062; NT: P = 0.0528) and had showed considerably higher values in general for LF/HF (averaged by treatments: 4.91 - 5.05) compared to the other horses (0.34 - 3.45). The lowest values for LF/HF were found in H1 and H5 (0.34 - 1.48).

Figure 5 shows the development of the parameters during the course of the treatments. The lowest values for SDNN and RMSSD were found during Week 2 in the treatment without turnout (NT; Days 7-10 for SDNN and Days 6-9 for RMSSD). The values for LF/HF varied to a great extent in all three treatments.

Some other correlations were also found. The higher the temperature (r = 0.27; P = 0.0089) and relative humidity (r = 0.33; P = 0.0017), the higher the LF/HF value was. The higher the value of SDNN, the higher the value of RMSSD was (r = 0.78; P < 0.0001) and the higher the value of RMSSD, the lower the LF/HF was (r = -0.58; P < 0.0001).

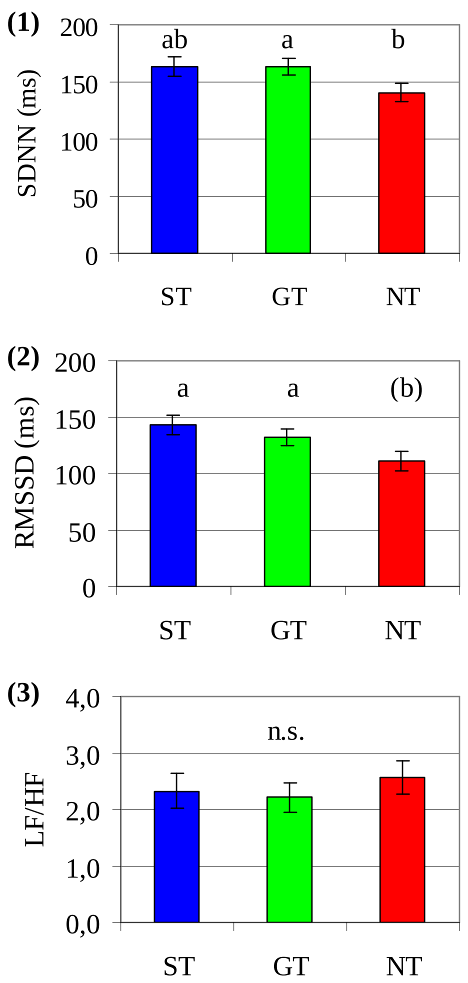

Fig. 4. Least squares means and standard errors of the HRV parameters SDNN (1), RMSSD (2) and LF/HF (3) subdivided according to treatment. [NT = no turnout; ST = solitary turnout; GT = group turnout; n = 90 (ST = 27; GT = 34; NT = 29); n.s. = not significant; a,b = least squares means within a parameter with different letters are significantly different (P < 0.05); (b) = least squares means differ tendencially (1 < P > 0.05)]

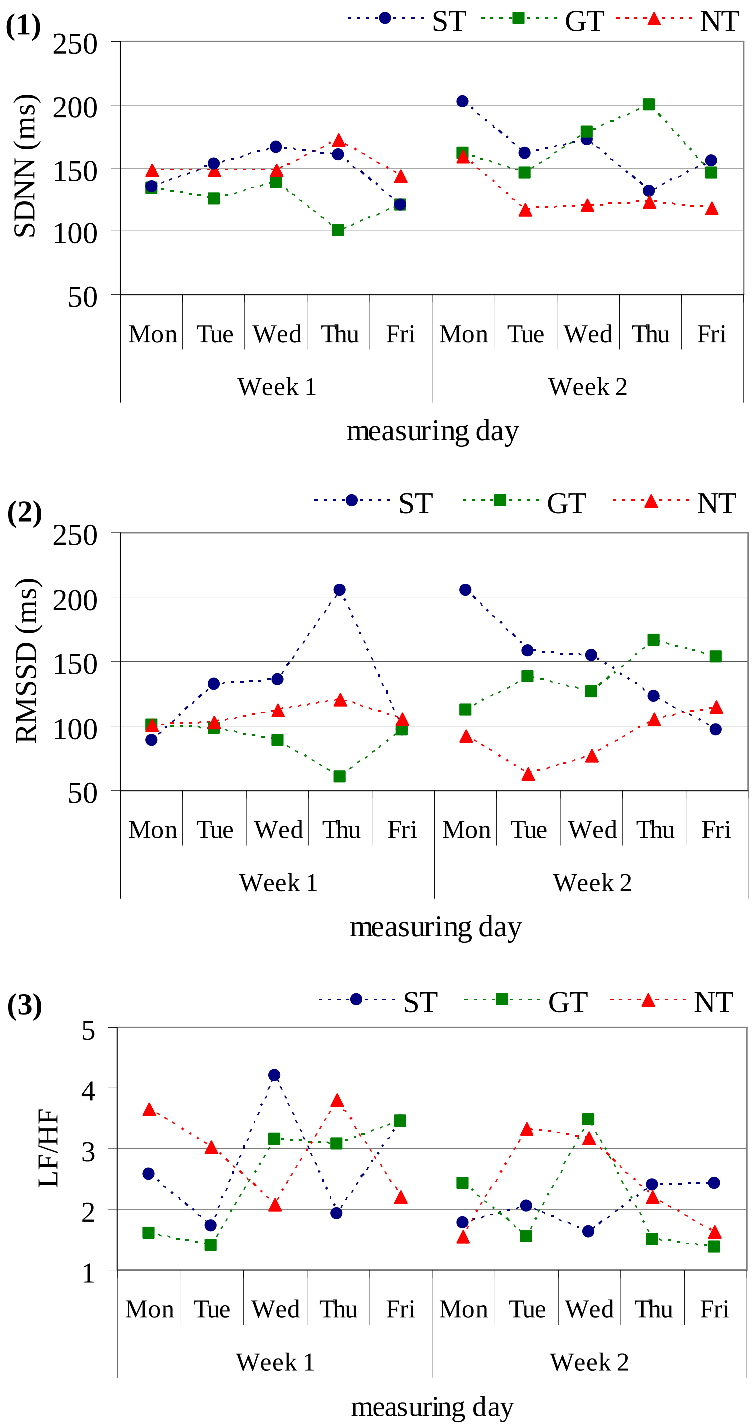

Fig. 5. Mean values of the HRV parameters SDNN (1), RMSSD (2) and LF/HF (3) on the individual days subdivided according to treatment. [ST = solitary turnout; GT = group turnout; NT = no turnout; n = 131 (3-6 measurements per day and treatment)]

3.3. Behaviour and distance covered during training

In Table 3, the horses' willingness to perform, urge to move, the distance covered and the duration of training in the treatments is presented. The treatments were not significantly different in any of these parameters. The individual horse had a significant influence on the distance covered, the average speed and the duration of training ($P < 0.01$). When the temperature was lower, the average speed was higher ($P = 0.0124$), while the distance covered and the duration of training were longer (*P* = 0.0015 and *P* = 0.0486, respectively). When the relative humidity was lower, the distance covered was longer and the average speed was higher (*P* = 0.0273 and *P* = 0.0091, respectively). The duration of training was longest in GT, a little shorter in NT and the shortest in ST. The distance covered was longest in GT and almost the same in ST and NT. The horses' willingness to perform was evaluated as being worst in the treatment NT. The scores in ST and GT hardly differed from each other. The urge to move was highest in the treatment GT; ST and NT hardly differed. The correlation analyses revealed a relationship between duration of training and distance covered (r = 0.74; *P* < 0.0001) and another between distance covered and average speed (r = 0.66; *P* < 0.0001). The willingness to perform was evaluated as being better when the duration of training was longer (r = -3.0; *P* = 0.0058). The urge to move tended to be higher when temperature was lower (r = -0.19; *P* = 0.0840).

Table 3: Means (M) and standard deviations (SD) of the behaviours "willingness to perform" and "urge to move" during training as well as the distance covered and duration of training subdivided according to treatment.

Variable	n	ST		GT		NT	
		M	SD	M	SD	M	SD
Willingness to Perform (Score)	86	2.82	0.79	2.80	0.85	3.01	0.76
Urge to Move (Score)	86	2.68	0.86	2.90	0.80	2.64	0.73
Distance (km)	82	4.28	2.13	4.69	2.05	4.30	1.88
Duration (Minutes)	82	33.21	12.84	39.59	15.47	35.48	9.40

n = number of values. ST = solitary turnout. GT = group turnout. NT = no turnout. Score (willingness to perform/urge to move): 1 = very good/very low, 2 = rather good/rather low, 3 = normal, 4 = rather bad/rather high, 5 = very bad/very high.

Regarding the individual horses, sporadic relationships were found. The urge to move was higher when temperature was lower in H3 (r = -0.57; *P* = 0.0205) and H5 (r = -0.47; *P* = 0.0492). When the relative humidity was higher, the urge to move was higher in H4 (r = 0.50; *P* = 0.0566), H5 (r = 0.64; *P* = 0.0043) and H6 (r = 0.51; *P* = 0.0722). H4 also had a lower average speed when the relative humidity was higher (r = 0.72; *P* = 0.0023). H1 showed better willingness to perform when the urge to move was lower (r = 0.58; *P* = 0.0489).

4. Discussion

4.1. Behaviour in the stable

The *total duration* of the observed behaviours (Fig. 2) show that the horses spent most of their time in the stall "standing alert", "standing occupied" or "dozing". In the present study, these behaviours were performed 66 to 71% of the observation time. Similar results have been documented in earlier studies [1, 18, 19]. The absolute time spent "lying" (2.1 to 2.5 hours) matches the findings of Littlejohn and Munro [20] and is a little less than the results of Kiley-Worthington [2] and Werhahn et al. [21]. This might have been caused by the selected observation time as the horses sometimes showed periods of "lying" after 6 am. The *total duration* of "lying" and "occupation" was influenced by the treatment. These behaviours seem to be complementary. The more "lying" was performed, the less "occupation" was observed and vice versa. The shortest time "lying" and the longest time "occupation" were observed in the treatment NT. In group housing, Caanitz et al. [22] also observed less lying in non-exercised horses compared to exercised horses and interpreted this finding as compensating for the energy expended during exercise. In the present study, the lack of exercise on pasture in the treatment NT might have caused the decreased time spent "lying". The time spent "occupied" might have been increased because the horses were not able to graze and occupy themselves in general on pasture and hence were more occupied in the stall. They also might have ingested more straw as they were not able to graze. Heleski et al. [23] observed more time spent lying in weanlings housed in single stalls than in group-housing systems. It was supposed that this observation was caused by a lack of activity and/or boredom in single housing on the one hand and by more privacy to rest (especially in low-ranking animals) on the other.

As the prevention of exercise leads to unspent energy and therefore to more restlessness [24, 25], it was expected that the horses would perform more "locomotion" in the treatment without turnout. This was only observed within Period 1. Generally, most "locomotion" was shown in Period 3 of the investigation. Although the temperature in Periods 2 and 3 was higher than in Period 1, a clear interpretation of this observation is not possible from the data at hand. The development of locomotion during the course of the observation time decreased considerably in all three treatments from 8 pm on (Fig. 3). Around that time, regular business in the stable and the whole yard was finished. Additionally, the horses had finished their hay and so resting behaviour might have gained in importance. Around 5.30 am, morning business started in the yard which might have led to the increase of locomotion as the horses were waiting for their feed.

In an earlier study [21], a more restless behaviour was observed in horses when no turnout was allowed. This was revealed by more frequent changes between the behaviours and more frequent aggression against the neighbouring horses. In the present investigation, this reaction was also expected but not found in the analysis of the behaviour in the stable, though a marginal effect was found in the day-to-day handling of the horses.

4.2. Heart rate variability (HRV)

Provided that certain conditions are maintained (e.g. measurements of the same duration, at the same time of day, in a similar state of activity; well documented correction of artefacts), the analysis of HRV in horses is evaluated as to be a sensitive measure of both physical and emotional stress responses [11, 26]. As these conditions were considered in the present study the results are seen as reliable. However, the measurements only give information about changes in the degree of stress within the experiment. As basal values in horses contain large inter-individual variations the results do not allow a safe evaluation of the absolute level of stress in the individuals [12, 27, 28].

The HRV analysis in the present study revealed a stressing effect of the prevention of free exercise. All three parameters (SDNN, RMSSD, LF/HF) indicated a lower HRV (i.e. decreased vagal regulatory activity) and therefore a greater degree of stress in the treatment NT compared to the other treatments on average (Fig. 4). This result matches the findings of Hoffmann [29], who showed that the degree of stress in horses goes down when additional exercise (turnout on pasture or in a horse walker) is offered. Considering that free-ranging horses spend up to 16 hours a day foraging, which happens predominantly at a walking pace [1, 2], this result demonstrates the importance of free exercise for horses.

The available data only permits speculation about the deviations found in the individual horses. According to the literature, external influences like age, sex, genotype, temperament, nutritional status, climate, (cardiac) disease, etc. have an effect on the regulation of cardiac activity [11, 16]. At a younger age, HRV is generally greater. This might have been a reason for the low LF/HF in H1 and H5 as they were the youngest horses in the group.

Extreme weather conditions (high temperature and relative humidity) caused a higher degree of stress on average. This result matches the findings of Berger et al. [30] and Mayes and Duncan [31], who found that high temperature and insects led to more restlessness and therefore to more stress. However, in the present study these relationships varied to such a great extent in the individual horses that no clear interpretation was possible. Great inter-individual differences were also found in earlier studies in horses which match the large ranges of the analysed values found in the present results [11, 12, 27, 28, 29].

The SDNN and RMSSD values in NT were lower in the second than in the first week (in the second week also lower than ST and GT), which might indicate that the degree of stress increases the longer free exercise is prevented (Fig. 5). These values tended to increase towards the end of the treatment in GT. No clear trend is visible for the parameter LF/HF during the course of the treatments. Particularly high or low values in all parameters on particular days were predominantly caused by outliers in individual horses, which cannot be explained by the available data.

Visser et al. [13, 32] found a relationship between temperament and HRV. In fact, horses characterised as highly responsive to changes in the environment had a lower HRV. Although Visser et al. [13, 32] measured HRV *during* the experimental situation and not at rest (as in the present study), their findings have been proved by the present study at least in tendency. For example, H2 was described as being little sensitive to changes in the environment and steady in behaviour and performance. The highest

HRV was measured in this horse compared to the other horses. The lowest HRV was measured in H4. This horse was considered to have a rather phlegmatic character but sometimes it reacted very intensely to specific changes in its environment.

4.3. Behaviour during training

As the horses showed very individual reactions, the treatment did not influence the horses' behaviour during training in any clear direction. As there are many factors influencing the behaviour during training and the evaluation by the riders is not quite objective, two explanations are supposable for this observation. On the one hand, it is possible that the treatment really did not influence the behaviour. On the other hand it is imaginable, that changes in the behaviour might not have been revealed clear enough by the recorded parameters. The average willingness to perform was slightly lower in NT than in the treatments with turnout. This result proves that free exercise has positive physical and mental effects in the horse [33]. A horse's good ability to move is the prerequisite for athletic use [33, 34]. Rivera et al. [35] also observed that young horses kept on pasture acclimatise easier to training and the use of equipment than horses housed in a stable. This observation was traced back to the fact that horses on pasture train their ability to adapt to new situations better than horses in a low-stimulus environment like a stable. The housed horses in the investigation of Rivera et al. [35] also showed more activity, such as jumping and bucking (these behaviour patterns are called "contumacy" in the present study), which was predicted by Hogan et al. [25] as being the result of unspent energy due to stabling. The horses' contumacy in this study led to a worse evaluation of willingness to perform as it was part of this parameter. The results therefore confirm the findings of Rivera et al. [35] and Hogan et al. [25] that a lack of free exercise has rather a negative effect on training.

Besides the risk of injury, free exercise is feared to decrease motivation for locomotion and so for training. In contrast, the present study revealed the highest urge to move in the treatment GT, which might have been caused by a better mental balance as a consequence of free exercise and social contact [33]. The duration and distance covered during training is dependent to a great extent on the rider and his/her conception of training. The willingness to perform was evaluated as being better when the duration of training was longer. This result disagrees with the results of Werhahn et al. [19] and Rivera et al. [35], who found that the training session was prolonged when the horses behaved restlessly and/or agitatedly. The riders in the present study had quite a tight time schedule for training all the horses they were responsible for and at times this led to a reduction in the length of the individual training sessions. Possibly, the evaluations of the willingness to perform were better when the duration of training was longer, because when the riders spent more time with one horse they were able to deal with the individual in greater detail, which might have led to better performance.

The sporadic correlations found in the individual horses (Section 3.3) provide indications about the temperament of the horses in some cases. H1, for example, was found to be easily excitable and had a great urge to move during training and turnout. This horse's willingness to perform was evaluated as being better when the urge to move was lower. H4 had a completely different reaction. This horse had a more phlegmatic character with a low urge to move, which was rather increased – leading to a better evaluation – when the relative humidity was higher. This might have been due to the fact that the relative humidity tended to be higher when the temperature was lower. The causes and effects of these relationships are obviously very individual and could not be clarified conclusively within this study.

5. Conclusions

In conclusion, this study shows that allowing or not allowing free exercise and its configuration (solitary or in groups of two) affects horses' behaviour in the stable and during training and also their degree of stress. The prevention of free exercise resulted in less lying and more occupation, which was interpreted as being a decreased demand for rest and as balancing the lack of occupation on pasture. When no turnout was allowed, all three HRV parameters at rest were lower indicating a higher degree of stress in this treatment. High temperature and relative humidity also caused higher values for LF/HF indicating the presence of stress. Group turnout resulted in the lowest degree of stress but the difference was only significant in SDNN compared to no turnout. The evaluation of training was best when turnout was allowed in groups but the evaluation did not differ significantly between the treatments.

Housing horses in single stalls restricts natural behaviour to a great extent. The present study demonstrates that management practices affect horses' behaviour and degree of stress during the whole day. Stress is supposed to be minimised to improve animal welfare in a housing system. Allowing free exercise and social interactions are invaluable tools to achieve this aim in horses and so should be facilitated by every horse keeper.

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