6-1-2012

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Recommended Citation
Young, T. Creighton, E., Smith, T., Hosie C. (2012) "A novel scale of behavioural indicators of stress for use with domestic horses". Applied Animal Behaviour Science, Volume 140 issue 1, pp. 33– 43.

A novel scale of behavioural indicators of stress for use with domestic horses.

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Abstract

Behaviour scores (BS) offer non-invasive, objective and easy to use ways of assessing welfare in animals. Their development has; however, largely focused on behavioural reactions to stressful events (often induced), and little use of physiological measures has been made to underpin and validate the behavioural measures. This study aimed to develop a physiologically validated scale of behavioural indicators of stress for the purpose of welfare assessment in stabled domestic horses. To achieve this, behavioural and physiological data were collected from 32 horses that underwent routine husbandry procedures. Principal component analysis (PCA) of the behavioural and physiological data revealed three meaningful components that were used as the basis of the scale. Analysis of video clips of the horses' responses to the husbandry procedures was undertaken by a panel of equestrian industry professionals using a free choice profiling (FCP) methodology. These results were added to the scale along with key definitions from relevant literature. Salivary cortisol levels were significantly correlated with the BS confirming

the scale was meaningful and reflected physiological stress. The scale offers an easy to use 'tool' for rapid, reliable non-invasive welfare assessment in horses, and reduces the need for potentially invasive physiological measures.

Key words

Horse; behaviour scores; cortisol; saliva; welfare assessment; non-invasive.

1.0 Introduction

Use of behaviour scores (BS) offers objective, immediate methods of welfare assessment in animals (Minka et al., 2009). They have been used, with varying degrees of success, to measure stress levels for the purpose of welfare assessment in various species (horses: Visser et al., 2010; Munsters et al., 2011; cats: McCune, 1994; Kessler and Turner, 1997; McCobb et al., 2005; Dybdall et al., 2007; goats: Minka et al., 2009; cattle: Maria et al., 2004b; ostriches: Minka and Ayo, 2008; chickens: Maria et al., 2004a). These scores have, however, been largely developed by focusing on expression of behaviours assumed to indicate stress rather than making use of established physiological indicators of stress to underpin them. In addition, BS are often used rather simply, ignoring all but 'negative' behaviours (e.g. Minka et al., 2009) and therefore losing potentially valuable information. Of the small number of behaviour scoring approaches to welfare assessment available, only the Cat Stress Score (McCune, 1994; Kessler and Turner, 1997) provides a scale of stress responses that can be used flexibly to assess welfare in different environments. This scale has not been physiologically validated, however.

Assessment of a stress response is clearly best carried out using a combination of both behavioural and physiological measures (Broom, 1991; Mason and Mendl, 1993; Dawkins, 2003). This provides a more comprehensive measurement of stress, and avoids drawing misleading conclusions that could be reached by taking just a single measure (Broom, 1991; Mason and Mendl, 1993; Dawkins, 2003). Previous success at correlating behavioural measures with physiological measures has, however, been mixed. Minka et al. (2009) established that certain behaviours and physiological indices of stress were related during the handling and loading of goats for transportation, but McCobb et al. (2005) were not able to correlate BS with urinary cortisol measures in cats. Clearly the behavioural and physiological measures must both be sufficiently sensitive and reliable to successfully correlate them for use in a behavioural scale for welfare assessment.

The only scale of BS available for use with domestic horses was developed to assess, specifically, whether horse and rider combinations were appropriate (Munsters et al., 2011). The scale was adapted from a scoring system used by Visser et al. (2010) to assess the temperaments of sports horses exposed to novel objects, not to evaluate stress responses. Some level of experience/expertise was needed to accurately assess temperament in this latter study. Physiological measures were not used to develop the score, although heart rate variability (HRV) was used when the score was tested.

The aim of the current study was, therefore, to develop an easy to use scale of BS that could be used to rapidly but reliably assess stress levels in domestic stabled horses. The scale was developed using both behavioural and physiological measures obtained from a wide range of horses (N=32) during naturally occurring daily routine husbandry procedures. Use of such husbandry procedures, that would have taken place irrespective of the study, was considered an ethical approach to data collection rather than artificially exposing horses to potentially stressful situations

Since assessment of welfare is most effective using multiple measures, analysis of the behavioural responses to the routine husbandry procedures used two different approaches. Behavioural responses were quantified in detail using The Observer (Noldus Information Technology Software Ltd) for 12 of the horses. Both quantitative and qualitative assessment of the behaviour of all study horses was made, by expert panel members, using the experimental free choice profiling (FCP) approach (Wemelsfelder et al., 2000; 2001).

We again used two measures for the physiological data collection, heart rate (HR) and salivary cortisol, to validate the BS scale. Both cortisol (Ralston et al., 1988; Toutain et al., 1995; McBride and Cuddeford, 2001; Covalesky et al., 1992; Stegaman and Jones, 1998) and HR (Reitmann et al., 2004; von Borell et al., 2007; Visser et al., 2008) are established indicators of stress in horses, and detect different aspects of the stress response. Both indices of stress were used here as their measurement can be achieved by non-invasive means, thus avoiding any further stress to the study horses.

2.0 Method

2.1 Subjects used for the study

The study aimed to ensure a wide range of horses was used to build the BS scale. The horses used in the study consisted of various breeds of stabled mares and geldings kept in similar management and exercise regimes, at four different locations (Table 1). All horses were housed in individual stables on either straw or shavings bedding, and received hay or haylage and water with up to two hard feeds i.e. mix or pellets, at around 07:00 h and 16:00 h. All horses were in light to medium work (receiving around two hours of exercise daily) throughout the study. When they were not being exercised all horses received up to four hours turn out to pasture daily, and remained in their usual daily management routine apart from undergoing routine husbandry procedures.

A total of 32 horses were used in this study and assigned to experimental groups on the basis of their availability (see Table 1 for details of location, age, gender and husbandry procedure). In summary, analysis of behaviour was completed from all 32 horses; nineteen of the horses were used for saliva collection for cortisol measurement, with a further 10 horses from location one used as a control group for this part of the study; eighteen horses were used for measurement of HR, with a further 10 horses from location one again used as a separate control group for this aspect of the study.

2.2 Husbandry procedures used for data collection

Horses were subjected individually to one of four different 10 minute husbandry procedures. They remained loose in their stables, except during grooming when they were loosely held by a familiar handler and had access to hay and water. A 10 minute period was deemed adequate to induce and measure a potential stress response, but not so long that habituation to the stressor should occur (Visser et al., 2001).

Procedure 1 - sound of electric coat clippers

Horses (from location 1 only, for logistical reasons) were exposed to the sound of electric coat clippers (Heiniger Handy Clipper, Switzerland) turned on to maximum clipping velocity. Typical sound emissions from such clippers were 80.1 decibels. Clippers were switched on and held by hand in an adjacent stable 3.66 metres away from the study horse.

Procedure 2 - social isolation

Horses (from locations 1, 2, 3) were caught from the field and returned to their usual stable. This process took no longer than five minutes, and horses showed no resistance to capture. The horses were stabled in the absence of any other horses on the yard for 10 minutes. At the end of the social isolation period the horse's usual neighbouring horse was returned to the adjacent stable.

Procedure 3 - grooming procedures

A head-collar and lead rope was fitted to the horse, and a familiar handler held the lead rope approximately half way along its length to restrain the horse loosely. Mane combing and mane pulling (a procedure used to thin and shorten the mane by taking small sections of hair back combing them and pulling out the remaining long hairs) then took place. Animals were used from location 1.

Procedure 4 - the sound of fireworks played on a CD

Police horses (from location 4 only) were used for this procedure as it involved the sound of fireworks played on a compact disc (CD), which was used as part of riot training with Police horses. The CD player was situated on a table outside the horse's stable 3.66 metres away.

The husbandry procedures were carried out over a number of weeks as the opportunities to collect data arose. Where the same procedures were carried out with a number of horses on the same yard e.g. exposure to the sound of electric coat clippers, one week was left between tests to minimise the effects of habituation on the horses that had not yet been sampled from. The control horses were chosen randomly from location one, as these had not been subjected to the husbandry procedures examined in the study for a minimum of eight weeks. This opportunistic data collection strategy meant that some procedures/control were tested in a single location, others across more than one. Location effects were tested for in the analysis to check that this had no confounding impact.

2.3 Behavioural measurement during the husbandry procedures

The behaviour of the subjects was recorded during all husbandry procedures using a Sanyo CCD/BW video camera (Sanyo Electric Co., Ltd, Osaka, Japan) secured at ceiling height in an appropriate position opposite the stable to gain an adequate field of view. The video camera was linked to a Mitsubishi HS-1024E time-lapse recorder (Osaka, Japan), set to three hour real time for recording of images onto three hour video tapes (BASF Vision Chrome Videocassette, BASF plc, Middlesex, U.K.).

2.3.1 Analysis of behaviour using The Observer

The first five minutes of the behavioural reactions exhibited during the husbandry procedures, for 12 of the horses, was analysed using The Observer 5.0. Behaviours were recorded using a pre-defined ethogram based on equine stable behaviour (Table 2). The ethogram had been compiled from six weeks of ad-hoc observation of race-horses and stabled riding horses, together with literature research (see Houpt, 1993; Winskill et al., 1996; McBride and Cuddeford, 2001; Strand et al., 2002; Heleski et al., 2002; Seaman et al., 2002; McDonnell, 2003)

2.3.2 Analysis of behaviour carried out by a panel of equestrian professionals

A panel of 13 professionals who had a working background with horses, and held a minimum of the British Horse Society (BHS) stage one qualification was convened. They were briefed on the nature of the study and asked to view a video of the initial two minutes of each horse's behavioural reaction to the husbandry procedures, and to provide a BS between zero and ten according to how stressed they perceived the horse to be. They were told that ten equated to an extremely stressed horse. The panel also had to describe, using their own descriptive terms, the horse's behaviour exhibited during the video. They, finally, were asked to state at which point on their subjective scale that they believed the onset of stress occurred in the horses undergoing the husbandry procedures.

2.4 Measurement of salivary cortisol concentrations during the husbandry procedures

Saliva was collected 60 minutes and 30 minutes prior to the start of the husbandry procedures, and then at the end of the 10 minute procedure and at 10 minute intervals up to 40 minutes. Forty minutes was chosen to provide enough time for peak cortisol to be reached following the onset of the potential stressor (the husbandry procedure). Plasma cortisol peaks in horses 30 minutes post exercise stress (Foreman and Ferlazzo, 1996; Marc et al., 2000; Hamlin et al., 2000), and 20 minutes following restraint stress (Hydbring et al., 1996).

Saliva was collected from the control group of horses to ensure that the swabbing procedure did not affect their stress levels. Collection took place at 60 minutes, 30 minutes, and 0 minutes before the husbandry procedure would have begun, and then at the same time intervals that the experimental group of horses had their saliva collected, except for the extra swab was taken at 0 minutes from control horses to provide a robust control measure.

Saliva was collected using sterilised flexi-swabs (Medical Wire & Equipment Co (Bath) Ltd) that were introduced into the corner of the horses' mouths first on the horse's left and then on the horse's right. The horses were allowed to manipulate the swabs using their tongues for approximately 20 seconds per introduction of the swab. The swabs were then placed into sterile 20ml plastic screw top containers, labelled and stored on ice until frozen at -20°C the same day to await cortisol extraction.

Saliva was extracted from the thawed cotton wool swabs by centrifugation using a Sorvall T.C. centrifuge (Thermo Scientific, Basingstoke, Hampshire, UK) for two minutes at 800g. The supernatant was then centrifuged using a Hettick Mikro 20 centrifuge (Tuttilgen, Germany) at 15,000g for two minutes. The supernatant was taken off using a pipette and frozen to await analysis. Salivary cortisol concentrations were quantified using a modified version of an EIA described by Smith and French (1997).

2.5 Measurement of heart rate (HR) during the husbandry procedures

HR was recorded from the experimental and control groups of horses at 60-second intervals for two minutes prior to the start of the husbandry procedure to provide a mean baseline HR. Recording of HR then continued at 60-second intervals for the first five minutes of the husbandry procedure for the experimental group of horses, and over the same time intervals, in the absence of a husbandry procedure, for the control group.

HR was recorded using a Polar HR monitor (S610i) (Polar Electro, Öy, Kempele, Finland). The HR monitor consisted of an electrode belt that picked up the electrical activity of the horse's heart, with a transmitter attached enabling wireless transmission of the HR to a wrist watch receiver. The belt was fitted around the horse's thorax with both electrodes sited to the left-hand side of the horse. One electrode was placed approximately 10cm below the withers, and the other about 10cm behind the elbow over the heart. Warm water and electrode gel (The Wyke of Shifnal, Shropshire) was used to optimise contact between the horse's skin and the electrodes. The wrist watch receiver was taped to a leather strap fastened around the horse's neck. All horses were given 10 minutes to habituate to the equipment (Reitmann et al., 2004).

2.6 Statistical analysis

Principal component analysis (PCA) was used to investigate whether there were any relationships between behavioural and physiological changes that took place during the husbandry procedures. 'Data reduction' was necessary to look for smaller sets of factors or components in the data (Pallant, 2004; Ennos, 2007) from which the scale of BS could be compiled. The percentage change in cortisol concentration from the median baseline value to the peak concentration was calculated for each horse. This percentage, together with the percentage duration of all behaviours included in the ethogram underwent PCA.

PCA of the cortisol and behavioural data exhibited during the husbandry procedures revealed correlation coefficients of 0.3 and above (following Pallant, 2004). An oblimin rotation of three factor solution was used to reduce the number of variables into meaningful components (Pallant, 2004). Each behaviour and change in cortisol concentration received a score for each component denoting whether the behaviour was performed or not, or whether change in cortisol was relevant. A median BS was calculated for each horse used in the study, as scored by members of the professional panel. The terms used by the panel to describe each horse's behaviour was pooled for horses with the same BS. Panel descriptions of behaviour were added to the relevant sets of factors or components revealed by the PCA, and the scale of BS for use with stabled domestic horses subsequently devised.

All HR and salivary cortisol data measured during the husbandry procedures and controls were log transformed to reduce heterogeneity of variance. The impact of location on each physiological measure was investigated to assess potential confounds caused by using subjects at different locations. To do this, levels of baseline cortisol and HR data were compared across the two locations in the case of

HR data using an independent samples T test, and across the four locations in the case of salivary cortisol data using a one-way between subjects ANOVA.

Any impact of the Polar heart monitor over time was explored during a control study by analysing HR at time zero and 60, 120, 180, 240 and 300 seconds as compared to the experimental timings, under control conditions. The impact of collecting saliva swabs on the HPA response was examined during a control study which assessed cortisol under control conditions at time 0, then 10, 20, 30 and 40 minutes later using a repeated subjects ANOVA.

Changes in HR data and levels of salivary cortisol were explored using General Linear Models (GLM) with sex, husbandry procedure and time of collection (i.e. before and after the procedure in terms of the HR data, and peak compared to baseline cortisol titres for the salivary cortisol data) as fixed factors. Post hoc analyses were conducted using Tukey test and alpha was set at 0.05.

To investigate whether the devised BS scale reflected physiological stress, median BS as calculated from the professional panel, and peak salivary cortisol following the husbandry procedures were investigated using Spearman's Rank Order Correlation.

3.0 Results

3.1 Behavioural data

PCA of the percentage change in salivary cortisol from baseline to peak, and percentage duration of state behaviour exhibited during the husbandry procedures identified three components in the pattern matrix. They were labelled no stress (factor 1), low stress (factor 3) and medium stress (factor 2) according to the type of behaviour and change in cortisol identified (Table 3).

Median behaviour scores were calculated for the study horses, and ranged between one and eight. The terms used by the panel to describe each horse's behaviour was pooled for horses with the same BS, and panel descriptions of behaviour were added to the three components revealed by the PCA.

Descriptions used for horses with a BS of one and two were added to the component labelled no stress, as the mean score representing the onset of stress as judged by the panel was three. Descriptions used for horses with a BS of three to seven were added to low and medium stress. The BS of five was used as the onset of medium stress, based on the type of behaviour included in the component extracted by the PCA. Descriptions used for horses with a BS of eight to ten formed a new category labelled high stress, because the PCA analysis did not include horses with a BS of this level. Relevant literature was also used to form this category. The scale of behavioural indicators of stress for use with stabled domestic horses was subsequently compiled (Table 4).

3.2 Physiological data

Both measures of HR and salivary cortisol were used to underpin the behavioural measures in the development of the scale of behavioural scores.

3.2.1 HR data

Baseline HR values were comparable across the two locations ($t = -0.660$, $df = 16$, NS) thus ruling out location as a confound in the study. There were, also, no significant changes in HR between baseline (mean HR 37.20 ± 8.34bpm) and the 'test' period (mean HR 38.98 ± 15.65bpm) in the control study suggesting that the presence of the Polar heart rate monitor over a period of time did not cause the horses any stress ($t = -0.381$, $df=9$, NS).

HR values were explored using a general linear model with sex, husbandry procedure and time of collection (i.e. before versus after the procedure) as the fixed factors. HR values were significantly raised following the husbandry procedures compared to baseline values (Figure 1) [F (1, 26) = 10.083, P < 0.0001].

There was no effect of sex on HR values [F (1, 26) = 0.261, NS], but there was a significant interaction between sex and husbandry procedure [F (1, 26) = 4.315, P < 0.05]. Further analysis using a t test showed that HR values before and after combined, for the clippers, were significantly higher for mares than the same values for geldings (t=-3.403, df =18, P < 0.003). In contrast for grooming, before and after HR values (combined) were similar for both mares and geldings (t = 1.294, df = 6, NS). Only geldings were exposed to the fireworks.

There was no effect of husbandry procedure alone on HR values [F (2, 26) = 2.444, NS], or interaction between husbandry procedure and sampling time [F (2, 26) = 0.621, NS], sex and sampling time [F (2, 26) = 0.169, NS], or between the three variables [F (1, 26) = 0.820, NS].

3.2.2 Salivary cortisol data

Baseline salivary cortisol titres levels were comparable across subjects in the four locations confirming that location was not a confound in our study [F (3, 18) = 1.824, NS].

Changes in physiological data collected from control horses were explored to ensure that the methods of data collection did not affect the horses. There were no changes in levels of salivary cortisol across the control study (mean ± s.e.m.) baseline 0.42 ± 0.12ng/ml; at 10 minutes 0.43 ± 0.2; at 20 minutes 0.35 ± 0.17; at 30 minutes 0.39 ±

0.15; at 40 minutes 0.49 ± 0.25) suggesting that the saliva swabbing was not stressful to our subjects [F (4, 32) = 0.821, NS].

There was a significant effect of husbandry procedure on levels of salivary cortisol [F (3, 22) = 5.644, P < 0.005]. Post hoc analysis revealed that levels of the hormone (baseline and peak concentrations combined) pertaining to exposure to the fireworks were significantly higher than those related to clipping (P < 0.01) and social isolation (P < 0.01), but not grooming (NS). Hormone values relating to grooming were higher than those relating to clipping (P < 0.05) and social isolation (P < 0.01).

There was also a significant effect of time (i.e. baseline cortisol versus peak concentration of cortisol) since hormone levels were significantly elevated following the husbandry procedures compared to prior to the procedures (Figure 2) [F (1, 22) = 6.077, P < 0.05]. There was, however, no interaction between husbandry procedure and sample time showing that despite gross differences in hormone values across the different procedures, the manner and magnitude of the change in cortisol levels pre and post treatment were comparable across the four husbandry procedures [F (3, 22) = 0.827, NS]. This was confirmed by two separate one-way ANOVAs which revealed comparable hormone levels across the husbandry procedures before [F (3, 14) = 3.035, NS] and after [F (3, 14) = 2.292, NS] the treatments. There was no effect of sex on hormone values [F (1, 22) = 0.645, NS], and no interaction effects, i.e. condition and sex [F (2, 22) = 2.184, NS], sex and time [F (1, 22) = 0.000, NS], condition, sex and time [F (2, 22) = 0.701, NS].

3.4 Correlating behavioural and physiological data

To ensure the final BS scale reflected increased levels of both behavioural and physiological stress, their relationship in response to the husbandry procedures was investigated. A significant correlation existed between median BS and peak salivary cortisol concentration measured during the husbandry procedures (r_s = 0.54, P = 0.02, n = 18) confirming that the final BS scale was a reflection of both behavioural and physiological stress.

4.0 Discussion

In this study a scale of BS has been developed to measure stress levels in stabled domestic horses for the purposes of welfare assessment. To do this, behaviour and physiology were each measured by two different techniques in a range of horses undergoing standard husbandry procedures. The physiological data were used to underpin behavioural measures. Physiological data revealed that husbandry procedures did significantly elevate HR. HR was greater for mares than geldings for the clipping procedure but overall patterns of change in HR before and after procedures was the same for both sexes. This shows that the husbandry procedures used here were perceived as stressful by the horses, and provides a biological validation of these procedures as stressful events. These data also demonstrate the sensitivity of HR as a reliable physiological measure of stress.

Similarly, cortisol measures demonstrated that the husbandry procedures had a significant effect on stress physiology. Importantly these data also showed that location (source of horses), sex and other factors did not affect cortisol measures. Again, this provides biological validation of these procedures as stressful events and demonstrates that salivary cortisol is a sensitive, reliable measure of stress. These two physiological measures can, therefore, be considered reliable to use to underpin the development of the scale of behavioural scores. Schmidt et al. (2010) recently also established use of HR and salivary cortisol together as sensitive parameters to detect stress in ‘routine’ transport procedures for horses.

Behavioural data (percentage durations) from each horse during the husbandry procedures was combined with percentage change in cortisol from baseline to peak in PCA analysis. The three components revealed by the PCA were combined with the expert panel descriptions to derive the scale of behavioural indicators of stress that make up the behavioural stress score this work has developed. To ensure the final scale of BS reflected increasing levels of behavioural and physiological stress, the relationship between behavioural and physiological changes in response to the husbandry procedures was investigated. Measures of salivary cortisol concentration following the routine husbandry procedures, and the median BS calculated for the same animal were seen to correlate. In doing this we have successfully combined two different behavioural approaches, underpinning them with two well-established physiological measures.

Whilst other studies have used BS these are often relatively simple measures of behaviours. For example Minka et al. (2009) use a number of 'negative' behaviours as a measure of stress, and Maria et al. (2004a) use a greater complexity of behaviours as an indication of less stress. In addition, these may involve a degree of subjectivity in their use (Minka et al., 2009; Munsters et al., 2011) or require sophisticated analysis to assess (Maria et al. 2004a). The BS indicators in our scale developed here are straightforward and do not require great experience of horses to be easily used. In addition, the behavioural descriptions provide a simple scale to enable a range of levels of stress to be measured. Of the small number of behaviour scoring approaches to welfare assessment published, only the Cat Stress Score (McCune, 1994; Kessler and Turner, 1997) provides a sliding scale of stress responses that can be used flexibly to assess welfare in different environments, as used by McCobb et al.(2005) and Dybdall et al. (2007). This latter scale however, has not been physiologically validated, and McCobb et al. (2005) were not able in their study to correlate BS with urinary cortisol measures in cats housed in traditional or enriched shelter environments. We believe our comprehensive approach to combining behaviour and physiology has resulted in a non-invasive, user-friendly, physiologically validated, behavioural stress score that can be used in a variety of environments to measure stress and assess welfare in domestic horses.

The work undertaken in developing this behavioural stress score also sheds interesting light on the role of stereotypies in assessing stress in horses. Abnormal or stereotypic behaviour was included in all components extracted by the PCA. 'No stress' horses showed an association with repetitive oral behaviour such as crib-biting, low stress horses exhibited weaving, and both low and medium stress horses carried out repetitive head movements such as head shaking or nodding. It has been suggested that performance of stereotypic behaviour may serve as a way of reducing stress levels, or as a way of horses' providing themselves with some sort of control over their environment (Cooper and Albentosa, 2005). This may explain the fact that horses perceived as experiencing no or low stress were exhibiting stereotypies. Weaving, which is indicative of chronic frustration in horses usually associated with attempts to gain social contact with other horses (Visser et al., 2008), was evident in medium stressed horses. This together with repetitive head movements suggests an increased level of frustration experienced by the horses in the low and medium stress groups. These findings suggest that stress assessment that takes a comprehensive view of all behaviour exhibited, rather than a focus on 'negative' behaviours, including stereotypies, is desirable. The behavioural stress score developed here now makes this possible for domestic horses.

5.0 Conclusion

The scale of behavioural indicators of stress developed in this study provides a quick and easy, reliable method of measuring the stress levels of domestic stabled horses to assess their welfare. It was developed using both behavioural and physiological measures, so the final behavioural scores that make up the scale also provide reliable indices of physiological change in response to stress. The relationship between behavioural and physiological changes inferred in the scale was further confirmed by the correlation seen between change in salivary cortisol and the same horse's behavioural score. This, therefore, reduces the need to measure various physiological parameters separately to validate the use of the scale and so makes it a valuable, cost-effective tool that could be used by horse owners and behavioural scientists alike.

6.0 Acknowledgements

We are grateful to the horse owners for allowing us to collect behavioural and physiological data from their animals. We also appreciated the support given to us by the stable staff at Welsh college of Horticulture, Granary Livery, Belle Vue Livery, and Merseyside Mounted Police unit, and by undergraduate students from the University of Chester who helped with data collection during the study. We also thank members of the professional panel who gave up their time to contribute to this study. Thanks are also extended to Dr. Rob Coleman for his help with the cortisol assay, and to Dr. Joanna Hockenhull for her assistance with this study.

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Table and figure captions.

Table 1. Details of the subjects used in the study (N=32).

Table 2. The ethogram used for The Observer configuration.

Table 3. Pattern matrix for PCA of the cortisol and duration of state behaviour recorded during the four different routine husbandry procedures using oblimin rotation of a three factor solution.

Table 4. A scale of behavioural indicators of stress in domestic stabled horses, as revealed by principal component analysis (PCA) and behavioural assessment completed by a professional panel.

Figure 1. Mean heart rate (bpm) ± 1.0 SE before (Baseline) and during the husbandry procedures (N=18).

Figure 2. Mean salivary cortisol (ng/ml) ± 1.0 SE before the husbandry procedures (Baseline) and at peak following the procedure (N=19).