Natural horsemanship: Round-pen training of horses

A thesis presented in partial fulfilment of the requirements for the degree of

Masters of Science
in
Physiology

At Massey University, Palmerston North, New Zealand

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2003

Abstract

The effect of round-pen training on the behaviour and physiological response (plasma cortisol concentration and heart rate) of 24 horses was examined using a stocks restraint stress test before, after and 3 weeks after round-pen training was carried out. Horses were allocated to treatment groups according to their ease of handling. Three treatment groups were formed, Control, Round-Pen Easy and Round-Pen Difficult (RP-D).

Before the treatment (round-pen training or control) there were no significant differences between the three treatment groups for plasma cortisol concentration and heart rate. Restraint in the stocks caused an elevation in plasma cortisol concentrations in all horses. The increase in plasma cortisol concentration was greater in the RP-D horses. A single round-pen training session was used as a treatment for the RP treated horses (Easy or difficult). Post-treatment most horses had a significant decrease in the time to enter the stocks, however, treatment had no significant effect on the plasma cortisol response, heart rate or behaviour of horses in each of the three treatment groups.

Round-pen training sessions were observed to see if the ease of round pen training was affected by either dominance rank or the behaviours observed during round-pen training. Despite the individual variation between horses, all horses followed a similar pattern of behaviour during round-pen training. There was no significant effect of social status on the ease of round-pen training.

The effect of dominance rank on the ease of handling, behaviours observed in the stocks and the plasma cortisol concentration during the pre-treatment stocks tests were examined. Horses that occupied the lower ranks were less easy to handle during the pre-treatment stocks test. The occurrence of some agitation and rest behaviours differed between horses of high and low dominance ranks. Dominance rank had no significant effect on the resting plasma cortisol concentration before treatment. Further research may clarify relationships between certain behaviours (head turning, head held up and defecation) and changes in plasma cortisol concentration during restraint, that could be used as non-invasive indicators of the onset of stress in the restrained horse.

Acknowledgements

To Associate Professor Kevin Stafford, guiding me through my Masters thesis, getting stuck in (literally) on my research project and making my postgraduate experience a fun and memorable task.

My second supervisor, Professor David Mellor for providing us all with a unique perspective and giving us the academic footing on which all animal welfare science students stand.

Tamara Diesch, for providing a wonderful research assistant even under the dustiest conditions, and for making the best behavioural observation notes I could ever hope to ask for.

Neil Ward and Phil Pearce for providing equipment, lab analysis and facilities for carrying out the research project.

To Robin Whitson for the use of the horses and facilities at the VLATU

Nicolas Lopez-Villalobos and Duncan Hederly for statistical consultation and assistance.

The IVABS Postgraduate research fund for providing the funding for my research project.

Sheila and Murray, for giving me guidance when I needed it and always being there to support me, and for assisting on the grammar side of my thesis writing.

Jayney, for being a good friend and providing me with creative energy; Graham for the use of his camera and offering to help with my stats stuff.

Ben and Leanne, a definitive source of time-out, fun, laughter and encouragement.

To Rissy, Jackson and Felix for posing in some of the photos.

Martine and her lovely horses for the photographs on chapters 4 and 5.

My sister, Heidi, for the competition and challenge you have provided me, throughout my life, and encouraging me to do what I do best.

My lovely flatmate Liz, for providing a source of rest and recreation.

Sharon, for being a good buddy and showing me that there is always two ways to do a thesis.

Kate Littin, Shauna Sylvester, Naigo Beausoleil, and the rest of the Animal Welfare Science and Bioethics group, for offering a sounding board and feedback the whole way through my thesis.

Chris, Caitlin and Ashleigh Hall, for helping with a bit of time out, a friendly ear, and reminding me that I am not really old yet.

Aunty Sandra, for offering encouraging words of wisdom, encouragement and support from a far

Dad and Miranda, for supporting me and helping motivate me throughout the work on my masters degree.

To Dave for taking care of mum so I don't need to or when I can't be there.

This thesis is dedicated to my Mum, Lynne, for making me the lover of horses that I am, making sure that I always know what to do, giving me the drive and determination to do whatever I set out to do, and shaping the person I am today.

And finally and most importantly, to Holly, Cheetah, Nugget and Kinder: the four brightest stars in my world that have taught me all I ever needed to know....
My horses...my teachers, my life, my love, my obsession and my inspiration.

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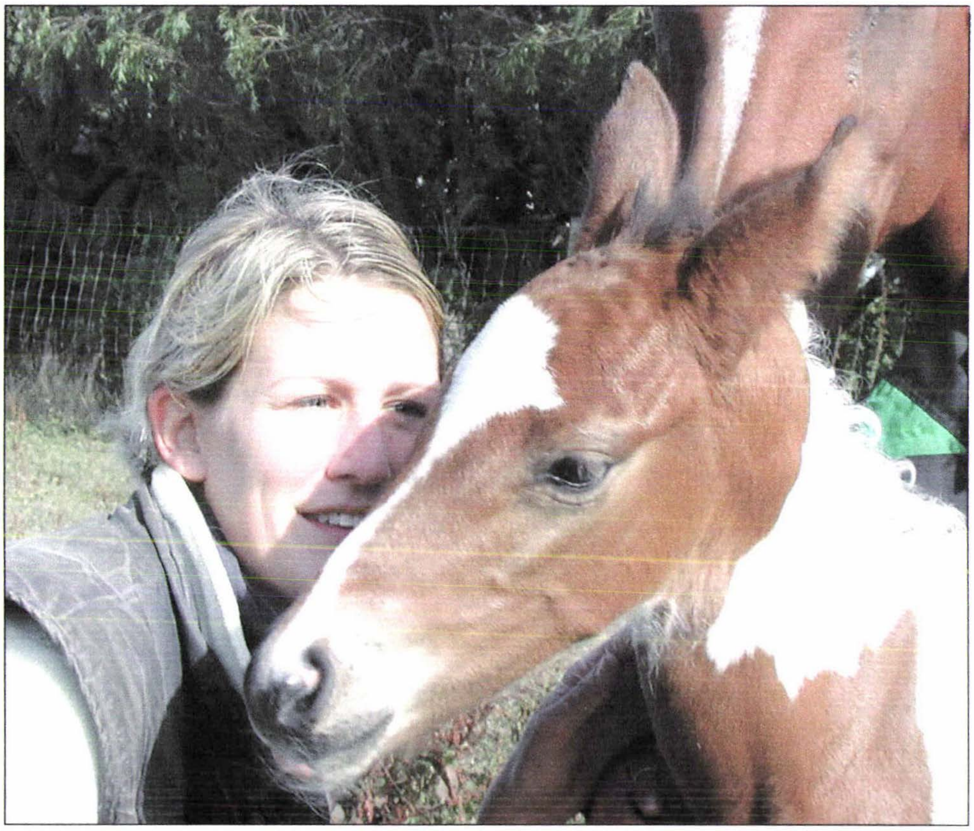

"There is something about the outside of a horse that is good for the inside of a man." Winston Churchill

1.1 Introduction

In the last century the horse changed from being a work animal to one whose primary use is in recreation. The importance of the horse and rider bond has received more attention and it has become important to consider the physical and psychological welfare of the horse as a companion animal. This has meant that certain practices involving horses that were accepted in the past are no longer considered appropriate.

Traditionally horse training and riding was male dominated. Recently there has been an increase in the proportion of female trainers and horse owners (Robinson, 1999). Female horse owners tend to be more affectionate toward their horse than male owners (Robinson, 1999). The increase in the number of females working with horses may be one of the reasons that Natural and other gentle horse training techniques have become hugely popular with horse owners and trainers around the world.

In most Natural horsemanship training techniques there is a step by step easy to follow approach (Parelli, 1993; Roberts, 1996). Thus persons with little horse handling abilities are able to follow the instructions and train their horses. Horse owners go to training days along with their horse, to watch and learn the methods. They can buy books, tapes and equipment so as to continue the training on their own (Parelli, 1993; Roberts, 1996). Thus it appears easier for the beginner horse owner to use easy solutions to form or improve their horse-human relationship. The major proponents of Natural, such as Monty Roberts, Pat Parelli and John Lyons, have come from traditional horse using backgrounds, such as rodeo, but are now promoting supposedly more effective and less aversive or "natural" ways to train horses.

However, it is unknown whether Natural horsemanship training methods improve the welfare of horses. This review looks at the background of horse training, and covers many of the factors that contribute to Natural horsemanship including the behaviour of the horse, domestication, the human-horse relationship, learning theory, how the Natural methods relate to learning theory, temperament and dominance.

1.2 Behaviour of the horse

Horses live in social groups called "bands". Bands consist of a small number of mares, their juvenile offspring, and usually a single dominant stallion (Linklater, Cameron, Stafford and Veltman, 2000). Within a band of horses a social hierarchy is formed. The dominant animal is usually one of the older mares. Living in a band is advantageous to the horse because it allows for better defence, increased chance of survival, and the opportunity for assistance in rearing young (Mendl and Held, 2001). It is likely that horses evolved to live in bands as an anti-predator behaviour (Goodwin, 1999; Houpt, 1979; Mendl and Held, 2001). A herd of horses comprises of a number of different bands, plus bachelor groups and incidental stragglers (Linklater *et al.*, 2000).

1.3 Domestication

It is thought that the horse was first domesticated around 4000-5000 B.C. (Levine, 1996). The first evidence of a selective breeding programme of horses comes from King Hamurabi of Babylon in 1700 BC. (Rubin, Oppegard and Hintz, 1980). Horses have been selectively bred to perform specific tasks and there are about 200 recognised breeds of horse. Some horses were selected for strength as draught animals, whilst others were selected for their speed or agility.

After their initial domestication horses were probably used for food and transport. Later the horse was ridden and became a means to transport people and to carry food and was used extensively by the military. As the need for horses as transport declined the use of horses for sport and recreation increased. In many countries horses are used for a variety of recreational activities such as racing, polo, show jumping, eventing, showing, dressage, hunting and recreational riding, and horses are often considered to be companion animals (Meehan, 1996).

1.4 Human-horse interaction

Many humans form close bonds with the animals they interact with. Dogs, cats and some farm animals may be given names and treated as companions depending on the familiarity and personal value their human handlers or owners have placed upon them. The attitude of humans towards companion animals may affect the behaviour of the animal. When the owner of a pony with behavioural problems changed their attitude toward the pony the behaviour of the pony improved (McNair and Hart, 1997).

The nature of the dominance that exists between horse and human has had little study, and it is unknown if it is the same as or just resembles the horse-horse relationship.

"Join-up" (Roberts, 1996) is one of the ways of accelerating the formation of a relationship between horse and human. The seven games taught by Pat Parelli are another method (Parelli, 1993).

A relationship may be forged in a few minutes or over a longer period depending on the nature of the horse and the person. Disruption of the human-horse relationship usually result in trauma and grief for both horse and human (Brackenridge and Shoemaker, 1996a; Brackenridge and Shoemaker, 1996b; McNair and Hart, 1997).

The human may be the only member of the pair that considers the bond to be serious. However, this is not to say that the horse does not feel some attachment to the human. Horses that live on their own may form a stronger bond with their human than horses that are living in groups of horses. This is due to the amount of time the horse spends in association with its equine herd in relation to the time spent with its human handler (McCall, Potter, Friend and Ingram, 1981).

When a person owns more than one horse they may form more significant bonds with specific individuals (Brackenridge and Shoemaker, 1996a). This may be due to the person having 'favourites' or it may be due to the length of time that the person has spent with each of the horses. In this sort of situation the preferred horse appears to be higher up in the group hierarchy because of their status in relation to the human. If a subordinate person owns a highly dominant horse the horse may dominate and control the person. In this type of situation it can be very dangerous for the owner, as they lack full control over their horse.

1.5 Learning theory

Learning is defined as the process of behaviour modification as a result of conditioning or prior experience (Anon). Horse training is a form of learning. Horses learn by habituation, operant conditioning, classical conditioning, discriminant learning, insight learning, observation, play or imprinting.

1.5.1 Habituation / Desensitisation

Horses live in a constantly changing environment and it is important that they are able to adjust to non-threatening and non-damaging stimuli. Habituation is a decrease in responsiveness to a given stimulus following repeated exposure (Houpt, 1998). Habituation is used to desensitise horses to novel stimuli that may be frightening but not painful (e.g. plastic bags, loud noises). This is done to make the horse safer to ride. This process is often referred to as "sacking". The horse is confined in a safe small area or held securely and the stimuli is introduced and repeated until the horse stops reacting to it.

1.5.2 Classical Conditioning

Classical conditioning is used to train an animal to perform behaviour when the original stimulus, that evoked the behaviour, is not present. The most famous examples of classical conditioning is Pavlov's dogs. Pavlov trained dogs to associate the sound of a bell with food, which caused an increase in saliva production. After the response was conditioned the dogs would salivate on hearing the bell without seeing the food (Figure 1-1).

An example of classical conditioning in the horse is the development of fear of a veterinarian. If a horse experiences pain when a veterinarian visits then a horse may quickly learn to fear a veterinarian.

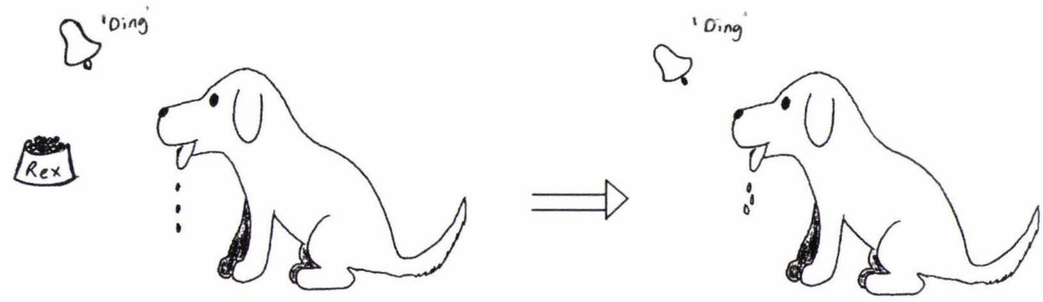

Figure 1-1 Classical conditioning

1.5.3 Operant Conditioning

A reinforcer or a punisher is delivered as a consequence of performing a behaviour. A common application of operant conditioning for horses is the use of automatic watering bowls. The horse depresses a lever at the bottom of the bowl and this behaviour is reinforced by the horse getting water (Figure 1-2). The ability for an animal to become conditioned depends on the stimulus, the task and the use of either punishment or reinforcement to back up the conditioning process. Several studies have shown that horses learn to press levers for food rewards (Dougherty and Lewis, 1991; Farmer-Dougan and Dougan, 1999). Operant conditioning is commonly utilised in horse training.

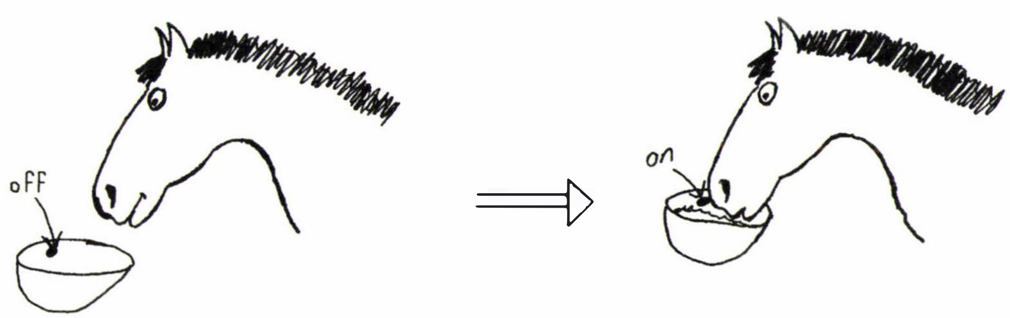

Figure 1-2 Operant conditioning: A horse using an automatic watering device to access water

Reinforcement and punishment are used to modify behaviours. If used correctly punishment and reinforcement can be use to efficiently train most animals. There are several factors necessary for the correct use of reinforcement and punishment.

Reinforcement

Reinforcement increases the likelihood of a behaviour occurring (Martin and Pear, 1992). Positive reinforcement is when a reinforcer, such as a food reward is used and a negative reinforcement involves the removal of something unpleasant, such as the release of pressure on a rope when the animal has submitted to the pressure (Mills, 1998a).

The following conditions must be met in order for reinforcement to be effective (Mills, 1998a):

Clearly identify the behaviour being reinforced, so as to not accidentally increase the occurrence of an undesired behaviour

Use a reinforcer that is appropriate for the situation

Reinforce only the desired behaviour

Reinforcement should occur immediately after the desired behaviour

Vary the reinforcement schedule after the stimulus – behaviour – reinforcer link has been established

There are problems with the use of reinforcement, these include (1) the accidental reinforcement of the wrong behaviour and (2) development of demanding behaviours or increasing aggressive behaviours, such as biting when a food reward has been used.

Clicker training combines classical and operant conditioning. A food reward is linked with a previously neutral stimulus, the clicker, so that the clicker becomes a positive reinforcer. Thus when a trainer is training a horse they are able to reinforce behaviours with a simple click. Clicker training has become a popular method of training in the horse world.

Punishment

Punishment is used to decrease the performance of a behaviour. Positive punishment is when something is applied as the punisher, such as a jerk on a lead chain and negative punishment is when something is taken away to punish the behaviour, such as the removal of attention or food (Mills, 1998a).

To use punishment effectively it is necessary to;

Choose an appropriate punishment for the behaviour and animal

Use it immediately after or during the behaviour that is to be punished

Change the punishment to prevent habituation, this may mean changing the punishment or its intensity

The delivery of the punishment must be consistent, that is the behaviour must be punished every time it occurs

Problems associated with the use of punishment include (Martin and Pear, 1992):

Punishment may not indicate the desired behaviour, and thus not help the animal make the correct decision next time

Improper application could lead to the horse being punished for the wrong behaviour

The animal may become desensitised to the punisher

Severe punishment may lead to an increase in other problem behaviours, such as fear and flight

The punishment may become associated with the person applying it and the animal may initiate avoidance or aggressive behaviours toward that person

The punishment may be seen as a reward if the behaviour they are performing is attention seeking

Punishment is used within animal groups to establish dominance (Clarke, Nicol, Jones and McGreevy, 1996). Therefore it can be useful for human trainers to understand how a horse is disciplined by its conspecifics, so that they may use this information to establish dominance over the horse.

Learned helplessness when a horse becomes reluctant to react to any stimulus, positive or aversive, due to fear of being punished, is sometimes associated with the excessive or incorrect use of punishment (Wolff and Hausberger, 1997).

1.5.4 Discrimination Learning

Discrimination learning is the ability to learn the differences between different visual, sound and tactile stimuli. Horses are able to discriminate between differences in colour (Fiske and Potter, 1979; McCall, 1989; Sappington, McCall, Coleman, Kuhlers and Lishak, 1997; Smith and Goldman, 1999), shape (Dougherty and Lewis, 1991), and pattern (Flannery, 1997; Hanggi, 1999; Mader and Price, 1980; Sappington and Goldman, 1994).

1.5.5 Insight Learning

Insight learning is also called concept learning. It is generally thought that only humans and the higher primates are capable of insight learning, and that it is not important in horses (Cooper, 1998). However, Sappington and Goldman (1994) suggested that horses are able to form simple concepts. Similarly Flannery (1997), found that horses were able to form concepts of sameness between patterns.

Insight learning occurs when the solution to a problem is reached on presentation of the stimuli, without trial and error occurring. If an animal is presented with a problem they can attempt to form a concept and solve the problem immediately.

1.5.6 Observational learning (Mimicry)

Observational learning occurs when an animal observes a demonstrator performing a task and is then able to successfully complete the task at the end of observation. Some people who keep horses in yards have noted that stable vices appear to be learned by observation of conspecifics performing them. Despite the anecdotal evidence, research (Baker and Crawford, 1986; Clarke *et al.*, 1996; Lindberg, Kelland and Nicol, 1999; Mills, 1998a) has failed to find significant evidence of observational learning in horses.

1.5.7 Play learning

Play learning is the name given to the learning that occurs by trial and error through interaction with conspecifics. In the horse, play behaviour is thought to play a role in the development of motor skills and learning social interaction skills (Waran, 2001). Play behaviour may be an important facilitator in the socialisation and development of bonds between individual members of a band. Occasionally play behaviour mimics the behaviours seen in adult members of the band, such as fighting and grooming, while other behaviours are exploratory, where the youngsters learn the limits of their abilities.

1.5.8 Imprinting

Imprinting was originally defined by Lorenz in 1935, as a process in which young birds bond and follow their mothers. Imprinting differs from other forms of learning in that (1) it generally occurs in a finite period immediately after birth, (2) it does not require reinforcement such as food reward, and (3) it is thought to be irreversible (Houpt, 1998). Imprinting is seen in many young mammals and birds that have been reared by humans, when the young animal follows the human as it might follow its mother.

Dr. Robert Miller has popularised imprinting in foals (Miller,1996). He claims that handling and 'imprinting' the foal in the first 3 hours after birth will cause an irreversible life long bond with the human to form. However the 'imprinting' done by Miller is largely based upon habituation and learned helplessness, the foal is restrained and exposed to various stimuli and not allowed to escape. Anecdotal evidence suggests that foal-imprinting is an effective training tool. Miller's technique includes training sessions in the first 3 months of life. Mal and McCall (1996) found that foals handled for 10 minutes each day in the first 42 days after birth were easier to handle than foals that were not handled until after 42 days old. It may not be the handling of the foal immediately after birth that is critical, but the regular gentle handling during the early development of the foal.

1.5.9 Factors which influence learning in the horse

Several factors influence learning in the horse. These include, prior experience, age, gender, breed, temperament and social status, as discussed below. When comparing the relative intelligence of different horses, all aspects of their temperament and management need to be considered (Mills, 1998a). An individual is the product of genotype, experiences and events that shape its current state of being. How learning ability is assessed may have an effect on the 'performance' of individuals; for instance if the task is one which is not natural then it may be more difficult for them to learn than a natural response (Mills, 1998a).

Prior experience

Regular quiet handling in the early weeks of life makes foals easier to handle later in life (Hanggi, 1999; Heird, Lennon and Bell, 1981; Levine, 1999; Mader and Price, 1980; Mal and McCall, 1996; Smith and Goldman, 1999). Mal *et al.* (1994) found that this early handling should take place in the first four months, but Mal and McCall (1996) found that handling was required in the first 42 days only. Early handling did not increase the rate of learning in a single-trial learning task (Mal *et al.*, 1994). It may be that horses that are less reactive appear to the trainer to be easier to train and hence learn faster than less well handled horses (Mills, 1998a).

The time spent training and the number of conditioning trials also influence the rate of learning in horses. Training was most effective when spaced over time, and too many training sessions in a short succession did not aid the learning of the horse (Mal, McCall, Newland and Cummins, 1993). Some authors (Rubin *et al.*, 1980) suggest that more short training sessions make for faster learning than one long training session. Short lessons may prevent the animal becoming bored and lessons may focus on specific points of interest.

Age

Young animals may learn faster than more mature animals and the age at the time of training may affect the rate and the ease of learning (Mader and Price, 1980). This may be due to the fact that the majority of learning occurs when an animal is young and the brains of young animals may be wired up for rapid learning. Mader and Price (1980) found that the performance in a learning test declines with age. However, Wolff and Hausberger (1997) found that there was no significant effect of age on the learning found more 'emotional' horses learned slower but that emotionality had no effect on the trainability of those animals. This suggests that while more emotional horse may take longer to train or they may need more assistance while learning, they are able to perform as well as less emotional horses. Differences in the learning ability within breeds and between different stallions lines have been demonstrated (Horohov *et al.*, 1999).

Social Dominance

Studies (Heird, Lokey and Cogan, 1986; Houpt *et al.*, 1982; ; Mal, McCall, Cummins and Newland, 1994; Mal *et al.*, 1993) have shown that a horse's social status has no significant effect on its ability to perform in tests of learning ability. However horses that are middle and bottom of the pecking order may have a greater sensitivity to stimuli or cues.

1.6 Traditional horse training

Historically the breaking and training of horses has been an aggressive activity. It depended on restricting the horses' movements and preventing the horse from fleeing the human trainer, and repeatedly exposing the horse to a series of stimuli. Such traditional methods include (1) confinement of the horses in a chute, (2) being saddled and ridden until bucking ceases, (3) being led into water or mud so that they are unable to run away, and (4) tying up, and being thrown the ground. Many traditional training methods involve physical and or psychological trauma in some form. Horse breaking sessions often involved the horse being physically restrained by having their limbs tied and or being thrown onto the ground. The horse might be beaten as punishment for not submitting to the demands of the trainer.

The lip-chain and many other traditional horse training aids are based on positive punishment. The horse performs an undesired behaviour, such as pulling away from medical treatment, and it receives punishment, a short sharp jerk on the lip-chain. However, some traditional methods such as "sacking-out" habituate the horse to many stimuli slowly and reasonably gently.

1.7 Natural horsemanship

Natural horsemanship is the term given to a number of techniques of horse training, and include "Round-pen training" and "Parelli Natural Horsemanship". Natural horsemanship is not a new concept, many trainers have been practicing this sort of horse training for years. The recent upsurge in popularity is due to changes in the mindset of the horse owning public who are probably no longer happy with having a horse that does as it is told, but wanting a willing equine partner.

1.7.1 Parelli Natural Horsemanship

Pat Parelli devised the Parelli Natural horsemanship programme (PNH). The training principles of PNH are supposedly based on the language of the horse. Parelli has organised the basic interactive behaviour of horses into seven 'games' horses play with other horses. These seven games are designed to get the horse used to touch and yield to pressure when asked by humans. The seven games mimic the grooming, interaction and punishment normally seen in bands of free roaming horses to establish dominance over one another. PNH aims to make the human the alpha horse by making the human win all the seven games (Parelli, 1993) and become dominant over the horse.

Parelli claims that punishment and predatory behaviours are not natural and not part of the PNH training system (Parelli, 1993). Punishment for non-submissive behaviour is common in the establishment of dominance over another individual (Clutton-Brock and Parker, 1995). Punishment can be either physical or psychological, and psychological punishment is the main type found in PNH training.

1.7.2 Round-pen training

Round-pen training is the name given to the method of horse training that utilises a round-pen to train horses. Monty Robert's process of Natural, called 'Join-up', is one version of round-pen training. It also involves the natural language of the horse and development of a human-horse bond. Roberts' claims to have developed his system of horse training through many hours of observing large groups of feral horses.

"Join-up" combines several types of learning theory, but for the most part is based upon punishment and reinforcement. Firstly, when the horse is doing what the trainer wants, i.e. coming to the trainer rather than avoiding him/her, the behaviour is positively reinforced by the trainer assuming a neutral body posture (Figure 1-3) and rubbing the horse's head. Secondly, when the horse does not do what the trainer wants, i.e. the horse does not stand with the trainer or does not look at the trainer, the behaviour is punished by the trainer assuming an aggressive body posture and forcing the horse to move around the round-pen (Figure 1-4). If the horse refuses to move in towards the trainer when made to change direction it is forced to continue moving around the round-pen. The trainer controls the speed and direction of the horse's movement around the round-pen and assumes a dominant role.

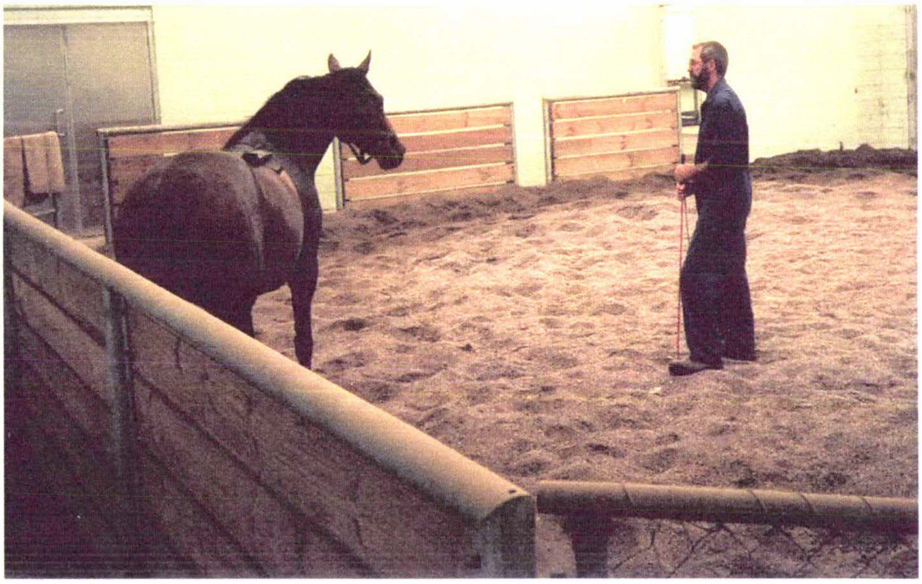

Figure 1-3 Round-pen training, negative reinforcement, the trainer has taken the pressure off the horse by assuming a neutral body posture and allowing the horse to stop moving, as long as the horse stays with the trainer

The power of the "join-up" process lies in the bond formed between human and horse. This bond results from the trainer exerting psychological dominance over the horse. The bond formed during the "join-up" may not be as stable or strong as a bond that has been formed over many hours of good quality handling, and it may not last for more than a short period of time.

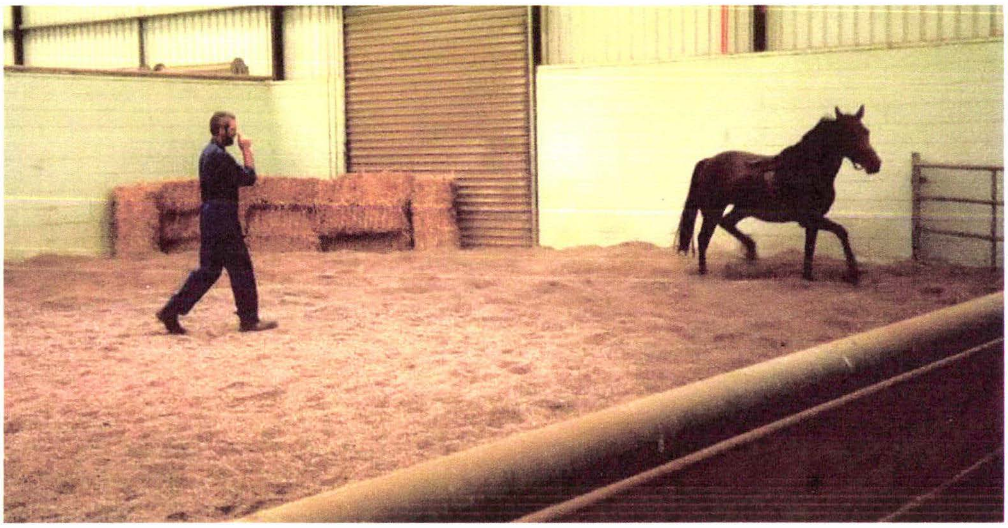

Figure 1-4 Round-pen training, positive punishment, the trainer driving the horse away and controlling the horse's direction of movement

"Join-up" is achieved by reinforcing submissive behaviour and the horse accepting the trainer as a surrogate band-leader. Submission in the "join-up" process is achieved in much the same way as a band-leader would establish dominance, for example by controlling movement, punishing undesired behaviours and or reinforcing desired behaviours. The method is called "join-up" because this is the name given to the end point where the horse and human have bonded and the horse willingly follows the human.

The trainer controls the horse's speed and direction of movement by a combination of posture and position. The trainer assumes an aggressive body posture, tall and square facing the horses, and positions himself in a driving position in relation to the horse (Figure 1-4) (Roberts, 1996). When the trainer wants the horse to turn he alters his position in relation to the horse's flight zone, so that he comes in front of the horse and the horse will turn away. As the "join-up" procedure continues the horse begins to show signs of submission, lowering of the head, a fixed ear hold on the trainer and licking the lips. Once these desired signs of submission are seen, the horse may be asked to stop and turn towards the trainer. If the horse turns towards the trainer the behaviour (looking at the trainer) will be reinforced by the trainer adopting a neutral posture and staying still (Figure 1-3). However if the horse does not look toward the trainer then it will be made to move around the ring until such time as it does look at the trainer.

1.8 The temperament of the horse

Temperament is defined as the psychological nature of a person or animal that defines its behaviour (Anon). Temperament is often used to describe various facets of an animal's personality that contribute to the way it acts in a given situation. The facets of personality that are most relevant during animal handling include, reactivity, handling ability and friendliness.

Reactivity or stability in novel situations has been called emotionality (Wolff, Hausberger and LeScolan, 1997). A reactive or highly emotional horse is likely to react to the sudden presentation of novel stimuli with fear and anxiety. It responds in the most basic manner, which is to assume that the stimulus is harmful and flee. Horses that are less reactive or emotional are less likely to respond to a novel stimulus fearfully. Personality and emotion have been assessed in a number of studies involving horses (Anderson, Friend, Evans and Bushong, 1999; Le Scolan, Hausberger and Wolff, 1997; Mills, 1998b).

Handling ability is important when deciding on the prospective use of a horse. Horses that are easy and safe to handle are less likely to cause injury to humans or other animals. Horses that are less reactive and more relaxed around humans and novel situations are likely to be easier and safer to handle. Horses that are easier to handle are less likely to require twitches, lip chains or anti-rearing bits when being handled which may reduce potential welfare compromise caused by rough handling.

The ability for a horse to interact easily with other animals and humans is an important element in temperament. Horses that interact easily with humans are often considered more suitable for use as companion or recreational animals (Schuppli and Fraser, 2000). Horses that are relaxed in the presence of humans are less likely to be stressed during routine management and handling procedures. In situations where only one horse is owned, its only social contact may be the person caring for it.

When scoring temperament the horse should be observed under conditions that reflect the normal situation in which that horse would be used (Anderson *et al.*, 1999; Le Scolan *et al.*, 1997; MacKenzie and Thiboutot, 1997; Mills, 1998a). It is important that the person scoring the temperament of the horse is familiar with horses and has had some experience with the horse to be scored (Le Scolan *et al.*, 1997; Manteca and Deag, 1993; Mills, 1998b). One way of scoring the reactivity of horses is to measure their response to a novel stimulus (Anderson *et al.*, 1999; Le Scolan *et al.*, 1997; MacKenzie and Thiboutot, 1997; Wolff *et al.*, 1997). This method is often used in selecting horses for therapeutic riding programmes where it is essential that the horse is extremely placid.

1.8.1 Factors affecting temperament

Breeding - Some breeds of horse are said to be pleasant, cooperative and of a nice temperament and others are easily excitable or volatile. Many of the tests (Le Scolan *et al.*, 1997; Visser, van Reenen, Hopster, Schilder, Knaap, Barneveld and Blokhuis, 2001; Wolff *et al.*, 1997) for reactivity have been carried out in groups from the same breed and similar ages and backgrounds so there is little scientific evidence of differences in temperament between breeds.

Prior experience – It has been shown that the way in which a horse is reared and handled from birth is likely to impact on its temperament and ease of handling as an adult (Heird *et al.*, 1981; Heird *et al.*, 1986; Jezierski, Jaworski and Gorecka, 1999; Mal and McCall, 1996; Mal *et al.*, 1994). Young horses that have had minimal contact with humans are likely to be more reactive than the same horses after several positive handling experiences (Jezierski *et al.*, 1999). However it is important that the experiences of early handling be postive, as negative experiences can cause the horse to develop fear of people, places or things associated with the experience (Jezierski and Gorecka, 1999).

Individual variation - All horses are individuals. Some of this variation can be explained or influenced by breed, sex, how the animals were reared or by the horses' prior experience (Manteca and Deag, 1994; Mills, 1998b). The background training of a horse could be used as a good predictor of the likely temperament that horse may have. However there are also many other factors which shape the temperament of each horse, including the amount and quality of handling it receives as a foal, and as a yearling.

1.8.2 Temperament affects the way horses learn and respond to stress

The temperament of a horse may have an impact on the ease with which it can be trained. Horses that are more highly reactive tend to learn less quickly than horses that have less reactive personalities (Heird *et al.*, 1986; Wolff *et al.*, 1997). Some breeds of horses that are known for their intelligence and ease of handling are generally horses with a relaxed and easygoing personality.

Horses that do not react rapidly to novel stimuli may have a lower stress response in novel situations (Mills, 1998b). Strains of mice, cattle and other animals have been selectively bred for temperaments, which allows for low stress during handling. It is possible that animals with a delayed or minimal stress response to stimuli may be better able to cope with changes or challenges in their environment. By decreasing the animal's responsiveness to stressful stimuli or dampening the physiological response to stressors the impact of stress on the health and well-being of the animal is reduced and related welfare compromise can be reduced (Manteca and Deag, 1993).

1.8.3 Scoring, measuring and observing temperament

The way a horse reacts towards or interacts with a person will have an effect on that person's judgement of the horse's temperament. For instance, a person may be biased towards a specific type of horse or regarding the type of temperament they assign to the animal. Most tests of emotionality or temperament in horses are based upon the horse's behavioural reactions to novel stimuli, such as an umbrella, walking across a series of different footings or an obstacle course (MacKenzie and Thiboutot, 1997). Tests of horses' temperament based upon opinion of observers after viewing an animal under given conditions are common (Anderson *et al.*, 1999). Many of the 'temperament' tests are subjective and there is no specific quantification of what temperament actually is.

It is difficult to group horses according to temperament type as there is a large amount of variation in behaviour between individuals (Manteca and Deag, 1993; Visser *et al.*, 2001). Individual horses may be friendly with one person and reactive and flighty with other people.

1.9 Dominance

The dominance status of a horse may have implications for the training of that horse in that more dominant horses may learn at a different rate and way to less dominant horses. In addition, the type of training method may need to be changed to incorporate the social rank of the horse. For example it may be pointless and potentially dangerous to try to force an alpha horse to do something, when more suitable training methods are available.

Hierarchies in horses tend to be linear with few complex interactions (Houpt, 1979; Weeks, Crowell-Davis, Caudle and Heusner, 2000). The relationship at the top of the hierarchy is often easily determined but near the bottom it may be more complicated (Houpt, Law and Martinisi, 1978). Stallions are not always dominant over mares (Keiper and Sambraus, 1986). Mature horses, older than 5 years, tend to be dominant over juveniles (Houpt, 1979; Houpt, 1979; Houpt *et al.*, 1978; Keiper, 1986; Keiper and Sambraus, 1986). Houpt, Law and Martinisi (1978) found no correlation between the age or size (weight and height) and the rank of a horse; however aggression was a important factor in determining status. Keiper and Sambraus (1986) found no correlation between the age, intra-group relationships or maternal position on the rank of juvenile or adult offspring. Ellard and Crowell-Davis (1989) found that the smaller younger mares were lower down the rank than the older larger mares.

Hierarchies tend to be stable when the horses within a group are constant (Houpt and Wolski, 1980). However, Keiper and Sambraus (1986) stated that in the feral herds of horses they studied the hierarchies were not stable over the four year time period they observed the animals. Keiper and Sambraus (1986) gave the rank order for three bands in 1978 and 1981. The horses in two of the bands appeared to be the same with only one or two older horses changing position. They were high up in rank in 1978 and after 4 years had been displaced as the new generation of leaders took over control of the band.

Dominant mares will punish subordinates and juveniles for undesired behaviours. A pair of individuals that have a bond formed between them may tend to spend a greater portion of their time together, grazing grooming and playing. Once dominance is established the alpha mare need only threaten lower ranking group members in order to gain access to resources or reprimand that individual (Houpt, 1979; Keiper, 1986; Weeks *et al.*, 2000).

In groups of domestic horses the hierarchy behaviour may be exaggerated (Weeks *et al.*, 2000), due to close confinement and competition for space, food and attention. In addition when specific members are removed for the group for extended periods of time the hierarchy has to be re-established on a regular basis.

Horses primarily communicate by body posture, but also use vocalisation, touch and smell to communicate (Keiper, 1986; Rubenstein and Hack, 1992; Waran, 2001). Communication based on body posture can range from being obvious (kicking or charging) to being very subtle (relaxation of the face and a change in the direction of attention) (Waran, 2001). Such postural changes are the basis of round-pen training of horses.

Aggressive behaviours are used to establish and maintain dominance within the hierarchy. The aggressive behaviours most often used include bites, threats to bite, kicks, other threats and chasing. Some submissive behaviours seen in younger horses, and sometimes in older horses, are snapping or clacking of the teeth (Houpt *et al.*, 1978) and agitation behaviours (Weeks and Beck, 1996).

Horses higher up the hierarchy are often more aggressive than those lower down (Araba and Crowell-Davis, 1994; Ellard and Crowell-Davis, 1989; Houpt *et al.*, 1978; Houpt and Wolski, 1980; Weeks *et al.*, 2000). Aggressive acts tend to be displayed to those lower ranked horses most closely related in rank to the aggressor (Araba and Crowell-Davis, 1994; Arnold and Grassia, 1982; Ellard and Crowell-Davis, 1989). Horses that occupy the lower ranks in the hierarchy are often subject to more agonistic interactions than higher ranked horses (Araba and Crowell-Davis, 1994; Arnold and Grassia, 1982; Houpt *et al.*, 1978).

Preferred associates often receive less aggression than the next nearest in social rank (Ellard and Crowell-Davis, 1989; Weeks *et al.*, 2000). Behaviours such as grazing, resting and grooming with each other serve to maintain a 'friendly' bond and reduce social tension between preferred associates. Time spent with preferred associates is unaffected by the dominance rank of the horses involved (Kimura, 1998). The formation and maintenance of preferred associate bonds appear to be critical to maintaining the group structure with a minimum of aggression (Waran, 2001).

1.9.1 Measuring dominance

There are two main methods used to measure social status within groups of horses. The first method involves observing free ranging animals and counting all aggressive interactions (Araba and Crowell-Davis, 1994; Arnold and Grassia, 1982; Keiper and Sambraus, 1986; Kimura, 1998; Weeks *et al.*, 2000). A matrix of each individual's wins or losses against each other member of the group is used to establish that individual's rank in the group. The second method is the pairing of individuals. The dominant horse is the one that controls access to the food (Houpt *et al.*, 1978; Houpt and Wolski, 1980). A combination of both methods (Ellard and Crowell-Davis, 1989) may give a better idea of the true nature of the dominance relationships within the group. Field observations are most often used for studying dominance in feral horses.

1.10 Animal welfare

In industrialised societies horses no longer serve an essential function and they are used for recreational purposes (Olsen, 1996). However, in some countries the livelihood of many people is still dependant on the horse and farmers use them for ploughing, farm work and transport (Olsen, 1996). Whatever the reasons humans keep and use horses, it is our duty to provide the best possible treatment for the animals in our care.

The definitions of animal welfare are numerous. In the present study animal welfare is considered to cover the physical and mental well-being of the horse. The welfare of any individual animal is variable and could be considered to be at some point on a continuum, from poor to high.

There are many different facets in the care of an animal which contribute to its welfare. These facets are sometimes called the five domains of welfare, which include environment, nutrition, health, behaviour and mental well-being (Mellor and Stafford, 2001). These domains are discussed below in relation to the horse.

Environment. The horse should be provided with a safe, comfortable environment and exposure to extremes of weather or temperature should be minimal. If the horse is exposed to an environmental challenge it should be provided with the freedom to move in order to meet its requirements for comfort.

Nutrition. Horses should be provided with sufficient quantity and quality of food and water to meet their nutritional and physical requirements. Depending on the age and use of the animal these needs will change over time, for instance during growth or pregnancy. The stabling of horses reduces the quantity of grass horses are able to consume. However many stabled horses are fed a concentrate diet that is formulated to provide for the nutritional and energy needs of the horse. Such diets though often consist of foods that may not normally form part of a horse's diet and may lack the variety that a horse would normally encounter. There are problems associated with feeding high concentrate diet to horses.

Health. Horses should be maintained in good health and injury free. The health of the horse can be consistently monitored and treatment provided readily. Ill horses tend to have impaired performance and decreased productivity, so it is in the best interest of the owner to maintain a high level of health.
Behaviour. Horses should be allowed to express a normal range of behaviours. The presence of abnormal behaviours can be interpreted as an indication of poor welfare. Abnormal behaviours such as self-mutilation and crib-biting, indicate poor welfare of that horse.

Mental state. Exposure of horses to stimulus that cause extreme fear or anxiety should be avoided or minimised. Some people might consider psychological stress to have a less significant impact than physical stress. In some situations such distinctions and ranking are not clear. For example, round-pen training may appear to be the better option when compared to methods of training where the horse is at risk of physical injury, but it is not obvious that if there is psychological distress caused by the round-pen training procedure that it is any less aversive than physical stress or injury.

All five domains contribute to the welfare of a horse. It is not likely that in any given situation that each of the domains contributes equally to the welfare of an animal. For example nutrition and environment may be of high quality but behaviour may be restricted. For an individual animal one domain may be more important than another domain.

The increased popularity of Natural training methods reflects the change in attitudes of people involved with horses; it is unlikely that the changes in attitude are due to societal objections to traditional methods.

Some traditional methods used in the 'breaking' and training of horses can result in physical injury and mental trauma, which can affect a horse's health and well-being for the rest of its life. The differences between the horses' natural environment and the environments in which humans keep them also represent potential sources of welfare compromise. Another welfare issue is the chronic stress caused by regular rough handling or restraint, and long-term ill effects of the breaking or training process. The purpose of the present study is to examine some of the behavioural and physiological effects of round-pen training on horses, and the impact on the welfare of horses of potential effects of this training.

1.11 Assessing welfare in the horse

There are three basic factors to be considered when assessing the welfare of horses (Figure 1-5). Firstly, sociology or how humans perceive how the horse feels or copes with a situation. Secondly, the horse itself: what can we learn about the welfare of the horse from its behaviour and physiology? Finally, the environment: what external factors affect the welfare of the horse, such as food, social interaction, shelter? These three factors will be discussed in the following sections.

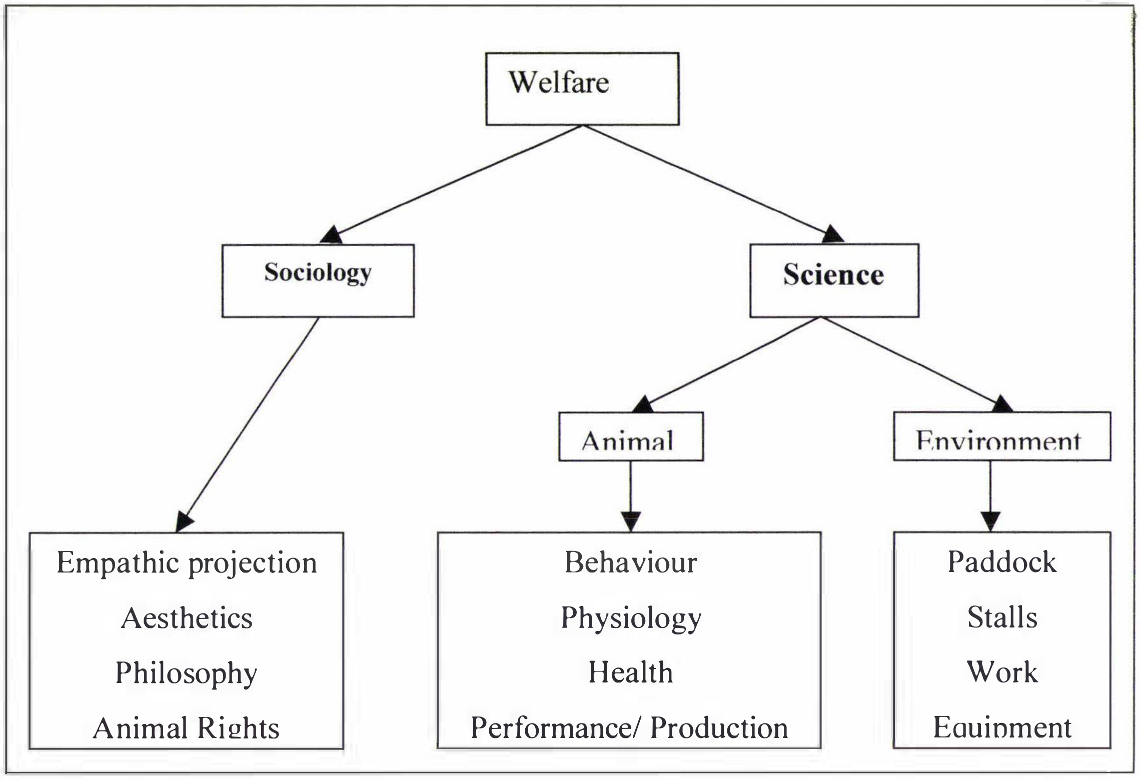

Figure 1-5 Aspects of the welfare of animals

1.11.1 Sociology

The sociological aspect of animal welfare depends on the interpretation of the animal's situation by human observers. The human viewpoint may vary depending on the moral standing, emotional involvement and experiences of the person involved. There are several different sub-categories of the sociological aspect of animal welfare, such as empathic projection, aesthetics, philosophy and animal rights.

Empathic projection is when the person assumes how the animal may feel based upon how that person would feel if they were in that situation. For example if I were a horse would I prefer to live outside or indoors? The main problem associated with this is that animals may not perceive situations in the same way as humans do.

Aesthetics looks at the appearance of the animal to assess welfare. For example if a horse is in a paddock with no grass and appears to be undernourished and unwell, it is assumed that the welfare of that horse is poor. The aesthetics of how animals are managed has become very important factor in the change in public opinion regarding the welfare of intensively farmed livestock. Aesthetics can be affected by empathic projection, through the person becoming emotionally involved in the situation. This may be one of the reasons why Natural training methods such as "join-up" have become so popular, with claims of non-aversive force being used and the horse being turned into a willing partner, and the fact that it appears to do no harm to the horse. Although the trainer may not physically restrain or harm the horse, it does not necessarily mean that the horse finds the procedure any less stressful than being tied up and forced into submission. Horses may find the social isolation, psychological pressure and physical activity associated with the "join-up" process a significant source of stress. Any short or long-term effects of "Join-up" on the behaviour and well-being of horses are unknown.

Philosophical reasoning allows us to be aware that animals, as sentient beings, are capable of experiencing pain or suffering. Also just because they are animals does not mean that they do not deserve equal consideration of their interests. Animal rights, takes the theory that animals are sentient beings capable of suffering and feeling emotion to an extreme. It suggests that animals have a right to be treated the same as we would expect humans to be treated. For example do we have the right to kill animals or force them to live in captive conditions, no matter how well they are treated.

1.11.2 Science

The scientific measurement of animal welfare uses the animal and the environment to assess the welfare of the animal. The information that can be gathered from horses includes behavioural changes, physiological changes, health, production/ performance and immunocompetency. The quality and input of different aspects of the environment have an impact on the horse's physical and mental well-being, and such aspects include the immediate environment the horse is kept in, the work the horse has to do and the people handling the horse.

Behaviour

Generally changes in behaviour are the first sign that a horse is trying to maintain its well-being (Matthews, 1992; Mench and Mason, 1997). When exposed to an adverse stimulus a horse will generally try to escape; if escape is not possible it will assume a range of other behaviours (for example, agitation and avoidance), to help it cope with the stressor. The measurement of behaviour requires no physicaly invasive measurement (Mench and Mason, 1997).

The behaviour of horses can be classified into three types, acute, reactionary and abnormal (Parker, 2003).

Acute – this is probably the most significant type of behaviour associated with the flight or fight reaction. Some examples of acute behaviour would be the performance of a normal behaviour either at a higher frequency than normal or not in their normal context (licking and chewing when no food is present). Another form of acute behaviour in horses is agitation (Weeks and Beck, 1996). Examples of agitation behaviours include rearing, pulling back, attempting to escape, threatening to bite or kick, and vocalisation.

Reactionary - The horse is exposed to a specific stimulus, which evokes a specific or known response.

Stereotypies – A behavioural pattern or repertoire that is repetitive, invariant and has no obvious goal or function (Mason, 1991). Some examples of stereotypies in the horse are stall walking, crib biting, wood chewing, wind-sucking and weaving. Equine stereotypies are apparently caused by frustration (Dellmeier, 1989), boredom, anticipation of activity (such as feeding or exercise) and inherited predisposition (Vecchiotti and Galanti, 1986).

Changes in a horse's normal repertoire of behaviours (frequency, duration, new behaviours, or abnormal behaviours) can be regarded as indications of change in that horse's well-being. The incidence of abnormal behaviours or the absence of a normal behaviour reflect changes in the horse's mental state and indicate that the animal's basic needs are not being met. For example horses experiencing social isolation stress may be off their food and display more locomotion (i.e. pacing) and other agitation behaviours (Araba and Crowell-Davis, 1994; Arnold and Grassia, 1982; Houpt *et al.*, 1978; McGreevy, Cripps, French, Green and Nicol, 1995; Waran and Henderson, 1998). Some examples of agitation behaviours include involuntary muscle tremor, lip flapping, weaving, pacing, pawing the ground, stamping the feet, swishing the tail, ear flattening, wrinkling the nose and head tossing or shaking (Weeks and Beck, 1996).

Changes in the frequency of certain behaviours that are normally seen may indicate that a horse is attempting to cope with a less than adequate environment. For example if a horse is sick it may eat less so there is a reduction in the foraging and ingestive behaviours.

When an animal finds a certain stimulus unpleasant or aversive then it may begin to avoid the source or locations associated with that stimulus. Avoidance behaviours may develop from punishment associated with that stimulus. The horse encounters a neutral stimulus such as a gate, but if the gate has accidentally become electrified the horse will then begin to associate the gate with getting shocked and hence avoid the gate. The cause of the aversion may be unknown and less knowledgeable owners might further reinforce the aversion by punishing the avoidance behaviour. For example the if the horses pulls back from the gate to prevent being shocked and gets punished by the handler for pulling back; the horses will further associate the gate with punishment and avoid it even more forcefully.

Problems with using behaviour to measure welfare

There are several problems associated with the use of behaviour to assess welfare. The first problem associated with the observation of behaviour is to know which behaviours being performed by a horse are indicative of distress (Mench and Mason, 1997). The display of abnormal behaviours is easy enough to use as an indicator of something being wrong, but may not give an indication of how aversive the horse finds that stimulus (Mench and Mason, 1997). Changes in the duration or frequency of behaviours that are normally seen may indicate changes in the horse's mental state due to stress or distress (Mench and Mason, 1997). The performance of some normal behaviours out of context, such as licking and chewing when no food is present, may be indicative of the horse experiencing problems (Mench and Mason, 1997).

Secondly the method used to record the behaviours should not affect the behaviours shown by the horses. Ideally the observer should be out of sight of the horse or video cameras should be used. All behaviours seen should be recorded. Some behaviours, such as steps, are easier to observe and record than others due to their being distinct, whereas other behaviours, such as licking and chewing, are not easy to count so timing the duration of the activity may be a better way to record such behaviour.

The next problem is to ensure the conditions where and when behaviour is being recorded are as close to normal as possible, so that behavioural measurements made accurately reflect the behaviours under normal management. For example, the presence or absence of conspecifics during a test may alter the results. In horses, isolation from herd-mates is a significant stressor (Keiper, 1986). However if the conditions of observation (i.e. "Join-up") would normally involve one isolated horse then such observations should be carried out with only one horse. If it is not suitable to have another horse there, due to space restrictions or problems with distraction, then the test horse should be habituated to being in the test area alone or conspecifics should be within visual range of the test horse; the presence of a relaxed companion there during handling may calm the horse during treatment (Wolfle, 2000).

The treatment group needs to be compared to a control group to know what the difference in behaviour is that results from the treatment. This control group may be horses observed in free ranging conditions, or observed before treatment, or a group of horses that remains untreated but is held near the test horses. In some circumstances when the control group may need to have some handling or restraint in order to take samples or make observations.

Physiology

In response to a stressor the body undergoes changes to the normal physiological function, which assists the animals ability to cope with the stressor. These changes are involuntary and controlled by sympathetic and parasympathetic divisions of the autonomic nervous system. The autonomic nervous system is activated by signals from the brain after the perception of a stressor or aversive stimulus.

Sympathetic division

Physiological changes controlled by the sympathetic division are heart rate, respiration, and endocrinological changes. These changes provide the animal with the necessary ability to escape danger and facilitate response to injury.

Heart Rate

The heart rate of an animal increases when exposed to a stressor (an aversive stimulus, or exercise) (McCann, Heird, Bell and Lutherer, 1988b; Minero, Canali, Ferrante, Verga and Odberg, 1999; Stewart, Foster and Wass, 2003; Waran, Robertson, Cuddeford, Kokoszko and Marlin, 1996). When heart rate data are used in conjunction with behavioural observations, they can be a useful measure between different stressors and how the animal interprets them (McCann, Heird, Bell and Lutherer, 1988a; Minero *et al.*, 1999; Pollard and Littlejohn, 1995; Stewart *et al.*, 2003; Waran *et al.*, 1996).

Respiration rate
The sympathetic nervous system causes the dilation of the airway, dilation of alveolar capillaries and an increase in respiration rate, so as to increase the supply of oxygen to the rest of the body (Bray, Cragg, Mackinght, Mills, and Taylor, 1994).

Endocrinology
Hormones are released into the blood stream to effect changes in metabolism, making energy available for the body to use for coping with the stress and recovering afterwards. There are two major endocrinological systems involved with the stress response in animals, the adrenaline /noradrenaline system and the Hypothalamic-Pituitary-Adrenal system (HPA) and the (Foreman and Ferlazzo, 1996).

Adrenaline and nor-adrenaline are released on exposure to the stressor. Blood plasma concentrations rise immediately and rapidly return to base levels. Adrenaline causes the following changes in the body: decreased flow of blood to the skin, gastrointestinal system and kidneys, and increased blood flow to the heart and skeletal muscle system, dilation of the pupil of the eye, increased airway diameter, increased blood glucose (Bray, Cragg, Mackinght, Mills, and Taylor, 1994). Adrenaline also causes a decrease in the production of saliva, which results in a dry mouth, a water conservation mechanism associated with the fight or flight response. There are other hormones that are sometimes used to measure the stress response in horses, including beta-endorphin (Canali, Ferante, Mattiello, Sacerdote, Panera, Lebelt and Zanella, 1996; Hydbring, Nyman and Dahlborn, 1996) and arginine vasopressin (Alexander, Irvine, Ellis and Donald, 1991; Nyman, Hydbring and Dahlborn, 1996).

The Hypothalamic-Pituitary-Adrenal system(HPA) is also activated in response to contact with a stressor. Stress causes the release of cortisol from the adrenal cortex into the blood stream of the animal (Bray, Cragg, Mackinght, Mills, and Taylor, 1994).

Changes in plasma cortisol concentration have been used to measure stress in the horse (Alexander and Irvine, 1998; Alexander *et al.*, 1991; Foreman and Ferlazzo, 1996; Guthrie, Cecil and Kotchen, 1980; Hoffsis, Murdick, Tharp V.L. and Ault, 1970; Houpt, Houpt, Johnson, Erb and Yeon, 2001; Hydbring *et al.*, 1996; Irvine and Alexander, 1994; Pell and McGreevy, 1999; Pitman, Ottenweller and Natelson, 1987; Snow and MacKenzie, 1977). The time from exposure to the stimulus, the duration of elevation above baseline, the rate of increase and the total change can be used to measure the response of the horse to certain stimuli.

Problems associated with the collection of blood for measuring plasma cortisol concentration in the horse

Taking a blood sample by venepuncture may be a source of pain and distress, which causes a rise in plasma cortisol itself. James *et al* (1970) found that venepuncture did not profoundly alter normal plasma cortisol concentration in ponies which were used to regular blood sampling. Alternatively animals can be cannulated to avoid injury caused by repeated venepuncture sticks or a remote sampling method could be used (Cook, Mellor, Harris, Ingram and Matthews, 2000).

Taking blood samples from the control group may involve some restraint, which may be stressful and alter the plasma cortisol concentration. If the stress of handling treatments was being measured, it may be better to have the control group remotely sampled (Cook *et al.*, 2000) under free ranging conditions.

Plasma cortisol is subject to a circadian cycle (Irvine and Alexander, 1994; Kurosawa, Takeda, Nagata and Mima, 1997). Cortisol secretion in undisturbed horses peaks between 6-9am and has a trough between 7-11pm (Irvine and Alexander, 1994). This cycle may become altered or shifted in horses that are in highly managed daily routines (Irvine and Alexander, 1994). Taking all the blood samples within a short time will help to negate the effect of time of day or samples may be taken over several days from the same horses at the same time each day to minimise the effect of circadian rhythm on plasma cortisol.

There is a time lag of about 20-30 minutes between when HPA triggers the release of cortisol and when plasma cortisol concentration maximum concentration. It is important that the sampling protocol allows for this so that the peak cortisol is measured at an appropriate time. It may be necessary to decide if plasma cortisol will provide an accurate indicator of the onset of stress or whether it should just be used to measure the intensity of the stressor (Mellor, Cook and Stafford, 2000). Other hormones such as adrenaline peak more rapidly but are shorter acting in the blood and their usefulness may vary depending on the situation (Mellor *et al.*, 2000).

The response of plasma cortisol concentration is not specific to one stressor, but integrates effects of all stressors interpreted by the horse, including uncontrollable external factors. Acute exercise (Alexander *et al.*, 1991; Foreman and Ferlazzo, 1996; James, Horner, Moss and Rippon, 1970; Snow and MacKenzie, 1977) is one of the major causes of plasma cortisol increase in the normal horse. Therefore the cortisol response of the horses seen during training may be an effect of both stress and exercise. Habituation to procedures associated with the treatment, removing external stimuli and resting the horses prior to testing will all aid more accurate measurement of plasma cortisol concentration. Exercise induced increases in plasma cortisol will remain elevated for at least 30 minutes following exercise having a minimum duration of 10 minutes (Snow and MacKenzie, 1977).

The cortisol response of different individuals may depend on each horse's interpretation of the stimuli and the variation in the physiological status of the horse. Factors that may contribute to variation in physiological response between individuals are: genetics, health, nutrition, weight, fitness, and hierarchical status. Pairing of similar individuals in control and experimental treatments (Manteca and Deag, 1994), or using larger groups or using groups of individuals from similar backgrounds (breed, age, management, sex) may help to minimise individual differences.

Parasympathetic nervous system

The parasympathetic nervous system is stimulated following the initial sympathetic response, which generally starts after the stress has ended. The parasympathetic response helps conserve energy and aid the body to recover after the stress.

Physiological changes associated with the parasympathetic response are generally opposite to the effects of the sympathetic response.

Some physiological changes associated with the parasympathetic nervous system are decreased heart rate and constriction of the airways, increased blood flow to the skin, kidneys and gastrointestinal system, increased enzyme secretion in the gut, increased gut motility, and increased saliva production. All of these changes appear to prevent further energy loss due to the high physical activity, aid digestion to provide energy input into the body, and to re-establish homeostasis in the body.

Health

Emotional and physical stress can cause horses to become depressed, lethargic, lose appetite and weight. Stress can limit a horse's ability to recover from illness and injury. Therefore horses that have had a poor record of health and that are exposed to regular, chronic stress may have more frequent lapses of illness. Horses involved in strenuous physical activity such as eventing or racing may take longer to recover, post activity, if their environment or situation is inadequate and causes stress.

Immunocompetency

Chronic stress can lead to the suppression of the immune system, which in turn can result in an increased susceptibility to diseases, especially diseases such as respiratory viruses and bacterial infection. It was shown that an intermediate stressor such as using a blindfold during restraint, can cause a horse's lymphocyte numbers to decrease (Blecha, 2000; Canali *et al.*, 1996).

Productivity

Problems with the quality of an animal's welfare can lead to a decrease in the productivity of that animal. Decreased growth in young stock, ill-thrift in adults, diminished athletic performance, and decreased fertility are all signs that the horse's nutritional needs are not being met or that their well-being are sub-optimal.

Environment

The areas of the horse's environment that are of most importance when it comes to welfare are their social interaction, housing, nutrition, and working conditions. All of these factors have an impact on their well-being. Generally the environments most domestic horses are kept in are sufficient to adequately provide for their needs.

Most horses require social contact in order to feel safe and comfortable; in the wild a lone horse is at risk of predation. Horses feel significant stress from social isolation (Zeeb and Schnitzer, 1997). In contrast, welfare compromise can also occur when horses live in large groups (Houpt *et al.*, 1978; Houpt and Wolski, 1980; Zeitler-Feicht, 1996) where there is inter-horse aggression, a greater risk of injury and the low ranking members of the group may not get sufficient food .

In New Zealand horses are kept in paddocks, open yards, covered yards and stables. Depending on upkeep, construction and management of any facility used to house horses the quality of the horses welfare can vary substantially in apparently similar management systems. Paddock-kept horses have most of their nutritional needs met with the pasture they graze, or with supplementary food, such as hay, when the pasture is insufficient.

A horse's working conditions depend on several factors; the handlers, equipment, exercise, and the amount of work. The skills and knowledge of the persons handling or managing the horse will affect the welfare of the animals. People with good training and handling abilities are less likely to cause distress or discomfort than are less knowledgeable persons in the same situation. Both equipment and facilities can be designed so that they are safe and easy to use. Exercise in itself can alter the physiological state of the horses to resemble that of a stressed animal, causing raised temperature, increased plasma cortisol and other changes. Stabled horses can develop abnormal behaviours if they do not receive enough exercise (Leuscher, McKeown and Halip, 1991; McGreevy *et al.*, 1995; McGreevy, French and Nicol, 1995).

1.12 Aims of the present study

The aims of the present study are to examine the behavioural and physiological effect of round-pen training on a group of horses used for the purposes of teaching veterinary students at Massey University. Also to be assessed is whether the dominance rank of a horse has any impact on the ease of handling, ease of training or the stress response of horses during restraint.

1.13 Hypotheses to be tested in the present study

Hypothesis 1: Round-pen training does not affect a horse's ease of handling, or physiological or behavioural responses to being led into stocks. Three treatments, Control (untrained) and two round-pen trained groups (easy and difficult to handle), were used to examine the effect of round-pen training on the physiology and behaviour of horses to round-pen training.

Hypothesis 2: The dominance status of a horse does not affect the ease of round-pen training or the behaviours seen in the round-pen.

Hypothesis 3: The dominance status of a horse does not affect the ease of handling, physiological response or behavioural response. The physiological and behavioural responses of horses during the stocks test, before treatment, were examined to see if there was any difference between horses in different dominance ranks.

Chapter 2 The behavioural and physiological response of horses restrained in examination stocks before and after Round-pen training

"Science is not a sacred cow. Science is a horse.
Don't worship it. Feed it."
Aubrey Eben

The welfare of an animal can be assessed through the monitoring of behavioural and physiological changes. Behaviours such as fight or flight suggest that an animal finds a situation aversive, but they may not define how aversive the stimulus is. Physiological changes may be a more accurate indicator of how aversive the animal finds a stimulus. Some common physiological measures used to assess stress and welfare in horses are plasma cortisol concentration (Alexander and Irvine, 1998; Guthrie *et al.*, 1980; Hoffsis *et al.*, 1970; Houpt *et al.*, 2001; Hydbring *et al.*, 1996; Pitman *et al.*, 1987) and heart rate (McCann *et al.*, 1988a; Stewart *et al.*, 2003; Waran *et al.*, 1996). Changes in behaviour or physiology outside the normal range may be indicative of stress or that the animal is failing to cope with the challenge it is facing.

The amount and quality of handling a horse has had and the quality of its previous experiences contribute to the ease with which it can be handled (Heird *et al.*, 1981; Jezierski *et al.*, 1999; Mal *et al.*, 1994). Round-pen training is one of the most popular methods to start the training of inexperienced horses and to reshape the behaviour of horses that are behaving inappropriately. It has been suggested that horses that have been round-pen trained are less difficult to handle (Roberts, 1996).

The aims of this study are to determine if round-pen training has any effect on the behaviour and physiological (plasma cortisol concentration and heart rate) responses of the horses being led into and standing in examination stocks.

2.2 Methods

2.2.1 Animals

The 24 horses used in this trial (Table 2-1) were held at the Massey University Veterinary Large Animal Teaching Unit (VLATU). All horses used in the trial were owned by the University. There were twenty-one mares and three geldings (Table 2-1) and they were of mixed age ranging from 5 to 15 years. Each horse was identified by a number freeze branded on the left or right shoulder. The weight of the horses was estimated by means of a "Weighband" (Dalton Supplies, Australia). The age of the horses was determined from the freeze branding on the right shoulder for thoroughbreds or by examining the teeth for unbranded horses and Standardbreds.

On the first day of the trial all of the horses were brought into the stocks and ease of handling and the time taken to get them into the stocks was noted. The time taken to enter the stocks, a handling score (Table 2-2) and the farm manager's previous classification of the horses were then used to allocate horses to treatment groups (Table 2-2). Horses that took a long time to enter the stocks and had high handling scores, for example, were more difficult to handle, were allocated to Round-Pen Difficult (RP-Difficult) treatment, while horses that entered the stocks quickly and had low handling scores were divided between Control and Round-Pen Easy (RP-Easy) treatment groups. Two horses from each treatment were then randomly allocated to one of four groups (Table 2-3 and Table 2-2). The horses in each group were then turned out to separate paddocks for two weeks, to allow them to settle into their new groups. The horses remained in these groups for the remainder of the study.

2.2.2 Treatment

The three treatment groups used in the study were Control, Round-Pen-Difficult (RP-Difficult) and Round-Pen-Easy (RP-Easy). The Control horses were placed in the yards and only handled for blood sampling and when in the stocks. The RP-Difficult and RP-Easy horses were held in the yards until it was time for their round-pen training session when they were brought into the round-pen, trained and then placed back into the yards until being led into the stocks (Table 2-3).

2.2.3 Stocks test procedure

The horses were lead into stocks (stocks test) on three separate occasions (Table 2-3), (1) pre-treatment, (2) post-treatment and (3) 3 weeks post-treatment The stocks test consisted of catching a horse in the yards and walking it into the stocks and then leaving it in the stocks for 60 minutes. During the 60 minutes in the stocks, the behaviour and heart rate of each horse was recorded and blood samples were taken from each of the test horses on several occasions. The pre (1) and post (2) treatment stocks tests were performed on the same day (Table 2-3). One trial group was tested and trained per day due to the round-pen training. It took four consecutive days to do all the training. At the end of each trial day the horses were turned out to pasture and left for a period of three weeks, after which the 3 weeks post-treatment (3) stocks test was performed.

Since there was no RP-training to be done on the day of the 3 weeks post treatment (3) stocks tests, all four trial groups were able to be tested on the same day (Table 2-3).

Table 2-1 Identification of the 24 horses used in the trial.

Horse Identification Number	Sex	Breed[1]	Age (years)	Colour	Weight (kg)
2	Mare	Tb X	15+	Bay	518
3	Mare	Sb	8-10	Brown	511
4	Mare	Tb	10+	Dark Bay	567
6	Mare	Tb	14	Brown	504
8	Mare	Tb	15+	Bay	560
10	Gelding	Tb	10	Chestnut	511
12	Mare	Tb	10	Brown	582
13	Gelding	Sb	10	Bay	511
14	Mare	Tb	19	Brown	490
15	Mare	Tb X	16	Dark Bay	546
20	Mare	Sb	10+	Dark Bay	511
23	Mare	Tb	14	Chestnut	498
24	Mare	Tb	9	Bay	498
25	Mare	Sb	10+	Bay	451
26	Mare	Tb X	8	Chestnut	490
27	Mare	Tb X	6	Dark Bay	458
29	Gelding	Tb X	10	Bay	532
33	Mare	Tb	4	Bay	524
45	Mare	Tb	6	Bay	498
51	Mare	Tb	11	Dark Bay	567
62	Mare	Sb	10+	Bay	504
90	Mare	Tb	13	Brown	582
92	Mare	Tb	11	Bay	504
115	Mare	Sb	8	Bay	511

[1] Tb = Thoroughbred, Sb = Standard bred, TB X= Thoroughbred cross

Table 2-2 Initial scoring and sorting of the horses used in the trial into treatment groups and trial day groups.

Horse Identification Number	Time to enter stocks (seconds)	Handling Score (1 easy – 5 difficult)	Farm Managers Classification	Treatment	Trial day
3	24	1	Good	Control	3
6	20	1	Good	Control	3
12	22	1	Good	Control	2
20	22	1	Good	Control	4
26	27	1	Good	Control	1
33	20	1	Good	Control	4
2	21	1	Problem	Control	2
51	23	1	Problem	Control	1
8	23	1	Good	RP-Easy	2
90	22	1	Good	RP-Easy	4
4	25	1.5	Good	RP-Easy	1
45	26	1.5	Good	RP-Easy	2
10	38	2	Good	RP-Easy	3
25	35	2	Good	RP-Easy	1
92	25	2	Problem	RP-Easy	4
14	20	4	Good	RP-Easy	3
62	18	1	Good	RP-Difficult	3
29	60	3	Good	RP-Difficult	3
27	157	4	Good	RP-Difficult	4
24	210	4.5	Problem	RP-Difficult	2
13	300	5	Problem	RP-Difficult	1
15	456	5	Problem	RP-Difficult	1
23	448	5	Problem	RP-Difficult	4
115	300	5	Problem	RP-Difficult	2

Note that horse number 62 was placed in the RP-Difficult treatment due to the manager's classification of this horse as well as its time to enter the stocks.

The order in which the groups were tested and trained was randomised, and only one group was brought in at a time to the yards (Figure 2-1). The horses were brought in from the paddocks quietly so as to not excite them. They were then separated into pairs in the yards (Figure 2-1, 1-6) and allowed approximately 30 minutes to settle. The heart rate monitors were then placed on the 6 test horses and they were allowed a further 30 minutes to adjust to the heart rate monitors, prior to the onset of the stocks test.

Three horses that were not part of the trial were also brought into the yards, one was left in the yards to provide company for the test horses and the other two were placed in stocks 1 and 5 (Figure 2-1) to act as dummy horses[2]. Dummy horses were used so that the first test horse was not walking into an empty stocks area as this might affect its response compared to the horses brought in after the first horse.

The test horses were caught in the yards, in a random order, a blood sample (-5 minutes) was taken and then the horse was led in to the stocks and the stocks test began. The time taken to get the horses into the stocks was the time it took for the horse to walk from the door (marked with an X in Figure 2-1) of the building until they were secured in the stocks and a tail rope was in place. On each day of the stocks tests the six horses being tested were led into the stocks in a random order and were led into the nearest unoccupied stocks. Once the horses were secured in the stocks a blood sample was taken (Figure 2-2) and their behaviour was recorded for 60 minutes.

Table 2-3 Organisation of trial, dates of stocks tests and treatment

Trial day	Treatment allocation*	Number (n)	Date of Pre-treatment Stocks test, treatment and Post-treatment stocks test	Date of 3 weeks post-treatment stocks test
1	Control RP-Difficult RP-Easy	2 2 2	14/12/01	16/01/02 3rd to be tested
2	Control RP-Difficult RP-Easy	2 2 2	19/12/01	16/01/02 2nd to be tested
3	Control RP-Difficult RP-Easy	2 2 2	17/12/01	16/01/02 1st to be tested
4	Control RP-Difficult RP-Easy	2 2 2	18/12/01	16/01/02 4th to be tested

* Control horses standing in yards, RP horses round-pen training in arena

[2] Horses number 1, 9, 21, considered to be 'tame' horses

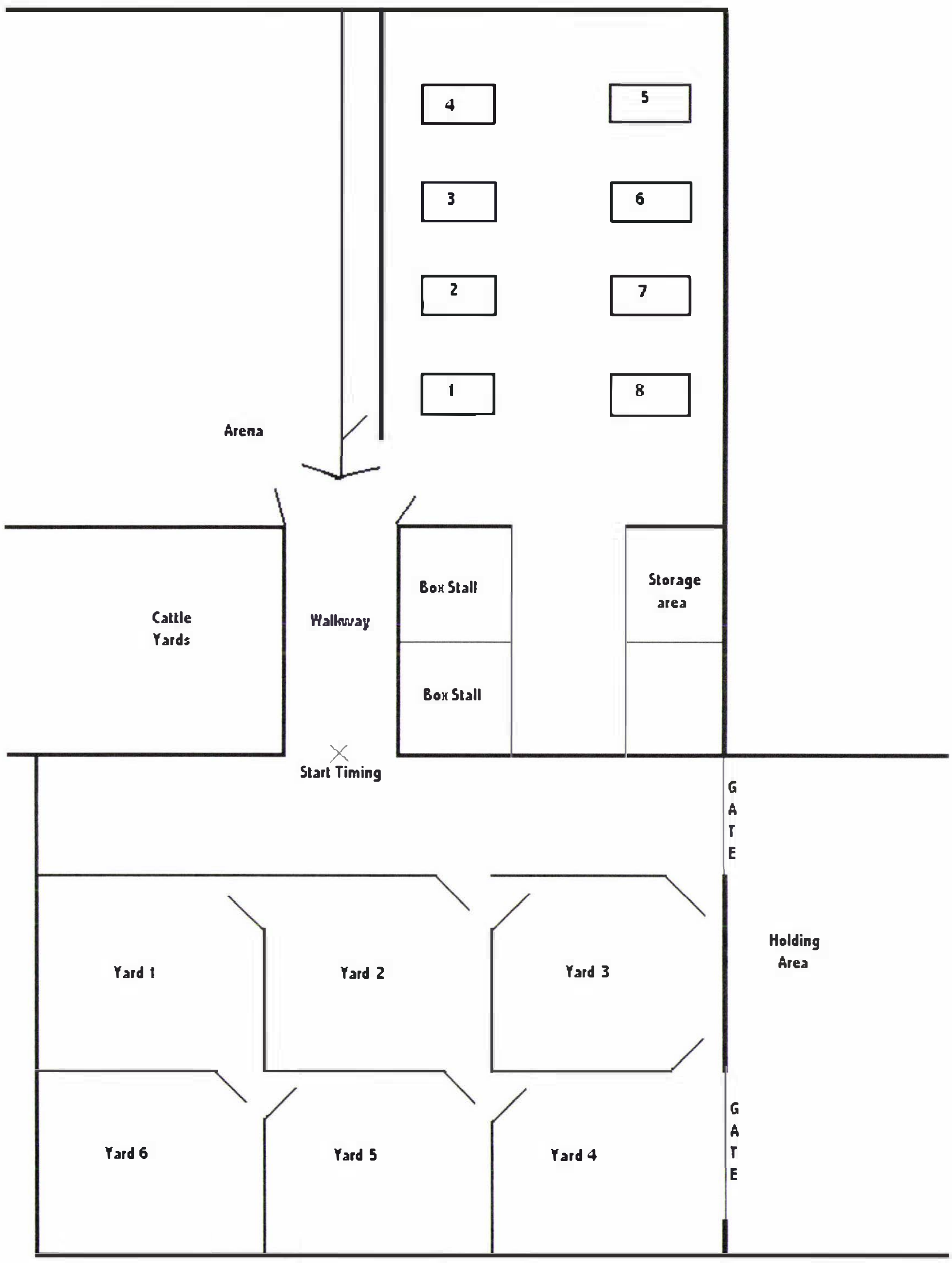

Figure 2-1 Diagram of Stocks and Yards used in the trial

2.2.4 Blood sampling

Blood samples were taken, by the same person, from each horse by jugular veinipuncture (Figure 2-2) at times –5, 0, 20,40 and 60 minutes. The samples were taken using sterile 0.9x25mm [20G1"] precisionglide needles (Becton Dickinson vacutainers systems, Belliver Industrial Estate, Plymouth, UK; ref no. 360214, lot 00B06, expiry 2005-02), mounted on a vacutainer system. A needle was screwed into the vacutainer holder after the grey rubber sheath covering the part of the needle that goes into the vacutainer tube was removed. The vacutainers collection tubes used were Sodium Heparin 10ml (Becton Dickinson, Franklin Lakes, NJ, USA; Ref no. 366480, Lot 0263678, Expiry 2002-09). Blood samples were taken, gently mixed and immediately placed on ice.

The blood samples were centrifuged in a Sorvall GLC-1 centrifuge at 4,000 RPM for 15 minutes. The plasma was then pippetted using disposable plastic pipettes (Samco ® transfer pipets; cat No. 225, purchase ID. *+H56822533*; Samco Scientific Corp., San Fernando, Ca., USA) into one 1.5ml yellow epindorf tubes (RayLab NZ Ltd., Auckland, NZ; code P4010-03, item code: 10301013, Batch 0 28 88) and the remainder of the plasma was placed in a 5 ml screw top vial (Sarstedt, SA, Austrailia; No. 60.9921.532, CH-B/lot No.00976, Exp 11-2003, sterile). The plasma samples were then frozen upright and stored at –20 degrees C in sealed, clearly labelled containers.

Figure 2-2 Taking a jugular veinipuncture blood sample

2.2.5 Cortisol analysis

The plasma cortisol analyses were carried out by one person on the same day in two batches. The plasma samples were defrosted on the morning of the cortisol assay (19 or 20/2/02). The plasma cortisol concentration was determined using a competitive binding radioimmunoassay (GammacoatTM [125I] cortisol radioimmunoassay; DiaSorin, Stillwater, Minnesota, USA). Standards and unknown plasma samples were incubated with cortisol tracer in antibody-coated tubes, in which the antibody is fixed to the test tube wall. After incubation the contents of the tubes were decanted and the radioactivity in the tube counted. A standard curve was prepared using 5 (human) serum standards ranging from 0-690nmol/L, that were provided with the assay kit. The tubes were counted using a gamma counter (LKB Wallac 1261; Stockholm, Sweden). The amount of radioactivity in each tube was inversely proportional to the concentration of cortisol in the plasma and the concentration was determined by comparison with a series of standard cortisol solutions.

The inter-assay coefficient of variations were 9.8% for 45 nmol/l and 6.8% for a control concentration of 245 nmol/l. the intra-assay coefficient of variation was 9.8%. The minimum detectable amount of cortisol in a plasma sample was 5 nmol/l.

2.2.6 Heart Rate Monitor

In the yards the horses were caught and held by one handler while a second assistant attached the heart rate monitor to the horse. One electrode was positioned on each side of the horse just behind the elbow (Figure 2-3). The sites for attachment of the electrodes (Figure 2-3) were washed using alcohol and an abrasive pad, to remove oil from the skin that may affect the attachment of the electrodes. The heart rate monitors (Polar Sports Tester NV; Polar Electronics Ltd.; Finland) were mounted on the horses in a backpack system (Figure 2-4) attached by an elasticised belly-band. The heart rate monitor transponder was attached to special electrically sensitive plate, which interfaced between the heart rate transponder and two copper wires attached to the electrodes.

The electrodes were Red Dot^{TM} monitoring electrodes with micropore tape and solid gel [silver/silver chloride] (3M; Ontario, Canada). The ECG electrodes were applied to the horses' sides using Loctite 454 instant adhesive (Loctite Australia, Caringbah, NSW) and extra electrode gel. The Loctite adhesive was required due to the tape on the electrodes being insufficient to hold the ECG electrodes in place during the activities of the trial.

After the ECG electrodes were attached the heart rate monitor recorder was activated and the time noted; the horses were then given 20-30 minutes to become accustomed to the hear rate monitor packs prior to the start of the stocks test. Heart rate was monitored from half an hour before testing until 5 minutes after the end of the stocks test after which the monitors were removed and the data down loaded to a computer.

The heart rate (beats per minute) was taken as a mean over 5 minutes at the following times during the stocks tests, -5, 0, 5, 30, 50 minutes. It was decided to take the last recording at 50 minutes rather than 60 minutes so as to not include changes due to increased activity at that time.

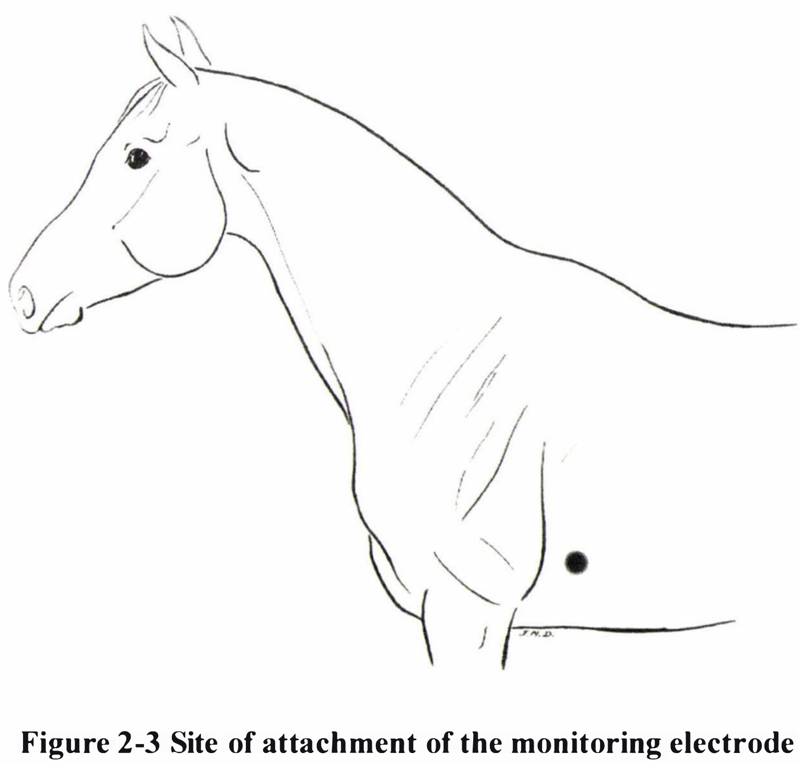

Figure 2-3 Site of attachment of the monitoring electrode

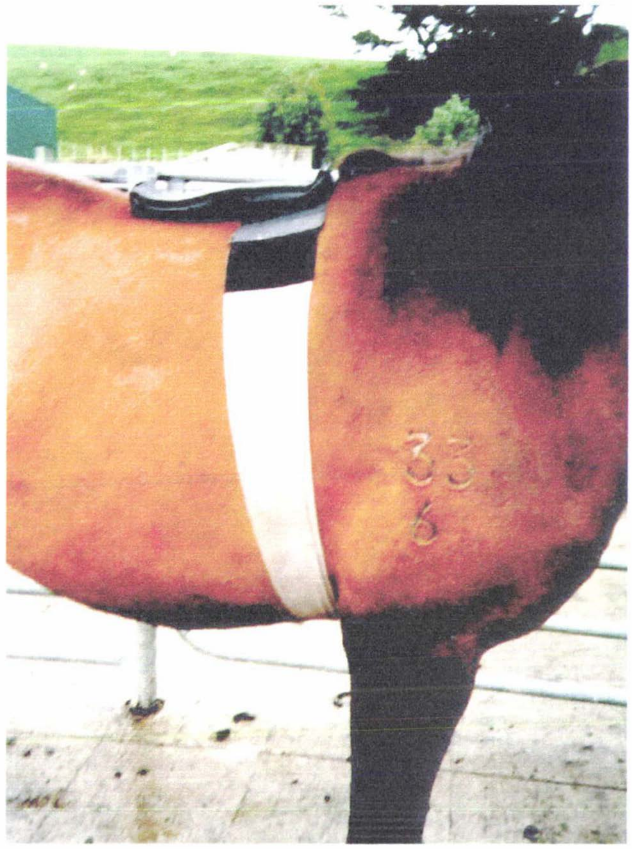

Figure 2-4 The heart rate monitor in a backpack attached to a horse

2.2.7 Behaviour Observations

An independent observer recorded the behaviour of each horse during six one-minute intervals at 0, 10, 20, 30, 40, 50, and 60 minutes after the horses were standing in the stocks. One minute recording times at 10-minute intervals were decided upon to allow for an array of behaviours to be observed and allowed for the same observer to record all horses in the stocks. All behaviours by the horses were recorded and some used in statistical analysis while others were noted for interest but no analysis was done. Where blood sample time and behaviour observations overlapped the behaviour was recorded after the blood sample had been taken. Continuous samples lasting one minute at each of these times were recorded on behaviour sheets. The behaviours were then tabulated and analysed statistically.

The observer stood off to the side either behind or in-front of the horse (Figure 2-5) where they were not in contact with the animal but they could observe virtually all behaviours.

Figure 2-5 Recording the behaviour of a horse in the stocks

The following behaviours were recorded:

Head bobbing or shaking: the head is moved up and down in a vertical plane either slowly (bobbing) or rapidly (shake)

Head turning: head and neck turn to the side more than 45 degrees from the straight position facing forward

Moving the front leg: the front leg is lifted off the ground and repositioned or a step is made

Resting the hind leg: the hind limb is rested with the foot tilted and the stifle flexed

Kicking or stamping the back leg: the leg and foot kick out or stamp on the ground in a fast sharp manner

Pawing with the front leg: the front leg is scraped over the ground or in the air, sometimes repeatedly. Despite the number of times the pawing motion is made the occurrence of the bout is recorded, so if the horse paws then rests (no pawing activity for more than one second) then paws again this equals two separate bouts. During the stocks tests the total numbers of pawing bouts was recorded.

Tail swishing: flicking or swishing of the tail. During recording a total number of tail swishes were counted.
Ears held back: the ears are held back (facing the rear) for at least one second
Licking and chewing: the horse makes licking and chewing motions with its mouth
Urination: the occurrence of urination during the time in the stocks
Defecation: the occurrence of defecation during the time in the stocks
Ear flicking: the ear is moved in a fast flicking motion
Tail held up: the tail is held in a position out from the hindquarters for more than one second
Head down: the head is held down and extended forward
Head held up high: the head is held above the normal plain of carriage for more than one second
Snort, Sniff and Yawn: These three behaviours were grouped. Snorting is a short rapid exhalation that is accompanied by a noise. Sniffing is where the horse investigates something with the nose and the nostrils are seen to move. Yawning is open-mouthed yawning behaviour.
Head rubbing: rubbing of the head on the poles and sides of the stocks
Pushing hindquarters against the rope at the back of the stocks: the horse steps back and pushes onto the rope at the back of the stocks.

In the analysis of the pawing, tail swishing, and head rubbing behaviours, the proportion of horses that performed at least one bout was used to compare the difference between treatment groups and the three stocks tests.

2.2.8 Statistical analysis

Plasma cortisol concentration and heart rate were analysed using the MIXED procedure in SAS (2001). The model included the fixed effects of treatment (Control, RP-Difficult, RP-Easy), sample time and their interaction, and the random effect of animal within treatment. The time to enter the stocks, head shake, head turning, licking and chewing, and ear flicking behaviours were analysed using the mixed procedure in SAS. Front leg move, hind leg rest and ears back behaviours were log-transformed and then analysed using the MIXED procedure in SAS (2001). Using the Akaike's information criterion, a variance component symmetry error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals. Least squares means and their standard errors were obtained for each treatment during each stocks test at –5, 0, 20, 40 and 60 minute blood sample times and at –5, 0, 5, 30 and 50 minutes for heart rate, and for the time to enter the stocks, head shake, head turning, licking and chewing, ear flicking, front leg move, hind leg rest and ears back behaviours. Urination, defecation, kicking, pawing, vocalisation, bum push on rope, tail swishing, tail held up and head rubbing were analysed as categorical data using the GENMOD procedure in SAS (2001). The proportion of horses and the standard error were obtained for urination, defecation, kicking, pawing, vocalisation, bum push on rope, tail swishing, tail held up and head rubbing during the stocks test.

This research project was approved by the Massey University Animal Ethics Committee on the 17th October 2001, ethics number 01/97.

2.3 Results

The physiological stress response, plasma cortisol concentration and heart rate, and the behavioural responses of horses to restraint in stocks are presented below as a series of questions and answers.

2.3.1 Plasma cortisol concentration

Were there any differences in the baseline (-5 minutes) plasma cortisol concentration pre-training between trial days?

The plasma cortisol concentrations of blood samples taken from the 24 horses before the initial stocks test were compared to see if there was any variation between the four trial Pre-treatment (and before the stocks test) days. There was a significant difference in baseline plasma cortisol concentration between day-four horses and the horses in the other three trial days (Table 2-4). However, the data were pooled in order to perform the statistical analysis and the data were adjusted for the effect of group. Least Squares Means were used in the results, to give the mean after adjustment for the effect of different trial days.

Table 2-4 The pre-treatment (-5 minute pre-treatment stocks test) plasma cortisol concentration (nmol/l) lease squares means ($\pm$ s. e.) of horses in each trial day

Trial day	Least Squares Mean (nmol/l)	Standard Error
1	142 [a]	17.3
2	163 [a]	17.3
3	155 [a]	17.3
4	196 [b]	17.3

Different letters denote significant differences (P< 0.05) between Means.

There were also significant concentration differences between groups at sample time -5 minutes 3 week post-treatment (Table 2-5). The concentration in the horses in groups one and three were not significantly different (P> 0.05) from each other but these concentrations were significantly different (P< 0.01) from those in horses in groups two and four and those in groups two and four were not significantly different from each other (P>0.05) (Table 2-5).

Table 2-5 The 3 weeks post-treatment stocks test (-5 minutes sample time) plasma cortisol concentration (nmol/l) lease squares means ($\pm$ s. e.) of horses on each trial day

Trial day	Least Square Means	Standard Error
1	106 [a]	13.7
2	160 [b]	13.7
3	108 [a]	13.7
4	168 [b]	13.7

LS means of treatment within the –5 minutes sample time during the pre-treatment stocks test. Different letters denote significant differences (P< 0.05) between Means

Did plasma cortisol concentration change during each stocks test?

Control Treatment

The plasma cortisol concentration in Control horses during the pre-treatment stocks test increased significantly (P<0.02) between –5 minutes (156 $\pm$ 16.8) and 60 minutes (205 $\pm$ 21.5) and between 0 minutes (157 $\pm$ 15.5) and 60 minutes (205 $\pm$ 21.5); the differences in plasma cortisol concentration between –5 and 0/20/40 minutes, and between 0 and 20/40 minutes, and between 20 and 40 minutes were not significant (P> 0.05) during the pre-treatment stocks test (Figure 2-6). The plasma cortisol concentration in the Control horses did not change over time during the post-treatment and 3 weeks post-treatment stocks tests (Figure 2-6).

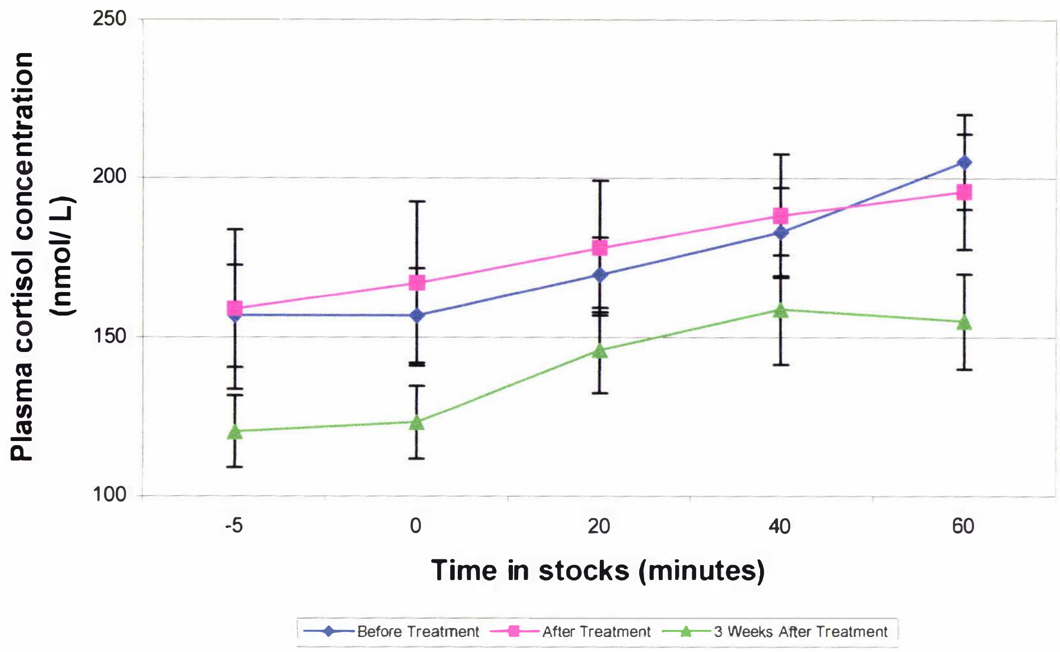

Figure 2-6 The plasma cortisol concentration of the Control horses during the pre-, post- and 3 weeks post-treatment stocks tests

RP-Difficult Treatment

In RP-Difficult horses there was a significant rise in plasma cortisol concentration during the pre-treatment stocks test between the following sample times: –5 (162 ± 16.4) and 20 (201 ± 15.1) ($P < 0.01$), -5 and 40 (225 ± 14.1) ($P = 0.004$), and –5 and 60 (228 ± 15.4) ($P < 0.001$) minutes (Figure 2-7). There were also significant increases ($P<0.01$) in plasma cortisol concentration between sample time 0-40 and 0-60 minutes (Figure 2-7). There were no significant ($P>0.05$) increases in plasma cortisol between sample times –5 - 0, 0 - 20, 20 - 40, 20 - 60, or 40 - 60 minutes during the pre-treatment stocks test (Figure 2-7).

Immediately post-treatment there were no significant changes ($P>0.1$) in plasma cortisol between any of the times (Figure 2-7) of the RP-Difficult horses.

During the 3 weeks post-treatment stocks tests there were highly significant increases ($P<0.01$) in plasma cortisol concentration between the following sample times, –5-20 or 40 or 60 minutes and 0 –20 /40 /60 minutes (Figure 2-7), but not between any other sample times.

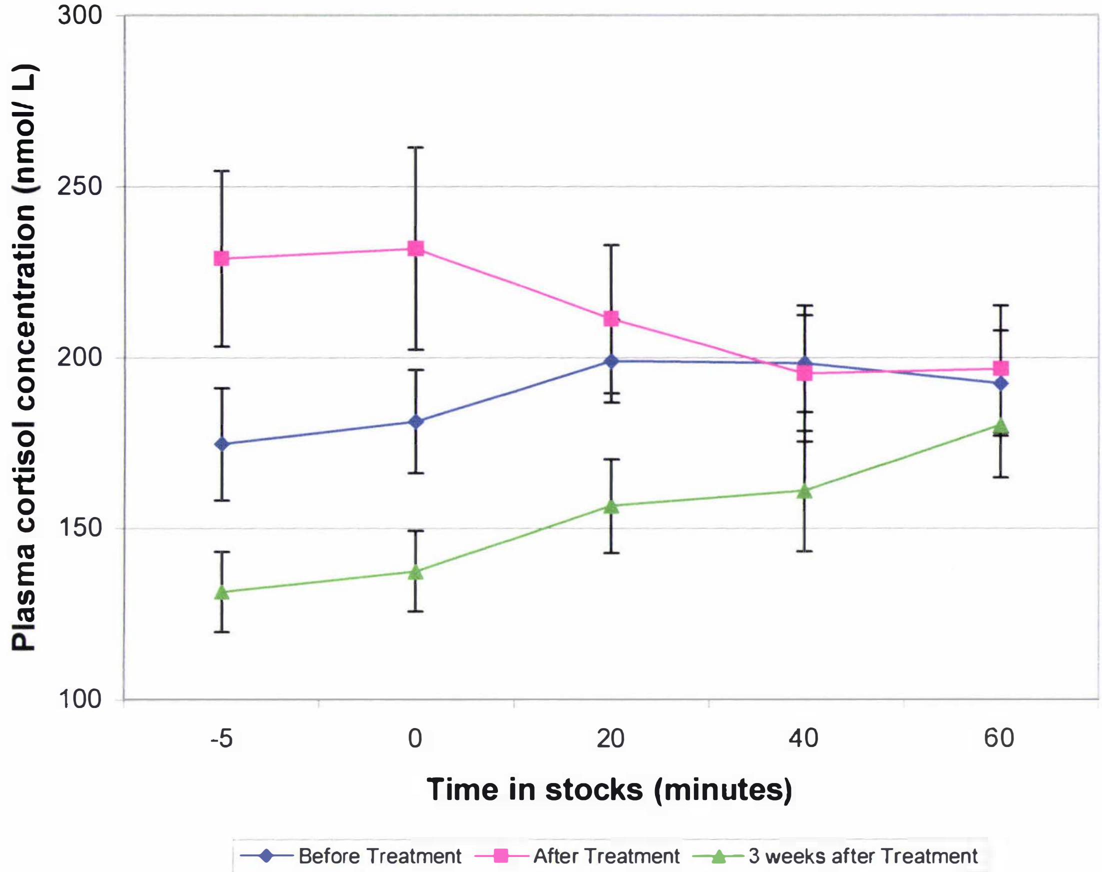

Figure 2-8 The plasma cortisol concentration in RP-Easy horses during the pre-, post- and 3 weeks post-treatment stocks tests

Were there any significant differences between treatment groups (Control, RP-Difficult, RP-Easy) during the pre-, post- and 3 weeks post-treatment stocks tests?

Pre-treatment stocks test

There were significant differences ($P<0.05$) in plasma cortisol concentration at time 20 minutes between the Control horses with a mean plasma cortisol concentration of 170 $\pm$ 11.8 nmol/L and the RP-Easy horses of 199 $\pm$ 12.0 nmol/L, but not the RP-Difficult horses ($P>0.05$) and at time 40 between the Control horses 183 $\pm$ 13.8 nmol/L and RP-Difficult horses 225 $\pm$ 14.1 nmol/L treatment groups ($P<0.05$). There were no differences ($P>0.05$) between any of the other treatment groups at any other sample times (Figure 2-9).

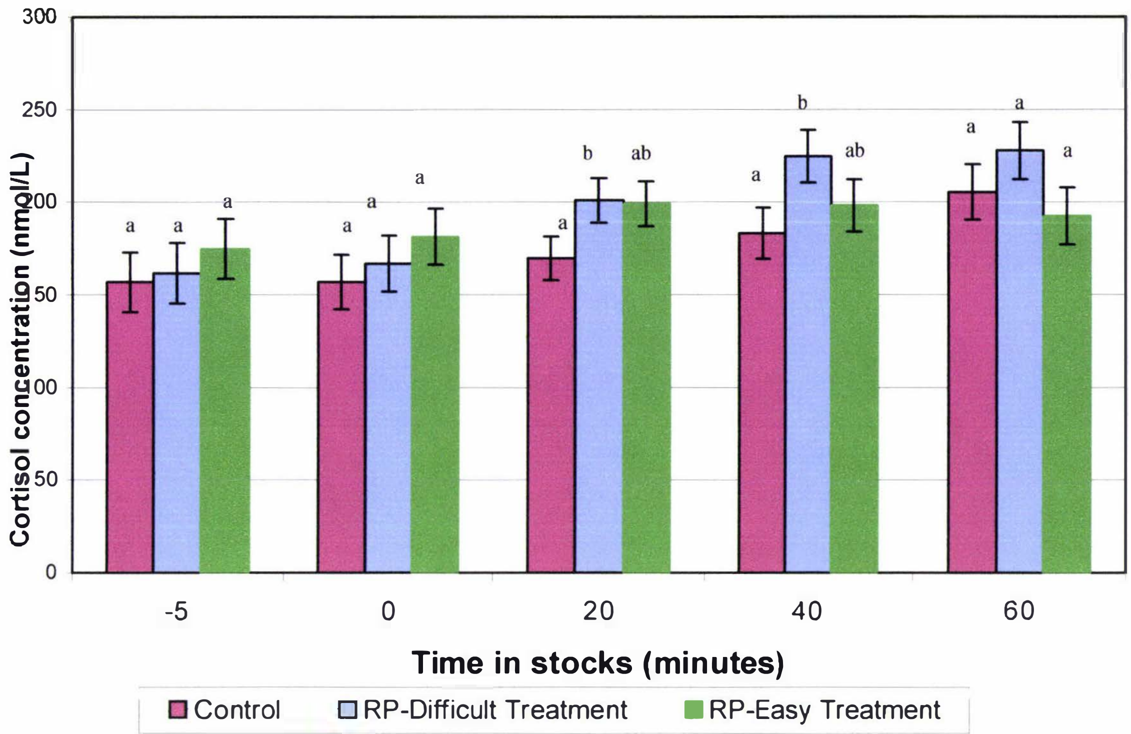

Figure 2-9 The difference in plasma cortisol concentration between the Control, RP-Difficult and RP-Easy horses during the pre-treatment stocks test
(Note letters that are different denote significant differences within each sample time)

Post-treatment stocks test

At the –5 minutes sampling time, post-treatment stocks test, there was a significant difference (P< 0.05) in plasma cortisol concentration between the Control (159 ± 25.0 nmol/L) and RP-Easy (228 ± 25.6 nmol/L) treatment groups (Figure 2-10). This was the only significant difference between the three treatment groups at any time in this stocks test.

3 Weeks Post-Treatment

There were significant differences (P<0.05) in plasma cortisol concentration 3 weeks post-treatment, between Control and RP-Difficult horses at the following sample times: 0, 20, 40 and 60 minutes (Figure 2-11). There were also significant differences (P<0.05) in plasma cortisol concentration between RP-Difficult and RP-Easy horses at sample times 20, 40 and 60 minutes (Figure 2-11).

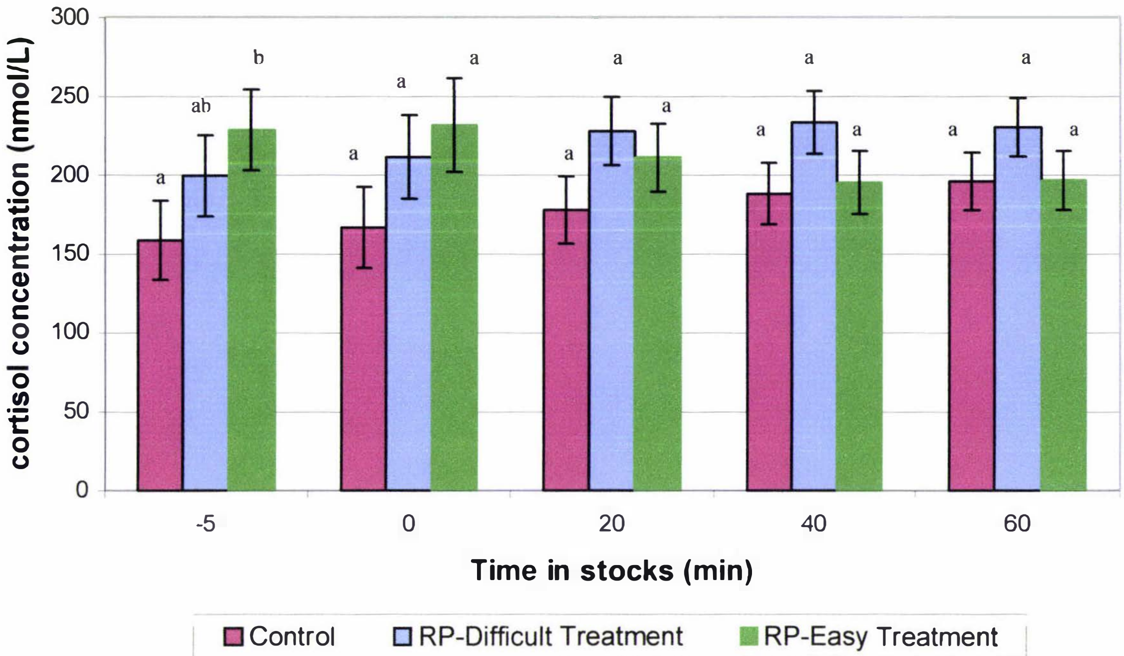

Figure 2-10 The difference in plasma cortisol concentration between the Control, RP-Difficult and RP-Easy horses during the post-treatment stocks test
(Note letters that are different denote significant differences within each sample time)

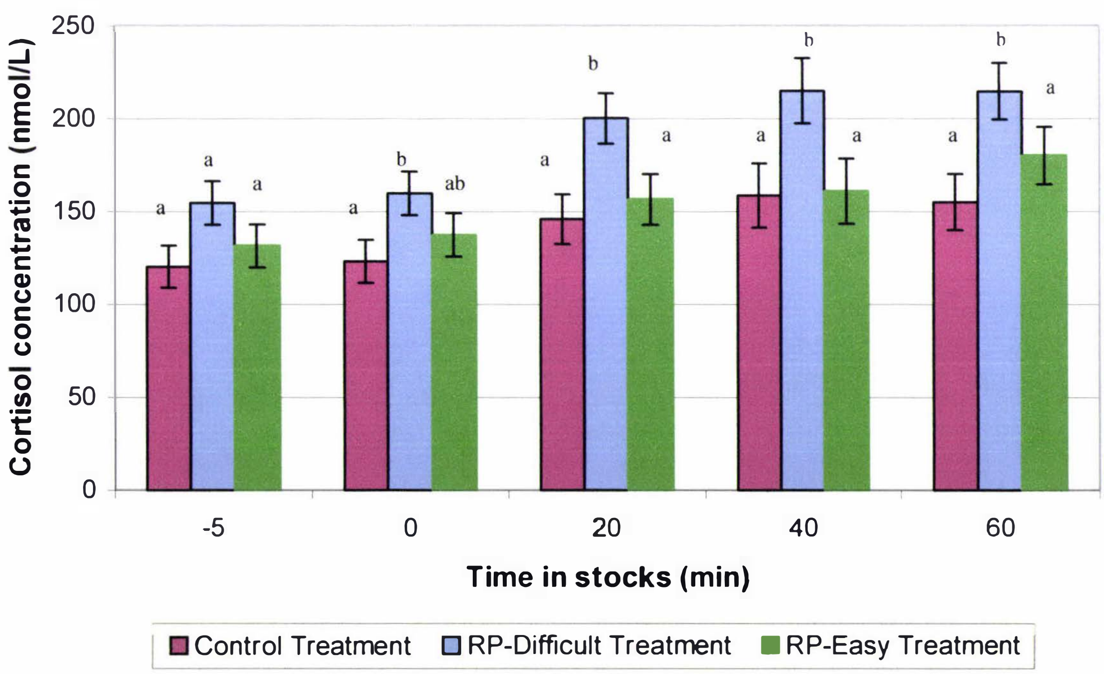

Figure 2-11 The difference in plasma cortisol concentration between the Control, RP-Difficult and RP-Easy horses during the 3 weeks post-treatment stocks test
(note letters that are different denote significant differences within each sample time)

Did round-pen training alter the plasma cortisol concentration of horses during the stocks test?

Control Treatment

In the Control horses the only significant change in plasma cortisol concentration was at sample time 60 minutes (P< 0.02) between the pre-treatment and 3 weeks post-treatment stocks tests. There was no significant differences (P> 0.05) between either the pre-treatment and post-treatment or between the post-treatment and 3 weeks post-treatment stocks tests for the 60 minute sample time (Table 2-6). There were no significant differences (P>0.05) between the pre-treatment and 3 weeks post-treatment stocks tests at any other sample time for the Control horses (Table 2-6).

Table 2-6 The plasma cortisol concentration (nmol/L) lease squares means ($\pm$ s. e.) of Control horses pre-, post- and 3 weeks post-treatment

Stocks test	Time	Least Squares Mean (nmol/L)	Standard Error
Pre-treatment	-5	157^{a}	15.0
Post-treatment	-5	159^{a}	25.0
3 Weeks post-treatment	-5	120^{a}	11.9
Pre-treatment	0	157^{a}	14.6
Post-treatment	0	167^{a}	25.7
3 Weeks post-treatment	0	123^{a}	11.9
Pre-treatment	20	170^{a}	11.8
Post-treatment	20	178^{a}	21.4
3 Weeks post-treatment	20	146^{a}	13.5
Pre-treatment	40	183^{a}	14.1
Post-treatment	40	188^{a}	20.2
3 Weeks post-treatment	40	159^{a}	16.2
Pre-treatment	60	205^{a}	15.6
Post-treatment	60	196^{ab}	18.6
3 Weeks post-treatment	60	155^{b}	13.9

L.S. Mean of treatment within sample time with different letters were significantly different (P< 0.05).

RP-Difficult Treatment

The only significant differences in plasma cortisol concentration in the RP-Difficult horses were between the post-treatment (209 $\pm$ 25.7 nmol/L) and 3 weeks post-treatment (155 + 11.9 nmol/L) stocks tests at sample time 0 minutes (P<0.1) (Table 2-7). There were no significant differences between stocks tests at any other time for the RP-Difficult horses, despite the average plasma cortisol concentration 3 weeks post-treatment being lower than pre-treatment (Figure 2-7).

Table 2-7 The plasma cortisol concentration (nmol/L) lease squares means (± s. e.) of RP-Difficult horses pre-, post and 3 weeks post-treatment

Stocks test	Time	Least Squares Mean (nmol/L)	Standard Error
Pre-treatment	-5	149[a]	15.0
Post-treatment	-5	199[a]	25.0
3 Weeks post-treatment	-5	148[a]	11.9
Pre-treatment	0	164[ab]	14.6
Post-treatment	0	209[a]	25.7
3 Weeks post-treatment	0	155[b]	11.9
Pre-treatment	20	198[a]	11.8
Post-treatment	20	219[a]	21.4
3 Weeks post-treatment	20	198[a]	13.5
Pre-treatment	40	221[a]	14.1
Post-treatment	40	223[a]	20.2
3 Weeks post-treatment	40	219[a]	16.2
Pre-treatment	60	221[a]	15.6
Post-treatment	60	224[a]	18.6
3 Weeks post-treatment	60	221[a]	13.9

L.S. Mean of treatment within sample time with different letters were significantly different (P< 0.05).

RP-Easy

In the RP-Easy horses (Figure 2-8 and Table 2-8) there were significant changes (P< 0.05) in plasma cortisol concentration over the three stocks tests, at several sample times (Table 2-8). At sample time –5 minutes there was a significant decrease (P<0.05) between pre-treatment (186 ± 15.0 nmol/L) and 3 weeks post-treatment (138 ± 11.9 nmol/L) and between post-treatment (229 ± 25.0 nmol/L) and 3 weeks post-treatment (138 ± 11.9 nmol/L) (Table 2-8). However the difference between the pre and post-treatment stocks tests were not significant (P> 0.05) (Table 2-8).

The RP-easy horses had a significant decrease (P<0.05) in plasma cortisol concentration between the pre-treatment or post-treatment and 3 weeks post-treatment stocks tests at sample times 0 and 20 minutes (Table 2-8). There were no significant differences between (P> 0.05) any of the three stocks tests for the 40 and 60-minute sample times for the RP-Easy horses (Table 2-8).

Table 2-8 The plasma cortisol concentration (nmol/L) lease squares means ($\pm$ s. e.) of RP-Easy horses pre-, post and 3 weeks post-treatment

Stocks test	Time	Least Squares Mean	Standard Error
Pre-treatment	-5	186 [a]	15.0
Post-treatment	-5	229 [a]	25.0
3 Weeks post-treatment	-5	138 [b]	11.9
Pre-treatment	0	183 [a]	14.6
Post-treatment	0	233 [a]	27.7
3 Weeks post-treatment	0	142 [b]	11.8
Pre-treatment	20	202[a]	11.8
Post-treatment	20	220[a]	21.4
3 Weeks post-treatment	20	158[b]	13.5
Pre-treatment	40	202[a]	14.1
Post-treatment	40	205[a]	20.2
3 Weeks post-treatment	40	157[a]	16.2
Pre-treatment	60	199[a]	15.6
Post-treatment	60	203[a]	18.6
3 Weeks post-treatment	60	174[a]	13.9

L.S. Mean of treatment within sample time with different letters were significantly different (P< 0.05) between tests within sample time.

2.3.2 Heart Rate

There were problems with the recording function of the heart rate monitors and there were a number of missing data points from the horses throughout the trial. During the pre- and post-treatment stocks tests the RP-Easy horses had many missing heart rate measurements, therefore the heart rate of the RP-Easy horses was not included in the analysis of the heart rate data for the pre- and post-treatment stocks tests (but the mean values are presented below in Table 2-9 and Table 2-10). However, the data from the Control and RP-Difficult horses were analysed and compared. During the 3 weeks post-treatment stocks test there were sufficient heart rate data points from the RP-Easy horses to be used in the analysis with the Control and RP-Difficult horses.

There was no significant difference (P>0.05) in the heart rate of horses in the Control or RP-Difficult horses during the pre-treatment (Table 2-9), or the 3 weeks post-treatment (Table 2-11) stocks test. There were no significant changes in heart rate during the course of the pre-treatment stocks test in the Control or RP-Difficult horses. There was a general trend for the mean heart rate of the Control horses to increase when being led into the stocks and to decrease over time during the stocks test. In the RP-Difficult horses there was a decrease in mean heart rate during the time they were led into the stocks.

Table 2-9 The mean heart rate ($\pm$ s.e.) (beats per minute) and range of heart rates (beats per minute) for the Control, RP-Difficult and RP-Easy horses during the pre-treatment stocks test

Treatment	Sample Time	Number of horses[3]	Mean heart rate (beats per minute	Range (beats/ minute)
Control	-5	5	27 ± 13.6^a	12- 49
Control	0	4	71 ± 74.5^a	23 –181
Control	5	7	56 ± 57.3^a	15 –181
Control	30	5	35 ± 13.4^a	20 - 50
Control	50	6	45 ± 24.4^a	19 – 88
RP-Difficult	-5	6	40 ± 14.1^a	19 – 53
RP-Difficult	0	3	29 ± 15.4^a	19 – 47
RP-Difficult	5	4	17 ± 8.4^a	10 - 27
RP-Difficult	30	4	16 ± 7.8^a	9 – 23
RP-Difficult	50	5	25 ± 15.6^a	11 – 52
RP-Easy	-5	3	29 ± 16.5	19 - 48
RP-Easy	0	2	35 ± 20.5	20 - 49
RP-Easy	5	3	30 ± 16.7	20 - 49
RP-Easy	30	3	41 ± 15.6	23 - 50
RP-Easy	50	3	44 ± 12.7	29 - 51

Letters that are different within treatment or within a sample time denote significant differences between means (P<0.05)

[3] The number of horses out of the 8 that were in each treatment group that contribute to mean heart rate

Table 2-10 The mean heart rate (± s.e.) (beats per minute) and range of heart rates (beats per minute) for the Control, RP-Difficult and RP-Easy horses during the post-treatment stocks test

Treatment	Sample Time	Number of horses[3]	Mean heart rate (beats per minute	Range (beats/ minute)
Control	-5	3	28 ± 3.8	24 – 31
Control	0	5	69 ± 91.2	21 – 232
Control	5	5	32 ± 9.8	21 – 48
Control	30	7	28 ± 11.0	14 – 48
Control	50	7	62 ± 74.5	15 –229
RP-Difficult	-5	6	98 ± 99.8	19 – 228
RP-Difficult	0	5	144 ± 109.0	25 – 228
RP-Difficult	5	5	143 ± 109.0	22 – 228
RP-Difficult	30	5	99 ± 105.6	17 –220
RP-Difficult	50	5	146 ± 110.8	20 - 234
RP-Easy	-5	2	111 ± 137.9	13 - 208
RP-Easy	0	1	208	208 - 208
RP-Easy	5	3	102 ± 92.1	48 - 208
RP-Easy	30	4	83 ± 84.7	22 - 208
RP-Easy	50	4	85 ± 83.3	25 -208

Letters that are different within treatment or within a sample time denote significant differences between means (P<0.05)

Table 2-11 The mean heart rate (± s.e.) (beats per minute) and range of heart rates (beats per minute) for the Control, RP-Difficult and RP-Easy horses during the 3 weeks post-treatment stocks test

Treatment	Sample Time	Number of horses[3]	Mean heart rate (beats per minute	Range (beats/ minute)
Control	-5	4	68 ± 75.4	20 – 180
Control	0	4	74 ± 55.9	22 – 152
Control	5	5	54 ± 47.9	25 – 139
Control	30	5	68 ± 78.7	24 – 208
Control	50	5	30 ± 5.9	23 – 39
RP-Difficult	-5	5	21 ± 4.5	16 –27
RP-Difficult	0	5	22 ± 5.6	16 –30
RP-Difficult	5	5	28 ± 13.4	17 – 50
RP-Difficult	30	6	63± 79.4	18 – 223
RP-Difficult	50	7	59 ± 73.3	22 – 223
RP-Easy	-5	5	33 ± 15.8	18 – 51
RP-Easy	0	5	73± 83.2	23 – 220
RP-Easy	5	5	71 ± 81.7	21 - 215
RP-Easy	30	5	56 ± 47.6	22 – 138
RP-Easy	50	6	45 ± 47.7	7 - 138

Letters that are different within treatment or within a sample time denote significant differences between means (P<0.05)

[3] The number of horses out of the 8 that were in each treatment group that contribute to mean heart rate

2.3.3 Behaviour

There was no effect of trial day on the occurrence of any of the behaviours within each treatment group, except for ear flicking. Hence the statistical model that was used to analyse ear flicking was adjusted to account for the effect of trial day.

Time to enter the stocks

There was no significant change ($P>0.05$) in the time taken to enter the stocks between the pre-, post- and 3 weeks post-treatment stocks tests for any of the treatment groups (Figure 2-12). Pre-treatment and post-treatment (round-pen training or standing in the yards), the RP-Difficult horses took significantly ($P<0.05$) longer to enter the stocks than the Control horses, but during the 3 weeks post-treatment stocks test this difference was not significant ($P>0.05$).

Table 2-12 Time to enter the stocks (seconds) prior to treatment allocation (pre-trial) and during the pre-, post- and 3 weeks post-treatment stocks tests.

Horse #	Treatment	Pre trial sorting	Pre-treatment	Post-treatment	3 weeks post-treatment
6	Control	20	12	23	26
33	Control	20	23	26	26
2	Control	21	23	24	28
12	Control	22	26	13	22
20	Control	22	20	21	20
51	Control	23	25	21	26
3	Control	24	22	19	22
26	Control	27	30	22	21
92	RP-Difficult	25	90	21	7
29	RP-Difficult	60	22	27	27
27	RP-Difficult	157	44	37	22
24	RP-Difficult	210	81	373	121
13	RP-Difficult	300	60	19	36
115	RP-Difficult	300	130	46	53
23	RP-Difficult	448	300	300	317
15	RP-Difficult	456	400	246	268
62	RP-Easy	18	23	19	20
14	RP-Easy	20	23	21	22
90	RP-Easy	22	19	21	22
8	RP-Easy	23	20	28	20
4	RP-Easy	25	61	32	27
45	RP-Easy	26	26	33	18
25	RP-Easy	35	40	23	22
10	RP-Easy	38	400	180	171

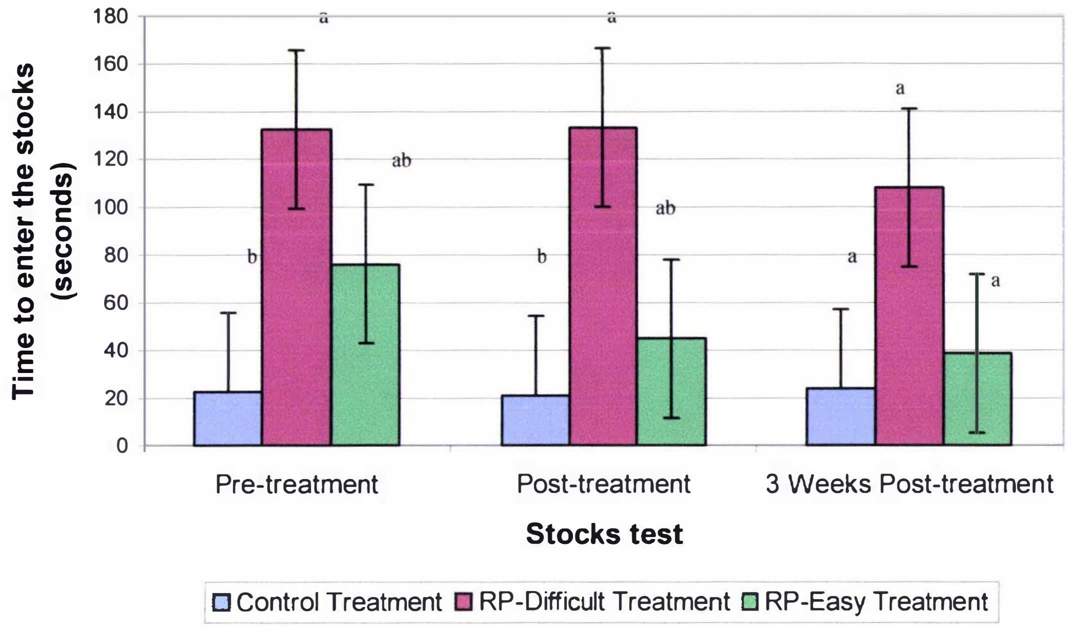

Figure 2-12 Mean (± s.e.) time to enter the stocks for the Control, RP-Difficult and RP-Easy horses during the pre-, post and 3 weeks post treatment stocks tests
(Different letters denote significant differences (P<0.05) between treatment groups within each stocks test)

Pawing behaviour during in the stocks tests

Horses pawed between 0 and 7 times during the stocks tests but not all horses that pawed in one stocks test pawed during any of the subsequent stocks tests. In all three stocks tests there was no significant difference in the occurrence of pawing between horses in the Control and RP-Difficult treatment groups (P>0.05); however fewer RP-Easy horses performed pawing behaviours than did either the Control (all three stocks tests) or RP-Difficult horses (the stocks tests immediately post- and 3 weeks post-treatment) (Table 2-13).

Tail swishing behaviour during stocks tests

Horses tail swished between 0 and 5 times during each stocks test. During the pre-treatment and post-treatment stocks tests there was no significant difference (P>0.05) between the three treatment groups for the number of horses performing tail swishing during the stocks tests. During the 3 weeks post-treatment stocks test the RP-difficult horses tail swished significantly (P<0.05) more often than either the Control or RP-Easy horses.

Post-treatment, more of the RP-Difficult horses and less of the RP-Easy horses tail swished, but these changes were not significant (P>0.05). The RP-Difficult horses had a significant (P<0.05) increase in the number of individuals that tail swished during the stocks tests 3 weeks post-treatment (
Table 2-14).

Table 2-13 Mean proportion (± s.e.) of horses that had at least one pawing bout during the pre-, post- and 3 weeks post-treatment stocks tests

Stocks test	Treatment	Proportion of horses that pawed during the stocks tests
Pre-treatment	Control	0.625 ± 0.17
Pre-treatment	RP-Difficult	0.25 ± 0.15
Pre-treatment	RP-Easy	0.125 ± 0.12
Post-treatment	Control	0.25 ± 0.15
Post-treatment	RP-Difficult	0.125 ± 0.12
Post-treatment	RP-Easy*	0
3 Weeks post-treatment	Control	0.125 ± 0.12
3 Weeks post-treatment	RP-Difficult	0.25 ± 0.15
3 Weeks post-treatment	RP-Easy*	0

Different letters denote significant differences (P<0.05) between treatment groups within each stocks test

Table 2-14 Mean proportion (± s.e.) of horses observed tail swishing during the pre-, post- and 3 weeks post-treatment stocks tests

Stocks test	Treatment	Proportion of horses tail swishing during stocks test
Pre-treatment	Control	0.25 ± 0.12^{a}
Pre-treatment	RP-Difficult	0.25 ± 0.12^{a}
Pre-treatment	RP-Easy	0.375 ± 0.15^{a}
Post-treatment	Control	0.25 ± 0.15^{a}
Post-treatment	RP-Difficult	0.25 ± 0.12^{a}
Post-treatment	RP-Easy	0.25 ± 0.12^{a}
3 Weeks post-treatment	Control	0.25 ± 0.15^{a}
3 Weeks post-treatment	RP-Difficult	0.875 ± 0.17^{b}
3 Weeks post-treatment	RP-Easy	0.125 ± 0.12^{a}

Different letters denote significant differences (P<0.05) between treatment groups within each stocks test

Head rubbing behaviour during stocks tests

The Control and RP-Difficult horses had no significant change in head rubbing during the stocks test post-treatment (Table 2-15). The RP-Easy horses had a significant increase in head rubbing behaviour between the post-treatment and 3 weeks post-treatment stocks tests (Table 2-15).

During the pre-treatment stocks test there was no significant difference between the number of horses observed head rubbing in any of the three treatment groups (Table 2-15). During the post-treatment stocks test the greatest number of head rubbing horses was seen in the Control group, but the difference was only significant between the Control group and the RP-Difficult treatment group (Table 2-15). During the 3 weeks post-treatment stocks test, more RP-Easy horses were head rubbing, than in either of the other treatment groups; the number of RP-Easy horses head rubbing was significantly different ($P<0.05$) from the number of RP-Difficult horses but not the number of Control horses. Head rubbing occurred 0 to 7 times and was seen most often in either Control or RP-Easy horses.

Table 2-15 Proportion of horses head rubbing during the pre-, post- and 3 weeks post-treatment stocks tests

Stocks test	Treatment	Total number of horses in treatment (n)	Proportion of horses observed head rubbing during stocks test
Pre-treatment	Control	8	0.500 ± 0.18^{a}
Pre-treatment	RP-Difficult	8	0.125 ± 0.12^{a}
Pre-treatment	RP-Easy	8	0.250 ± 0.15^{a}
Post-treatment	Control	8	0.625 ± 0.17^{a}
Post-treatment	RP-Difficult	8	0.125 ± 0.12^{b}
Post-treatment	RP-Easy	8	0.250 ± 0.15^{ab}
3 Weeks post-treatment	Control	8	0.375 ± 0.17^{ab}
3 Weeks post-treatment	RP-Difficult	8	0.125 ± 0.12^{a}
3 Weeks post-treatment	RP-Easy	8	0.625 ± 0.17^{b}

Different letters denote significant differences ($P<0.05$) between treatment groups within each stocks test

Front leg step during the stocks tests

There was a significant difference ($P<0.01$) in front leg steps between RP-Easy (mean=5 steps) and RP-Difficult (mean=19 steps) horses pre-treatment, but all other inter-treatment differences were not significant ($P>0.05$). There were no significant ($P>0.05$) changes in the frequency of front leg steps over all three stocks tests for the Control and RP-Easy horses (Figure 2-13). Overall the RP-Difficult horses had a notable decrease in the total number of front leg steps, between the pre-treatment (19 steps) and 3 weeks post-treatment stocks tests (5 steps), of although this was not significant ($P=0.9$) (Figure 2-13).

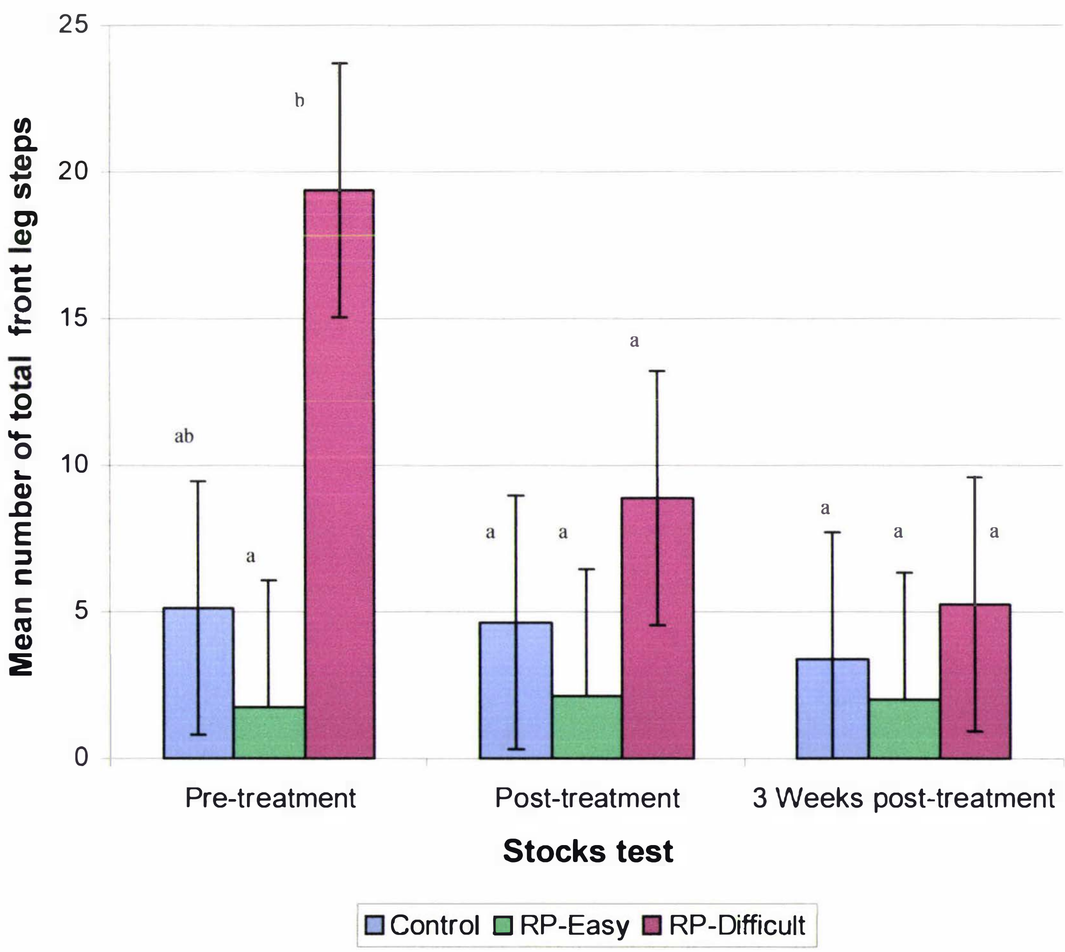

Figure 2-13 Mean (± s.e.) number of total front leg steps during the pre-, post- and 3 weeks post-treatment stocks tests
(Note different letters denote significant differences ($P<0.05$) between treatments within stocks test)

Head down

There was no difference (P>0.05) in the number of head down behaviours observed, within any of the three treatment groups between the pre-, post or 3 weeks post-treatment stocks tests (Figure 2-14). Pre-treatment there was a significant difference (P<0.01) between the Control and RP-Easy horses. During the post-treatment stocks test there was a significant difference in the mean number of head down behaviours between Control and RP-difficult horses (P<0.005), and between the Control and RP-Easy horses (P<0.001) (Figure 2-14). In the post-treatment stocks test there was a significant difference (P<0.05) between the mean number of times the Control horses (4 times during sampling) and the RP-Difficult horses (2 times during sampling) held their heads down.

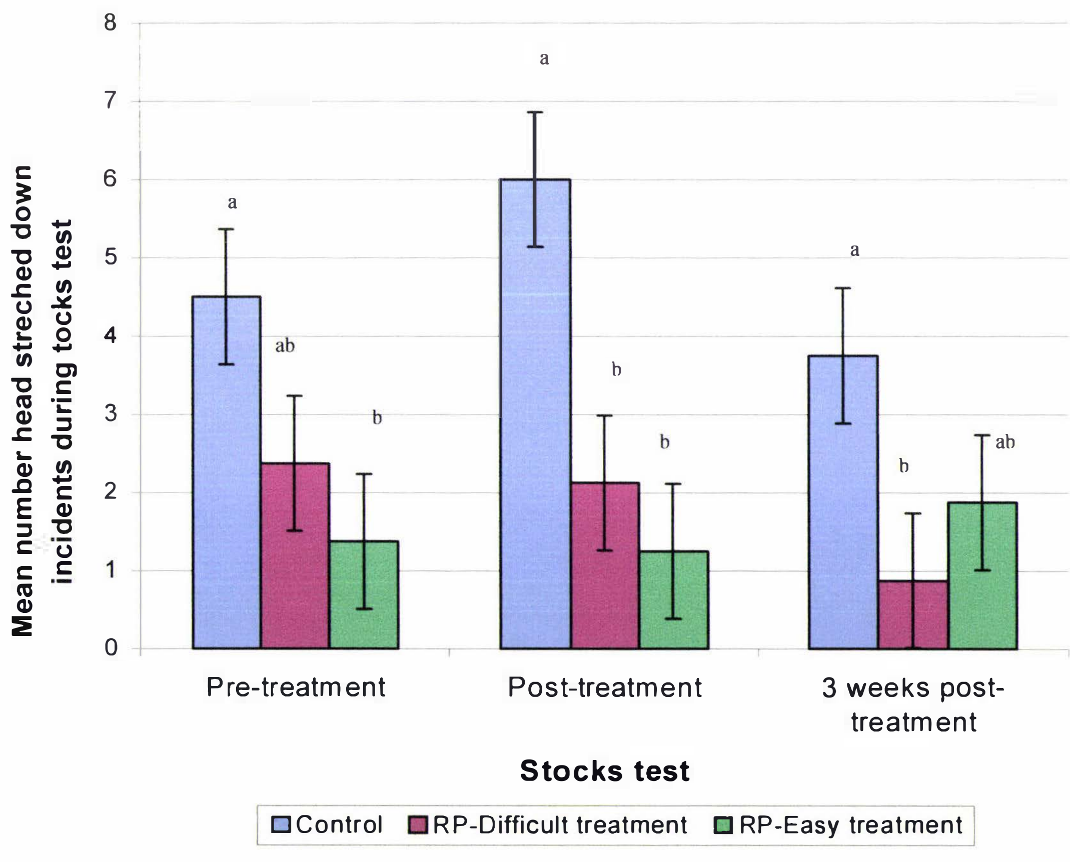

Figure 2-14 Mean (± s.e.) number of head down during the pre-, post- and 3 weeks post-treatment stocks tests
(Note different letters denote significant differences (P<0.05) between treatments within stocks test)

Ear Flick

There was no difference (P>0.05) in the number of ear flicks observed within any of the three treatment groups during the pre-, post- or 3 weeks post-treatment stocks tests (Figure 2-15). The Control horses had a significant increase in the total number of ear flicks between the pre-treatment and 3 weeks post-treatment (P<0.05) and the post-treatment and 3 weeks post-treatment (P<0.02) stocks tests (Figure 2-15).

The RP-Easy horses had a significant increase in the number of ear flicks between post-treatment and 3 weeks stocks tests (P<0.01) (Figure 2-15). There were no significant (P>0.05) changes in ear flicking behaviour between any of the three stocks tests for any other treatment/tests combination (Figure 2-15).

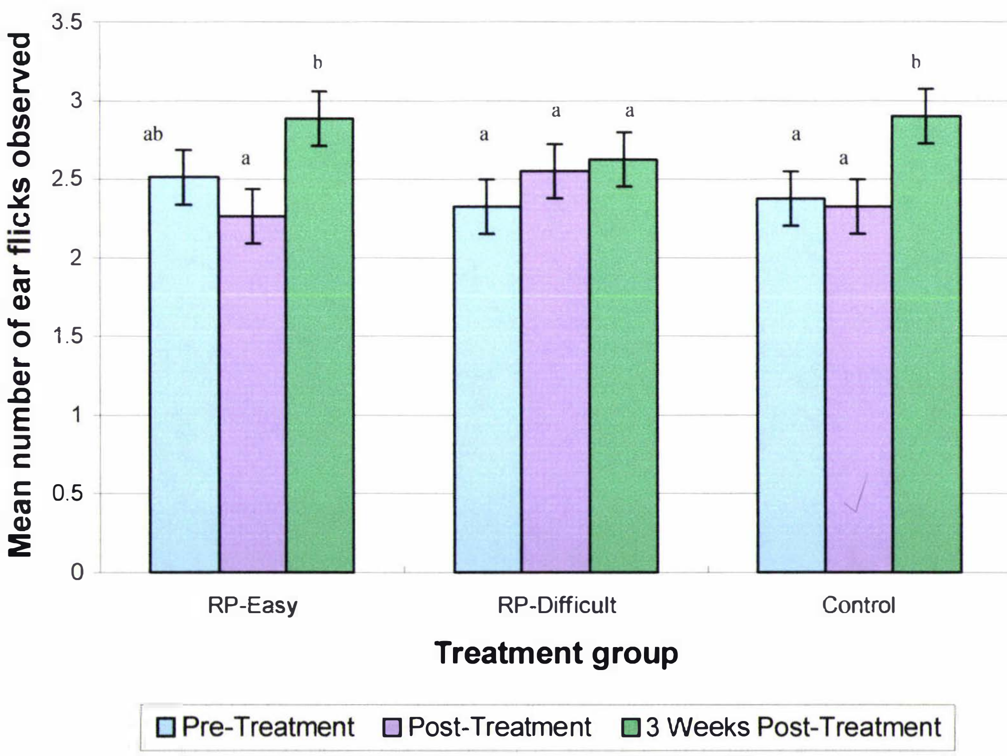

Figure 2-15 Mean (± s.e.) ear flicking during the pre-, post- and 3 weeks post-treatment stocks tests

(Note different letters denote significant differences (P<0.05) between stocks test within treatment group)

Snort, Sniff and Yawn

There was a significant difference (P<0.05) in the number of snort, sniff and yawns between the Control and RP-Easy horses in the post-treatment stocks test (Figure 2-16), but there were no other significant difference (P>0.05) between the three treatment groups between any of the stocks tests. There was no significant difference (P>0.05) within each of the three treatment groups pre- and post-treatment (over the course of the stocks tests) between tests for the frequency of these behaviours (Figure 2-16).

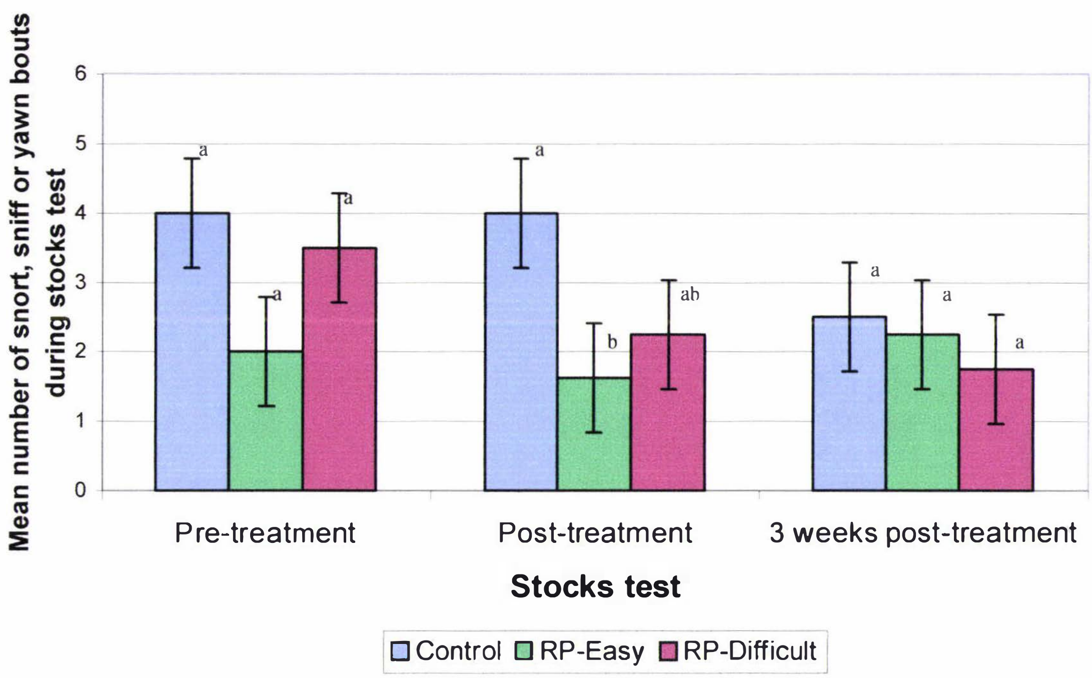

Figure 2-16 Mean (+ s.e.) total number of snort, sniff and yawn observed during the pre-, post- and 3 weeks post-treatment stocks tests
(Note different letters denote significant differences (P<0.05) between treatments within stocks test)

Other Behaviours

The following behaviours occurred commonly among the horses while standing in the stocks but when they were analysed and it was found that there was no statistical significance (P>0.05) for test or treatment effects and in all cases there was no interaction between the stocks test and treatment. These behaviours included head shaking, licking and chewing, hind leg rest, head held up, urination, kicking with hind leg, vocalisation, pushing on the tail rope of the stocks, and tail up.

Ears held back had significant differences between trial day groups ($P<0.01$) but no significant differences between tests or treatment ($P>0.05$).
Defecation differed significantly ($P<0.05$) between trail days, but had no significant differences ($P>0.05$) between tests or treatment group.
The pushing on the rope of the stocks only occurred in 2 horses, both of which were in the RP-Difficult treatment group.

2.4 Discussion

In this study the plasma cortisol concentrations and heart rates were similar for each of the treatment groups pre-treatment. Plasma cortisol concentration increased during the time that the horses were held in the stocks and there was an increase in heart rate as the horses were led into the stocks. The plasma cortisol concentrations of RP-Difficult horses were significantly greater by 20 minutes after the start of the stocks tests compared to the Control horses in which they took 60 minutes to increase significantly. There was no difference in plasma cortisol concentration between the Control and RP-Easy horses during all three stocks tests. Post-treatment there was a greater difference in plasma cortisol concentration between the Control and RP-Difficult horses. Treatment had no effect on the increase in heart rate response when horses were led into the stocks. The time to enter the stocks decreased after treatment and although this was not significant in the RP-Difficult horses the reduction in the time to enter the stocks meant that after treatment there was no significant difference between the Control and RP-Difficult horses.

There was no change post-treatment in occurrence of agitation behaviours (pawing, front leg step and tail swishing) in the Control or RP-easy horses, but post-treatment the RP-Difficult horses had an increase in the number of tails swishes observed during the stocks test. The performance of agitation behaviours was similar in the Control and RP-Difficult horses (except post-treatment for the tail swishing behaviour) and between the RP-Difficult and RP-Easy horses (except pre-treatment for the front leg step and pawing behaviours and 3 weeks post-treatment for tail swishing). The front leg step and tail swishing behaviours of the Control and RP-Easy horses were similar for all three stocks tests, but different for all three tests for pawing.

Ear flicking, which was thought to be an active behaviour rather than a relaxed behaviour, was not different between treatment groups for any of the stocks tests, and the Control and RP-Easy horses had a significant increase post-treatment (during the 3 weeks post-treatment stocks test).

There was no difference post-treatment for any of the three treatment groups for the relaxation behaviours (head rubbing, head down and snort/sniff/yawn). However there were differences between some of the treatment groups for all three behaviours, (1) Control and RP-Difficult horses had different levels of head down behaviour from each other post-treatment, (2) the Control and RP-Easy horses showed different amounts of snort/ sniff/ yawn behaviour post-treatment and head down behaviour pre-treatment, and (3) the RP-Difficult and RP-Easy horses showed different amounts of head rubbing post-treatment.

The plasma cortisol concentrations and heart rates of horses in each of the different treatment groups were similar before the stocks tests began and before the treatments were carried out. This suggests that these 24 horses had similar physiological states before the start of the trial. Hence the differences between the treatment groups in plasma cortisol concentration is likely to be due to the difference in temperament and experience that make the horses harder to handle or makes them interpret the stocks tests as being more aversive than other horses in the easier to handle treatment groups (Control and RP-Easy). It is likely that the lack of difference in plasma cortisol concentration between the Control and RP-Easy horses was due to these animals being similar based upon their selection criteria, but that they were allocated to the Control or RP-Easy treatment groups randomly. However it is interesting to see that round-pen training did not affect the plasma cortisol concentration response of the RP-Easy horses, which may be due either to the round-pen training not having an effect on plasma cortisol concentration or to the one round-pen training session not being sufficient to alter plasma cortisol concentration response to a stressor.

The differences in plasma cortisol concentration between the trial groups were probably due to either differences in activity in the different groups prior to testing or to uncontrollable external factors, such as noise or movement. The experiment was designed to allow horses one-hour to settle once they were in the yards prior to the onset of testing. It was hoped that this would be sufficient to minimise any residual effects of mustering the horses from the paddocks. Hoffis *et al.* (1970) found that plasma cortisol concentration remained elevated for 30 minutes following acute exercise. Bringing the horses in from the paddocks did not involve acute exercise, however the persistent elevations in plasma cortisol concentration suggest that the horses did not settle for at least one hour before the heart rate monitors were attached. Hence it may be possible to reduce the effect of trial day by allowing a longer time for the horses to settle. The other alternative is to perform the stocks test for all the horses in one day, but this may still lead to variation between groups due to effects of circadian rhythm or external factors, as well as being impractical if the number of horses to be tested is large.

The increase in plasma cortisol concentration during the stocks test could be due to the activity of the horses while being led in to the stocks, as observed in the RP-Difficult horses, or it may have been due to the horses finding restraint in the stocks stressful, as apparently occurred with the Control and RP-Easy horses. In the RP-Difficult horses, the rapid increase in plasma cortisol concentration, which became significant by 20 minutes, suggests that the activity or stress associated with getting the RP-Difficult horses into the stocks caused the increase in plasma cortisol concentration. This suggestion is supported by the observation that it takes 20-30 minutes for plasma cortisol concentration to become elevated in horses (Hoffsis *et al.*, 1970). Moreover, the elevation in heart rate during the time the horses were led into and held in the stocks is similar to the findings of (Waran *et al.*, 1996). Furthermore the longer time to enter the stocks for the RP-Difficult horses reinforces the notion that this procedure was stressful, which was similar to other studies (McCann *et al.*, 1988a; Stewart *et al.*, 2003).

The plasma cortisol concentration of the Control horses increased during all three stocks tests but the increase was only significant after 60 minutes in the stocks during the pre-treatment stocks test, which suggests that the Control horses may have started to become habituated to the handlers and the procedures involved in the stocks test and as such were finding them less stressful.

The RP-Easy horses also exhibited a rise in plasma cortisol concentration during the pre-treatment and 3 weeks post-treatment stocks tests, but this was not significant. This suggests that being in the stocks was a stressor for the RP-Easy horses, which contrasts with the Control or RP-Difficult horses which apparently found it less stressful. The RP-Easy horses showed a decrease in cortisol concentration following acute exercise during the post-treatment stocks test during the first 20 minutes, which may have been due to residual elevated concentration after acute exercise following round-pen training. Snow and MacKenzie (1977) found that plasma cortisol concentrations remained elevated for at least 30 minutes following acute exercise. It was anticipated in the present study that allowing the horses to rest for between 30 minutes and 1 hour after round-pen training would be enough time for the elevated concentrations following acute exercise to return to near resting levels. The RP-Easy horses then had a plateau between 20 and 60 minutes of the post-treatment stocks test, which likely to be reflective of the stress of being restrained in the stocks.

Treatment had no significant effect on the response following acute exercise of the Control horses, but decreased the pre-test (resting) response following acute exercise in both the RP-Difficult and RP-Easy horses, and did not alter the overall cortisol response during the stocks test. This is what we would expect as the Control horses did not undergo round-pen training during the treatment phase of the present study, hence their response in each of the stocks tests should have been the same except for the effect of habituation (Pitman *et al.*, 1987).

There was no difference in the heart rate response of horses in the Control and RP-Difficult treatment groups during the stocks tests. Due to missing data points the RP-Easy horses were not included in this analysis. It was not expected that round-pen training would reduce the heart rate response of going into the stocks. This was because the increase in heart rate is controlled by the autonomic nervous system (Bray, Cragg, Mackinght, Mills, and Taylor, 1994). The heart rate response to a stressor is usually elevation (sympathetic control) followed by a return to normal (parasympathetic control) as the horses becomes used to the change in their surroundings (Stewart *et al.*, 2003), provided that the animals surroundings remain constant. Hence being brought into the stocks is the stressor, and once the horses have adjusted to being in the stocks, the heart rate decreases.

The change in heart rate during the stocks tests was not significant for any of the treatment groups. This may be caused by the large errors encountered during the recording of these data. The problems of getting the heart rate monitors to record consistently meant that many data points were lost and data that were recorded may not have been accurate. The same equipment could be used again, but it might be better for the recording portion of the device to be on the outside of the backpack, so that the observer could note down the heart rate at a set time for each horse as backup for electronic recording. Alternatively it might be better to investigate the use of another recording system.

The round-pen training improved the handling ability, i.e. it reduced the time to enter the stocks, of the RP-Difficult and RP-Easy horses. Although the decrease in the time to enter the stocks in the RP-Difficult horses was not significant, it meant that before treatment there was a significant difference between the Control and RP-Difficult horses; post-treatment this significant difference was gone.

Since most of the horses used in the present study were familiar with the stocks at the VLATU, where the horses are normally restrained for teaching veterinary students, using the time to enter the stocks with the VLATU horses may not have been the best method for assessing handling ability. Nevertheless with the large range in time to enter the stocks it could be said that several of the horses did not find being led into the stocks a benign experience. The horses that are kept at the VLATU facility have generally been selected for ease of handling, enter the stocks and stand quietly, and have a reasonably calm or well-mannered temperament. Hence a different handling situation or use of horses that were less familiar with the VLATU stocks may have been a better approach to this experiment.

The lack of differences in the behavioural variables post-treatment correspond with the lack of change in the plasma cortisol concentration and heart rate after training, suggesting that round-pen training had no effect on the response of horses during restraint. It was unknown what effect round-pen training may have had on the behavioural responses of horses during restraint in stocks, but it was anticipated that bonding with humans may reduce the occurrence of agitation behaviour and increase resting behaviours. The present study has shown that the behaviour of the horses in each of the treatment groups in the stocks was unpredictable and while some horses had an increase in agitation behaviour they also had an increase in rest behaviours at the same time, results which evidently contradict each other.

There was no difference in behaviours during the pre-treatment stocks test except in the pawing and front leg step behaviours. In the pawing behaviour the RP-Easy horses pawed less than horses in the Control and RP-Difficult treatment groups. The RP-Difficult horses had more front leg steps than either of the other two treatment groups. Pawing is often regarded as a frustration or agitation behaviour in the horse (Weeks and Beck, 1996), and it was expected that it would be seen most often in the RP-Difficult horses and not the RP-Easy horses. The pawing displayed by the RP-Difficult horses and Control horses may be an expression of their agitation.

Treatment had no effect on the occurrence of behaviours except pawing, tail swishing and ear flicking. The RP-Easy horses had a decrease in pawing after undergoing round-pen training, which suggests that the RP-Easy horses were less agitated post-treatment. The RP-Difficult horses had an increase in tail swishing post-treatment, which may suggest that the RP-Difficult horses were more agitated during the stocks test post-treatment. The Control and RP-Easy horses had an increase in ear flicking post-treatment; this change is most likely due to some change in the surrounding environment, such as increased noise or movement.

2.5 Conclusions

Round-pen training improved handling ability even after only one treatment. Round-pen training was effective at improving the handling ability of difficult to handle horses, although this effect may have been greater after multiple training sessions. Round-pen training had no effect on the measured physiological response of horses to a stressor. In most cases there was no change in either agitation behaviours (pawing, tail swishing and front leg steps) or relaxation behaviours post-treatment. The RP-Difficult horses had no reduction in agitation behaviours post-treatment, unlike the RP-easy horses, which had a decrease in agitation behaviours post-treatment. There was little difference between treatment groups prior to the first stocks test.

Chapter 3 Behavioural aspects of round-pen training and effect of dominance rank on ease of join-up

"God forbid I should go to any heaven where there are no horses."

R.B. Cunningham Graham

3.1 Introduction

Round-pen training

"We will not hit, kick, jerk, pull or tie or restrain..." (Monty Roberts, 1996). Round-pen training has been used for many years but it has received much publicity in the last few years due to its promotion by horsemen such as Monty Roberts and John Lyons. These individuals have become equestrian celebrities through association with famous horse enthusiasts such as Queen Elizabeth, widespread media coverage, as well as the publication of books (Roberts, 1996) and 'how-to' video's. Other proponent's of these and similar methods of horse training, commonly called "Natural", include: Pat Parelli, Tom Dorrance and Ray Hunt (Miller, 2000).

It is often stated that Natural relies on "natural" communication between horse and trainer (Roberts, 1996). The trainer acts as the alpha 'mare' and punishes the horse that is being trained for not being with and bonding with the trainer. Roberts (1996) claims that developing a trust with a naïve horse encourages it to accept saddle, bridle and rider within 30 minutes of initial handling and that the behaviour of horses can be modified and they can be made to behave better (Roberts, 1996).

The three basic characteristics of Natural horsemanship training methods include, 1) the human is dominant over the horse (Roberts, 1996), 2) the use of punishment and reinforcement (Mills and Nankervis, 1999), and 3) a bond is formed between horse and human (Roberts, 1996).

Dominance

There is some doubt over whether or not a true dominance relationship can be formed between a human and a horse, with the human being dominant over the horse. Roberts (1996) claims that during round-pen training the human trainer assumes the social position and behaviours of the alpha mare, over the horse that is undergoing training. It is unknown if the horses perceives the dominance relationship between horse and human in the same way as a dominance relationship between two horses. It is unclear whether the subordinate horse accepts the human as its leader or if it tolerates such treatment from fear of being punished further, indicated for example, by learned helplessness (Mills and Nankervis, 1999).

Punishment and reinforcement

Monty Roberts (1996) states that his method of round-pen training uses no pain and that it is non-aversive. It is true that the method is free from physical pain as the horse is not injured during the procedure. However, the use of psychological stressors to punish unwanted behaviours is aversive. There are no ropes used to restrain the movement of the horses in the round-pen. However, the fences that enclose the round-pen visually isolates the horse from conspecifics (Jezierski and Gorecka, 1999), so that their use provides the restraint that allows the trainer to control the horse. The horse is not 'free' to move within the confines of the round-pen, as it must do what the trainer wants it to do and this may represent severe psychological pressure.

Round-pen training is relatively non-aversive in comparison to some training methods that are outdated and not used by many horse trainers. Today these methods, such as tying and throwing the horse and forcibly riding the horse until it stops bucking, are no longer considered to be acceptable as they may result in substantial physical and psychological trauma for the horse. These methods are the origin of the term horse breaking, where the horse was submitted to various treatments until their 'spirit' was broken.

In Natural horsemanship the term breaking or breaking-in have been replaced with 'starting' (Parelli, 1993; Roberts, 1996). Round-pen training relies heavily upon positive punishment and positive and negative reinforcement. Punishment is often an aversive stimulus that is applied to reduce the occurrence of a behaviour (Houpt, 1998). The severity or intensity of a punisher can be varied. A positive punisher is an aversive stimulus that is applied to the animal to reduce the occurrence of a behaviour, such as causing the horse to move away from the trainer. A negative reinforcer is the removal of a stimulus that the animal likes, for example the removal of attention, while a positive reinforcer is giving the animal a stimulus it likes, such as a pat or a food reward.

Bond formation

There is evidence that supports the formation and effects of bonds between humans and animals (Brackenridge and Shoemaker, 1996c; Hausberger and Muller, 2002; Jezierski and Gorecka, 1999; McNair and Hart, 1997; Meehan, 1996; Robinson, 1999). The formation of a bond between a horse and handler could benefit both the horse and rider. These benefits may include, company for horses living on their own, reduced stress, easier and safer handling and reduced risk of injury to both horse and rider.

In general the bond formed between a horse and its owner is not transferable to other humans. It develops over time and when established is generally stable. Bond formation is accelerated during round-pen training and may not have the same strength as human-horse bonds formed naturally over long-term contact. Thus the bond formed during round-pen training may not be stable and unless the contact with the horse is followed up and maintained the bond is likely to be lost.

Aims of the present study

There have apparently been no studies on the physiological effects and the behavioural responses of horses during round-pen training.

The present study aimed to (1) examine the cortisol response of horses to round-pen training, (2) determine what behaviours horses display in response to round-pen training, and whether or not the occurrence of those behaviours indicates that "join-up" is soon to be anticipated, (3) to examine the potential impact of a horse's social status on the ease of round-pen training, and (4) to see if there is any difference in the ease of round-pen training and the behaviours seen in the round-pen in horses of easy to handle and difficult to handle temperaments.

3.2 Methods

The data presented in the present study were generated during the round-pen training that the horses underwent as reported in Chapter 2. Also included here in this analysis are the observations and recordings from the Control horses, which were round-pen trained after the completion of the pre-treatment stocks test (Chapter 2). This chapter investigates the behavioural aspects of round-pen training and examines the relationship between the occurrence of certain behaviours and the ease of round-pen training, as well as the effect of a horse's social rank on the ease of round-pen training.

3.2.1 Animals

The horses that underwent training were the same horses that were used in Chapter 2 (Table 2.1). Prior to the onset of training horses were brought into the yards and had heart rate monitors placed. The horses were then subjected to a pre-treatment stocks test and the round-pen training commenced. Four horses were trained on each day with two other horses acting as untrained Control animals. The Control horses were trained on two consecutive days after the completion of the 3 weeks post-treatment stocks test (Chapter 2, Table 2.3).

3.2.2 Heart Rate Monitor

The procedures used for measuring heart rate were the same as used in chapter 2.

3.2.3 Blood sampling

The methods used to collect and process the blood samples were the same as those used in chapter 2. Blood samples were taken from each horse [either the Round-Pen Easy (RP-Easy) or Round-Pen-Difficult (RP-Difficult) treatments] immediately prior to the start of training (time 0), on the completion of training (variable times depending on the time taken to train the horses), and 30 minutes after the end of training. Blood samples were taken from the Control horses on the days that they were round-pen trained, at the start of training, at the end of training and 30 minutes post-training.

3.2.4 Cortisol analysis

The analysis of plasma cortisol concentrations was carried out using the same methods as outlined in Chapter 2.

3.2.5 Round-pen Behaviour Observations

An independent observer recorded the behaviour of each horse during the round-pen training session. A video camera was used as back-up and it recorded the horses movements and behaviours during the round-pen training session. Each round-pen training session began when the horse was released in the arena and the trainer took up position. An initial test was performed to determine if the horse was already "bonded" to the trainer. The trainer released the horse and walked from the door of the arena to the other side of the arena, to determine if the horse would follow the trainer prior to the onset of the round-pen training session. The horses were then trained according to the round-pen training protocol (below). When the horses passed the criteria for 'join-up' the training session was concluded and the time noted. The observer (O in Figure 3-1) sat outside the arena near the video camera (C in Figure 3-1) so that a clear view of the whole arena and each horse's movements could be observed.

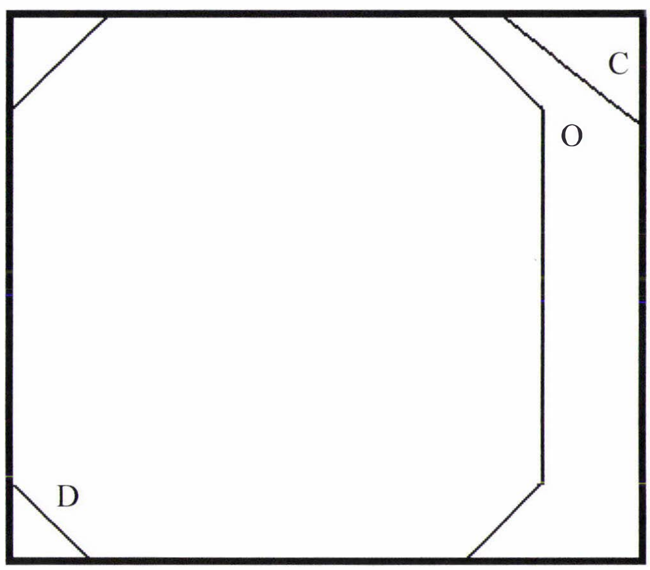

Figure 3-1 Diagram of round-pen training arena and the position of the Camera (C), Observer (O) and Door (D).

The following behaviours were observed and recorded:

Time to join-up: The time from the start of training until the successful completion of the round-pen training session (join-up)

Incident of licking and chewing: The horse makes a licking and chewing motion with the tongue. Every bout of licking and chewing that lasted for 2 or more licks was counted as one incident.

Time to first lick and chew: the time from the start of training until the first observation of licking and chewing behaviour (above).

Time to ear hold: This is the time until the inner ear, nearest the trainer, was held pointed at the trainer for more than 10 seconds.
Defecation: Each individual occurrence of defecation was counted as one incident.
Urination: Each individual occurrence of urination was counted as one incident.
Time to first turn: The time from the start of training until the first turn was made
Time to first contact between horse and trainer: The time from the start of training until the first contact between horse and trainer was made, this first contact may or may not be successful for join-up.
Who initiated first contact: Horse initiated contact is when it approached the trainer. The trainer initiated contact was when the trainer approached the horse.
Assisted: When after several failed contacts and attempts to join-up, the trainer assisted by leading the horse to show it what was expected of it. Horses that were not assisted followed the trainer without any aid.
Tail swishing: The horses flicks or swishes the tail to both sides of the body.
Vocalisation: Each time a horse vocalised was recorded as one incident
Clacking: An oral behaviour that appeared to be similar to licking and chewing, but an audible clacking or grinding of the teeth could be heard. Each individual occurrence was recorded.
Kicking: Each time the horse kicked out with a back leg was recorded and the position of the horse relative to the trainer noted.
Pawing: Each time the horse made a pawing or digging motion with a front leg was noted, as well as the horse's position in relation to the trainer.

The term "join-up" is used here as the criterion to define the successful completion of the round-pen training session. "Join-up" occurs when the horse has submitted to the trainer and willingly approaches, stays with and follow the trainer wherever he/she moves. Prior to the successful completion of round-pen training (join-up), the horses may make one or more attempts to contact the trainer, however when the trainer approaches the horse to touch it, or asks the horse to follow him/her, the horse will move away and the training must continue.

The training was deemed successful when the horse freely followed the trainer; walking in a figure 8 (performing two changes of direction), then walking from the door to the observers corner directly opposite, and standing quietly next to the trainer for a period of 30 seconds. The horse was then placed back on a lead rope and taken back to the yards for a post-training blood sample to be taken. The horse was allowed to stand quietly in the yards and a further blood sample was taken at 30 minutes post training. The whole time the horse was in the yards it had space to move freely and had access to fresh water.

3.2.7 Dominance test

The horses were the same as those used in the round ring trial. They were in their same groups for 10-12 weeks prior to the dominance trial. The only exceptions to this was the two spare horse (numbers 18 & 19) which were in with trial group one and which were removed two weeks prior to the performance of the dominance tests; since the horse groups were changed frequently and since a period of time was given to allow resettling of the groups, it was thought that the removal of these two horses would have a nominal effect on the social structure of that group.

The horses from one group were brought into the yards and identified by either distinguishing marks or by a mark applied to the withers and rump using a stock marker (Super Sprayline; Donaghys, Christchurch, New Zealand). The horses were placed in a yard measuring 8x10m and all interactions were observed for 15 minutes.

The horses were then placed in separate yards and fasted for 4-5 hours before the dominance tests were performed. The dominance test was based upon a similar study by Houpt et. al. (1978) and consisted of 5 minute long contests between two horses for a small quantity of red clover hay. The hay was served in a small shallow plastic trough (30x50x10cm). Two stopwatches were used to record the time each horse was in control or had access to the food. The horse that controlled the hay for longer was considered to be the dominant horse. Any aggressive actions made by either horse were recorded during the test time. Aggressive actions included (Houpt *et al.*, 1978): threats to bite (movement of the head with ears back or baring of teeth, in the direction of the opponent), threats to kick (movement of the hindquarters in the direction or lifting of a hind leg toward the opponent), actual bites, actual kicks and chases (more than 3 steps away from the bucket towards opponent).

The horses were kept in separate pens after the group observation period until the end of the paired tests. The horses required for each test were led into the test area and given one minute to settle. The hay was then placed in the test arena, at a point along the fence near the observer. The horses' eating and behaviour were then observed for five minutes. The hay was removed and the horses placed back in their holding pens for a short rest period, unless that horse was to be used in the next test.

3.2.8 Statistical analyses

Social rank was established, within each trial group, as the horses with the greater number of wins in the paired food competition test; in the event of a tie the horse that controlled the food longer or which showed the most aggressive behaviours was declared the winner. The horse in the alpha position was given rank 1 and the least dominant horse rank 6. The behaviours observed during round-pen training were then tabulated (Table 3-2) and analysed statistically. The age, weight, sex, treatment, and the behaviours (rate of lick and chew, time to first lick and chew, time to inner ear hold, trail group, time to first turn, rate of turn, rate of defecation, time to first contact with the trainer, horse or trainer initiates contact, assisted to meet criterion, rate of tail swishing, vocalisation, pawing, and kicking) were analysed using analysis of variance (the GLM procedure in SAS) to see if there were any significant differences between either treatment groups or social ranks. Due to the variable time to "join-up" for all the horses the change in plasma cortisol concentration was measured as the total change in plasma cortisol from the initial sample at time 0 minutes, until the end of the training session. This change in plasma cortisol concentration (nmol/l) was then divided by the total time taken to "join-up" and a rate of cortisol increase was measured.

3.3 Results

3.3.1 Round-pen training

The heart rate data were not analysed here due to problems with the recording of the heart rate for some of the horses.

Description of the behaviours during the round-pen training sessions

All horses successfully completed round-pen training. The protocol used here for the round-pen training, was designed to make all the training sessions as uniform as possible, while being flexible to meet the needs of each individual horse. Differences in individual temperament led to each horse's round-pen training session being unique. This explains some of the differences in time taken to "join-up" and the difference in the behaviours observed in the round-pen.

The eight horses in the RP-Difficult group either took less than 30 minutes (n=6) or over two hours (n=2) to "join-up". One of the RP-Easy horses took 120 minutes to join up, while all other RP-Easy horses took either less than 30 minutes (n=4) or 30-60 minutes (n=3) to "join-up". Most of the Control horses took less than 30 minutes (n=7) and one took 59 minutes.

The times taken to successfully "join-up" and some commonly occurring behaviours (time until first occurrence and total number during round-pen training) are shown in Table 3-1. Several other behaviours not shown in Table 3-1 were seen in only a few of the horses. These behaviours included vocalisation, teeth-clacking, kicking and pawing. Due to the low number of animals that displayed these behaviours they are described in the results but not analysed statistically.

Vocalisation occurred twice in horses number 6 and 23, once in horse 25 and three times in horse 26. Generally vocalisation occurred early in the training sessions where the horse in the round-pen was newly separated from its herd-mates and was calling out due to being isolated from other horses. The horses that vocalised tended to weigh less (mean weight 478 $\pm$ 27kg) than horses that did not vocalise (mean weight 526 $\pm$ 30kg) in the round-pen.

Teeth clacking occurred 10 times in horse 45 and twice in horse 27 during round-pen training. Teeth clacking occurred when the horses were moving and they appeared to be licking and chewing, except a definite 'clacking' noise was heard when they moved their jaws. The two horses that clacked took longer than 120 minutes to complete training and they were among the younger horses in the trial, both being six years old.

Horses 15 and 33 kicked out once during their round-pen training session, while horse 20 kicked out 7 times during round-pen training. It is thought that horses 15 and 33 were kicking out in response to the training rope being moved in behind their field of vision and startling them, causing them to kick out in defence, although they were not hit by the training rope. It was thought that horse 20 had poor vision and her kicking out was a defensive reaction toward any movement on her left side, where she was not able to see properly.

Horse 13 had a single bout of pawing that lasted less than 5 seconds and horse 20 had one extended bout that lasted for about 10 seconds. These pawing bouts occurred when the horses were asked to stop and turn by the trainer, and the horses were both facing the trainer in the centre of the arena when the pawing was carried out. Pawing was seen in one alpha ranked horse and one beta ranked horse. Of the two horse that pawed both took 17 minutes until their first contact, one was the horse that contacted the trainer and the other the trainer contacted the horse, except that horse number 51 took longer (20 minutes) to make contact with the trainer.

Table 3-1 Horse identification, dominance rank, trainer, time to "join-up" and behavioural observations during round-pen training

Horse #	Dominance rank	Treatment	Trainer *	Time taken to join up (minutes)	Time to first lick and chew (minutes)	Bouts of lick and chew	Time to first inner ear hold (minutes)	Total number of defecations	Time to first turn (minutes)	Time to first contact (minutes)	Who initiates contact (Horse (h) or trainer (t))	Assisted	Bouts of tail switching
2	1	C	P	18	1	24	1	4	3	5	T	No	2
3	2	C	P	8	9	4	2	3	1	3	H	No	0
4	3	E	K	22	1	20	1	2	1	5	H	No	0
6	1	C	P	12	4	9	1	1	1	3	H	Yes	0
8	3	E	K	34	3	60	1	3	2	13	T	Yes	5
10	5	E	P	14	2	12	1	2	1	10	H	Yes	0
12	4	C	P	14	3	10	1	1	3	9	H	No	9
13	2	D	P	19	2	31	1	4	3	17	H	No	0
14	6	E	P	23	1	16	1	2	2	7	T	Yes	30
15	6	D	K	134	6	109	1	4	2	12	T	Yes	4
20	1	C	K	56	3	59	2	3	1	17	T	Yes	22
23	5	D	P	9	1	14	1	1	2	3	H	No	1
24	6	D	K	4	1	9	1	0	1	4	H	No	2
25	1	E	P	56	6	34	2	3	5	13	H	No	7
26	5	C	P	19	4	15	1	5	1	5	H	Yes	5
27	4	D	K	152	4	250	2	0	2	14	T	Yes	8
29	3	D	P	22	2	44	1	0	1	7	H	Yes	4
33	2	C	P	17	4	4	1	2	1	2	H	Yes	4
45	5	E	K	120	4	163	1	3	1	8	T	Yes	32
51	4	C	P	42	1	23	2	1	5	20	T	Yes	44
62	4	D	P	17	2	23	1	5	1	8	T	Yes	0
90	3	E	P	31	4	63	1	2	2	8	H	Yes	13
92	6	E	P	25	2	55	2	1	2	11	H	No	39
115	2	D	K	18	1	34	1	0	4	7	H	No	0

* P = Philippa Mello, K=Kevin Stafford trained horse

Difference in *"join-up"* time and behaviours between treatment groups during round-pen training

There was no significant difference (p>0.05) between the three treatment groups for any of the behaviours except the rate of licking and chewing (Table 3-2). Horses in the RP-Control group (0.7 licks/minute) had a significantly (p<0.005) lower rate of licking and chewing than horses in RP-Difficult group (1.6 licks/ minute) (Table 3-2). There was no difference (p>0.05) in the rate of licking and chewing between the RP-Control and RP-Easy, or RP-Easy and RP-Difficult horses (Table 3-2). There were no other significant differences (p>0.05) in the occurrence of behaviours or the time to "join-up" between treatment groups.

Cortisol response to round-pen training

Round-pen training caused an increase (P<0.05) in plasma cortisol concentrations (from time 0 minutes until the end of training), whereas the Control horses while standing in the yards had no change in plasma cortisol concentration (-0.19 nmol/l/minute change). Horses in the RP-Difficult (3.7 nmol/l/minute increase) and RP-Easy (4.3 nmol/l/minute increase) groups had greater increases in plasma cortisol than did the Control horses (P<0.05). When the Control horses were round-pen trained they exhibited a greater increase (P<0.0001) in plasma cortisol concentration (RP-Control, 9.3 nmol/l/minute) than occurred when they were just held in the yards during the main part of the trial (-0.19 nmol/l/min). There were no significant (P>0.05) relationships between the rate of increase of plasma cortisol concentration during round-pen training and any of the behaviour variables observed during the round-pen training sessions.

3.3.2 Dominance order

Dominance test

The results of the paired food competition dominance test are shown in Table 3.1. There was no significant correlation between the age (r=0.076; p=0.72), weight (r=-0.21; p=0.33), or gender (p=0.86) and dominance rank with in each group. In most of the groups the hierarchy was linear.

Table 3-2 Mean and standard deviation for trial and treatments for times and behaviours observed in the round-pen

Treatment	Trial	Control	RP-Difficult	RP-Easy
Number (n)	24	8	8	8
Time to "join-up" (minutes)	37 ± 40	23 ± 17	47 ± 60	41 ± 34
Time to first contact (minutes)	9 ± 5	8 ± 7	9 ± 5	9 ± 3
Time to first turn (minutes)	2 ± 1.3	2 ± 1.5	2 ± 1.1	2 ± 1.3
Time to start licking and Chewing (minutes)*	3 ± 2	4 ± 3	2 ± 2	3 ± 2
Rate of licking and chewing (incidents/ minute)*	1.2 ± 0.6	0.7 ± 0.3 [b]	1.6 ± 0.4 [a]	1.3 ± 0.6 [ab]
Time to inner ear hold (minutes)	1.3 ± 0.4	1.4 ± 0.5	1.1 ± 0.4	1.3 ± 0.5
Rate of defecation (defecations per minute)	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.1 ± 0.1

* significant differences between means of different treatments ($P<0.1$). Means with the same letter are not significantly different from each other ($P>0.05$)

Were the manager's 'Bad' horses dominant over his 'Good' horses?

The horses that were in the VLATU managers difficult group of horses were not uniformly dominant in the trial groups (Table 3-3). There was no significant relationship ($p>0.05$) between the manager's classification as either an easy to handle or difficult to handle horse and the dominance rank achieved by that individual. Therefore the reason for their being difficult to handle may not be due to social status.

Table 3-3 Identification, group, dominance rank, sex, age, weight and treatment allocation for the 24 horses used in the trial

Horse Number	Trial Group	Dominance Rank	Sex	Age (years)	Weight (kg)	Treatment
25	1	1	Mare	10	451	RP-Easy
13	1	2	Gelding	10	511	RP-Difficult
4	1	3	Mare	10	567	RP-Easy
51	1	4	Mare	11	567	Control
26	1	5	Mare	8	490	Control
15	1	6	Mare	16	546	RP-Difficult
2	2	1	Mare	15	518	Control
115	2	2	Mare	8	511	RP-Difficult
8	2	3	Mare	15	560	RP-Easy
12	2	4	Mare	10	582	Control
45	2	5	Mare	6	498	RP-Easy
24	2	6	Mare	9	498	RP-Difficult
6	3	1	Mare	14	504	Control
3	3	2	Mare	10	511	Control
29	3	3	Gelding	10	532	RP-Difficult
62	3	4	Mare	10	504	RP-Difficult
10	3	5	Gelding	10	511	RP-Easy
14	3	6	Mare	19	490	RP-Easy
20	4	1	Mare	10	511	Control
33	4	2	Mare	4	524	Control
90	4	3	Mare	13	582	RP-Easy
27	4	4	Mare	6	458	RP-Difficult
23	4	5	Mare	14	498	RP-Difficult
92	4	6	Mare	11	504	RP-Easy

Note that the Control horses were considered to be the same as the RP-Easy horses during the sorting, and were allocated to either the Control or RP-Easy treatment groups randomly

Effect of dominance on time to "join-up" and behaviours during round-pen training

There were no significant ($p>0.05$) differences between the six different dominance ranks for the time to join-up, or for most of the behaviours seen during round-pen training (Table 3-4). It was found that more high-ranking horses had a lower rate of tail swishing than low-ranking horses ($r=0.39$; $p=0.05$) during round-pen training.

Table 3-4 Mean (± standard deviation) of each dominance rank for the behaviours observed during round-pen training

Variable	Trial	Rank 1 (alpha)	Rank 2	Rank 3	Rank 4	Rank 5	Rank 6
Number	24	4	4	4	4	4	4
Age	11 ± 3	12 ± 3	8 ± 3	12 ± 2	9 ± 2	10 ± 3	14 ± 5
Weight *	518 ± 35	496 ± 31 [a]	514 ± 7 [ab]	560 ± 21 [b]	528 ± 57 [ab]	499 ± 9 [a]	510 ± 25 [ab]
Time to “join-up” (minutes)	37 ± 40	36 ± 24	16 ± 5	27 ± 6	56 ± 65	41 ± 53	47 ± 59
Time to first contact (minutes)	9 ± 5	10 ± 7	7 ± 7	8 ± 3	13 ± 6	7 ± 3	9 ± 4
Time to first turn (minutes)	2 ± 1.3	3 ± 2	2 ± 2	2 ± 1	3 ± 2	1 ± 1	2 ± 1
Time to start licking and Chewing (minutes)	3 ± 2	4 ± 2	4 ± 4	3 ± 1	3 ± 1	3 ± 2	3 ± 2
Rate of licking and chewing (incidents/ minute)	1.2 ± 0.6	0.9 ± 0.3	1.1 ± 0.8	1.7 ± 0.5	1.1 ± 0.5	1.1 ± 0.4	1.5 ± 0.9
Time to inner ear hold (minutes)	1.2 ± 0.4	2 ± 1	1 ± 1	1 ± 0	2 ± 1	1 ± 0	1 ± 1
Rate of defecation (defecations per minute)	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	0.1 ± 0	0.1 ± 0.1	0.1 ± 0.1	0
Rate of tail swishing (incidents/ minute)	0 ± 0.4	0.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.2	0.4 ± 0.5	0.2 ± 0.1	0.8 ± 0.7
Clacking	0.5 ± 2.1	0	0	0	0.5 ± 1	2.5 ± 5	0
Vocalisation	0.3 ± 0.8	1 ± 1	0 ± 0	0	0.8 ± 1.5	0.5 ± 1	0
Kicking	0.4 ± 1.4	2 + 4	0 ± 1	0	0	0	0 ± 1
Pawing	0.1 ± 0.3	0.3 ± 0.5	0.3 ± 0.5	0	0	0	0 ± 0

* significant (P<0.1). Within each variable different letters denote a significant difference between the mean for each rank (P<0.05).

3.4 Discussion

3.4.1 Round-pen training

All the 24 horses displayed a pattern of behaviour similar to that described by Roberts (1996) during the round-pen training. There were however differences in behaviour between individuals and treatment groups. Some behaviours, such as licking and chewing, defecation, inner ear hold, tail swishing and contact with the trainer, were observed in most individuals but the occurrence varied between animals.

Some behaviours, such as kicking, pawing, clacking teeth, and vocalisation were only seen in a few horses, but they may provide insight as to whether or not the horse was interpreting the round-pen training to be stressful.

There was no affect of treatment on the time to join-up. This suggests that the temperament of a horse does not affect its response to round-pen training. However, the method used to allocate horses to the difficult or easy treatments was based upon their handling rather than their reactivity.

The difference in the incidence of licking and chewing between the Control horses and the RP-Difficult horses during the round-pen training may be due to the length of time those horses stayed in the round-pen, the Control horses taking less time on average than the RP-Difficult horses. Licking and chewing during round-pen training may be part of a physiological response to exercise or stress (i.e. decreased saliva production), rather than a psychological sign of submission as suggested by Roberts (Roberts, 1996). Thus horses that were exercised for longer (i.e. a longer time in the round-pen) would be expected to have a lower rate of licking and chewing than horses that were in the round-pen for less time.

3.4.2 Cortisol response to round-pen training

In all of the horses plasma cortisol concentration increased during round pen training. It was not possible to determine whether this was due to exercise or the stress associated with the training procedure or both. The stress response measured during round-pen training is likely to be a composite of the effects of exercise (Alexander *et al.*, 1991;

Foreman and Ferlazzo, 1996; Snow and MacKenzie, 1977), isolation stress (Jezierski and Gorecka, 1999) and psychological stress from the trainer.

The difficult horses (RP-Difficult) had a lower cortisol response per minute (3.7nmol/l/minute) than the Control horses (9.3nmol/l/minute) and this could be due to the latter finding the round-pen training more stressful. However if plasma cortisol concentration reached maximum concentration during 20-30 minutes after the start of training, and reached a plateau at those values then those horses that completed training quickly would have had a greater cortisol concentration change per minute than horses that took longer to complete training.

It would have been better to take samples at more regular intervals, but this would have interfered with the training procedures and may have influenced the time to "join-up". The plasma cortisol concentration of horses exercising in a group might provide data showing the effect of exercise on the rate of increase of plasma cortisol concentrations during round-pen training and this could then be compared with the results found here.

The lack of any significant relationships between the behaviours observed during round-pen training and the rate of increase in plasma cortisol concentration does not mean that there were no correlations at all because the increase in plasma cortisol concentration may not have been influenced by exercise.

3.4.3 Dominance order

Linear hierarchies were formed between the 6 horses in each of the four trial groups. The hierarchy was determined only by using paired food competition test, as there were too few interaction between horses during the larger yard observation and it was not possible to determine the relationship between individuals. This lack of interaction may be due to the horses being familiar with each other and having no need for agonistic interactions in the yard because there was not enough food or space for all individuals. Using the paired food competition test is an established method (Araba and Crowell-Davis, 1994; Ellard and Crowell-Davis, 1989; Houpt *et al.*, 1978) to determine dominance relationships between horses. However the dominance hierarchy formed around a food competition may not necessarily reflect the true nature of the group when out in the paddock.

There was no significant relationship between the physical characteristics of the horse (age, weight, gender) and the horses' status in the social structure of their group. The weights of the horses in ranks 1 and 4 were significantly less than those of horses in rank 3 only, but not different from those in the other ranks. The horses in rank 1 had the lowest mean weight of all six dominance rank groups. This is in contrast to other studies where the dominant horses were the heaviest (Houpt *et al.*, 1978).

There are problems in determining the hierarchy in small groups. Firstly, circular hierarchies may be formed with no clear alpha individual, and secondly sometimes there are too few antagonistic interactions between individuals in the group during a whole group observation to determine the exact relationship between individuals. This was the case with our group observations, where not all of the horses interacted with all other members of the group, and in one case all of the horses stood quietly and no interactions were observed at all.

Temperament may be a major factor in determining status. Hoput, Law and Martinisi (1978) found that aggression and temperament had the greatest influence over hierarchy within the horses they studied. The alpha horses used in the present study were either overtly aggressive or they had a more subtle control over the group. For instance, horse number 2 in group 2 was the alpha animal in her group and her control appeared to be uncontested despite the fact that she did not display any overt bite or kick threats; she just moved in and the opposing horse moved out of her way.

Horses that were classified by the manager as more difficult to handle were no more likely to be dominant in their new groups than horses that were easy to handle. The difficulty involved in handling those horses may have been due to the handler not being able to gain control over the horses' behaviour and not to the aggression between horses. Leading horses into the stocks may not be the best and only measure of handling ability, however it is an activity that these horses were required to do on a regular basis; therefore it was a true test of a typical handling situation for them.

Effect of dominance on time to "join-up" and behaviours during round-pen training

There was no significant correlation between the dominance rank of a horse and the total time to join up or the time until the first lick and chew. It was thought that dominant horses would take longer to complete round-pen training as success of this training relies on the horse showing signs of submission to the human trainer and more dominant horses may be less willing to become submissive to the trainer. However, lower ranked horses actually took longer to "join-up" than horses that were more highly ranked. The three horses that took the longest (more than 60 minutes) to successfully complete training were horses that were placed 4th, 5th and 6th in the hierarchies of their respective trial groups. This may be due to the horses in our trial having similar temperaments and dominance levels. There may have been little difference between the temperament of the high and low ranking individuals in the present study. Also there may have been some difference between the dominant individual in each of the four trial groups, hence it may have been better to run the dominance study looking at the dominance rank of individuals in relation to the group as a whole. This was not done due to the complicated and lengthy time such a dominance trial would have taken to carry out. It is possible that most of the differences in handling ability between low and high ranked individuals was due to the lower ranked horses being more reactive than the higher ranked horses, which is displayed as nervous behaviour during round-pen training. The higher reactivity in the lower ranked horses meant that these horses were more nervous and responded more slowly to round-pen training than less reactive horses. It has been seen in cattle that more dominant cows were less reactive and hence easier to handle (Plusquellec and Bouissou, 2001).

The lack of any significant correlations between many of the behaviours observed during round-pen training and the dominance rank of a horse does not prove that there is no relationship between social dominance and those behaviours. Correlations may be found if further studies used larger groups or a measure the relative dominance of all the horses together was used. There is also the possibility that certain behaviours that were expected to have some sort of relationship with the dominance, such as licking and chewing or pawing, may not have any relationship with the social status of horses.

3.5 Conclusions

The round-pen training of horses follows a predictable pattern of behaviours. However the pattern varies between individuals. There was no difference in the time to "join-up" of easy or difficult to handle horses, but there was a difference in the behaviours observed in the round-pen. Horses that showed a willingness to bond with the person came in and attempted to establish contact with the trainer, and joined-up more readily than horses that declined contact with the trainer. Social status had no apparent effect on ease of join-up.

Chapter 4 Effect of dominance on the behavioural and physiological response during the pre-treatment stocks test and the relationship between change in plasma cortisol concentration and behaviours in the stocks

"Did you ever see an unhappy horse? Did you ever see a bird that had the blues? One reason why birds and horses are not unhappy is because they are not trying to impress other birds and horses." Dale Carnegie

4.1 Introduction

Dominance in horses has been studied in relation to the social structure of feral groups (Goodloe, Warren, Osborn and Hall, 2000; Linklater, Cameron, Minot and Stafford, 1999; Linklater *et al.*, 2000; Rutberg and Keiper, 1993; Zharkikh, 1997), and the management of captive horses (Keiper and Sambraus, 1986). Dominance in horses is one aspect of temperament. Social status has no effect on the learning ability of horses (Araba and Crowell-Davis, 1994; Arnold and Grassia, 1982; Beaver, 1986; Ellard and Crowell-Davis, 1989; Heird *et al.*, 1986; Houpt *et al.*, 1978; Houpt *et al.*, 1982; Houpt and Wolski, 1980; Mal *et al.*, 1994; Mal *et al.*, 1993; Van Dierendonck and De Vries, 1995; Zeitler-Feicht, 1996; Zharkikh, 1997).

In horses, dominance rank is established by monitoring agonistic interactions between individual animals (Araba and Crowell-Davis, 1994; Arnold and Grassia, 1982; Ellard and Crowell-Davis, 1989; Hagg, Rudman and Houpt, 1980; Houpt *et al.*, 1978; Houpt and Wolski, 1980; Keiper and Sambraus, 1986; Mader and Price, 1980; Van Dierendonck and De Vries, 1995; Zharkikh, 1997). Araba and Crowell-Davis (1994) found a strong positive correlation between the aggression score of an individual horse and that horse's social rank in the group.

The effect of social rank on the ease of handling has not been studied in horses. In cattle, cows that have been selected for dominance are easier to handle than cows that have been selected for submissiveness (Plusquellec and Bouissou, 2001). The handling tests used for cows often involve the ease of confinement by a single human without the use of force and is a measure of flight distance (Fisher, Morris and Matthews, 2000). More dominant animals are easier to restrain under such circumstances. In the horse, handling tests usually involve the ease of manoeuvrability or the horse's reaction to certain stimuli (Heird *et al.*, 1981). The effect of dominance has not been tested in the horse in relation to the ease of handling or reactivity. It may be expected that more dominant horses would be less reactive and perform better in temperament tests measuring reaction to novel stimuli (Mal *et al.*, 1994; McCann *et al.*, 1988a).

The basal plasma cortisol concentration of an individual may be influenced by its relationship with the other members of the group. In baboons, plasma cortisol concentration has been positively correlated with dominance rank (Sapolsky and Ray, 1989). Coe *et al* (1979) found that the most dominant squirrel monkey had the highest plasma cortisol concentration but had the lowest stress response. McGuire, Brammer and Raleigh (1986) found that in Vervet monkeys plasma cortisol concentration did not differ between low and high ranking males in stable groups, but in newly formed groups the plasma cortisol concentration was highest in the most dominant individuals as they established their position in the new group. There was no difference in the basal plasma cortisol concentration of rhesus monkeys, but lower ranking males had more variable cortisol concentrations than high-ranking males (Bercovitch and Clarke, 1995).

The social rank of an individual has an effect on the physiological response of that individual to stress and the ability to cope with stressful events as in cows (Plusquellec and Bouissou, 2001), pigs (Otten, Puppe, Kanitz, Schoen and Stabenow, 1999) and monkeys (Coe, Mendoza and Levine, 1979; Gust, Gordon, Hambright and Wilson, 1993; McGuire, Brammer and Raleigh, 1986; Saltzman, Schultz-Darken and Abbot, 1996; Sapolsky and Ray, 1989). This has not been demonstrated in the horse. Cows selected for being dominant had a smaller cortisol response to surprise than cows selected for submissiveness (Plusquellec and Bouissou, 2001). Dominant pigs had a higher cortisol response than subordinate pigs during an introduction to a non-familiar pig (Otten *et al.*, 1999). In the horse it is unknown whether dominance rank has any effect on the physiological response to stress, however it might be assumed that if dominant horses are less behaviourally reactive then they might have less of a stress response during restraint. Conversely dominant horses may have a grater stress response to restraint situations due to their having a higher 'drive' to control their surroundings than may less dominant horses.

The effect of dominance status on the behaviour of horses during restraint has not been studied. If dominant horses are less reactive, then they may display more resting and less agitation behaviours during restraint.

Behaviour has been used as a non-invasive indicator of welfare in many different species of animals. In calves (de Passille, Rushen and Martin, 1995), high frequencies of defecation and vocalisation associated with movement, including walking, represent a fear response to novel and social stress. Cows have a higher rate of defecation and urination when being milked in an unknown room and during social isolation (Rushen, Munksgaard, Marnet and de Passille, 2001).

Studies of stress in restrained horses have used plasma cortisol concentration (Hydbring *et al.*, 1996; Irvine and Alexander, 1994; Lagerweij *et al.*, 1984; Minero *et al.*, 1999; Pitman *et al.*, 1987), heart rate (Jezierski and Gorecka, 1999; Jezierski *et al.*, 1999; McCann *et al.*, 1988b; Minero *et al.*, 1999; Stewart *et al.*, 2003; Waran *et al.*, 1996) and behavioural changes (Hydbring *et al.*, 1996; Lagerweij *et al.*, 1984; McCann *et al.*, 1988a; Minero *et al.*, 1999; Waran *et al.*, 1996) as indicators of stress. The social position of a horse may affect the physiological and behavioural responses seen in response to acute stress such as restraint or novel stimuli.

The purposes of the following study were to: 1) determine if a horse's dominance rank affected the ease of handling, its stress response during stocks tests, or its behavioural response in stocks, and 2) to see if there was any correlation between the behaviour and the plasma cortisol response of a horse during the stocks test.

4.2 Methods

The behavioural data and plasma cortisol concentration reported in Chapter 2 were used in conjunction with the dominance rank noted in Chapter 3 to examine the relationship between the dominance rank and the ease of handling and the response to stress in the horses used in the trial.

4.4.1 Data

The data collected during the studies presented in Chapters 2 and 3 re-analysed here to see if there was any effect of dominance rank on the occurrence of behaviours or the plasma cortisol concentration seen in the pre-treatment stocks test. Then the data from all three stocks tests were analysed to see if there were any correlations between the plasma cortisol stress response and the occurrence of behaviours during the stocks test. Therefore the horses used, stocks test procedures, measurements made, blood sampling protocol, and the dominance tests were the same methods and data used were the same as Chapters 2 and 3.

4.4.2 Behavioural Observations

The following behaviours were recorded:

Head bobbing or shaking, head turning, moving the front leg, resting the hind leg, kicking or stamping the back leg, pawing with the front leg, tail swishing, ears held back, licking and chewing, urination, defecation, ear-flicking, head down, head held up high, snort, sniff and yawn, head rubbing, and pushing hindquarters against the rope at the back of the stock.

4.4.3 Statistical analyses

Within each group the dominance rank was established with the most dominant horse being given rank 1 and the least dominant rank 6. The behavioural observations for each horse during each of the three stocks tests (pre-, post- and 3 weeks post-treatment) were tabulated and the data analysed. The behaviours observed during the pre-treatment stocks test were analysed using the MIXED procedure in SAS (2001). The model included the fixed effects of social status, stocks test, their interaction, and the random effect of animal within treatment. The correlation coefficients were calculated and the Wilcoxon signed rank test (CORR procedure in SAS (2001)) was used to determine if there was a relationship between change in plasma cortisol concentration and the time to enter the stocks or the other behaviour variables (head shake, head turning, licking and chewing, ear flicking, front leg move, hind leg rest and ears back, urination, defecation, kicking, pawing, vocalisation, bum push on rope, tail swishing and head rubbing).

4.3 Results

Does dominance rank affect the ease of handling?

Ease of handling was defined by the time it took to lead each horse into stocks (Table 4-1). During the pre-treatment stocks test horses in rank 5 took longer to enter the stocks than horses in ranks 1, 2, 3, 4, and 6, but this difference was significantly different ($P<0.05$) only with ranks 1-4 (Table 4-2).

Table 4-1 Initial sorting and scoring of horses used in the trial (from Table 2.2)

Horse Number	Time to enter stocks (seconds)	Handling Score	Farm Managers Classification	Treatment	Trial Group	Dominance rank
25	35	2	Good	A	1	1
2	21	1	Problem	Control	2	1
6	20	1	Good	Control	3	1
20	22	1	Good	Control	4	1
13	300	5	Problem	B	1	2
115	300	5	Problem	B	2	2
3	24	1	Good	Control	3	2
33	20	1	Good	Control	4	2
4	25	1.5	Good	A	1	3
8	23	1	Good	A	2	3
29	60	3	Good	B	3	3
90	22	1	Good	A	4	3
51	23	1	Problem	Control	1	4
12	22	1	Good	Control	2	4
62	18	1	Good	B	3	4
27	157	4	Good	B	4	4
26	27	1	Good	Control	1	5
45	26	1.5	Good	A	2	5
10	38	2	Good	A	3	5
23	448	5	Problem	B	4	5
15	456	5	Problem	B	1	6
24	210	4.5	Problem	B	2	6
14	20	4	Good	A	3	6
92	25	2	Problem	A	4	6

Table 4-2 Mean time to enter the stocks within dominance ranks during the pre-treatment stocks test

Dominance Rank	Stocks Test	Number of horses	Mean time to enter stocks (seconds)	Standard Deviation	Range (seconds)
1	Pre-treatment	4	24 [a]	11.8	12- 40
2	Pre-treatment	4	59 [a]	50.7	22- 130
3	Pre-treatment	4	31 [a]	20.4	19- 61
4	Pre-treatment	4	30 [a]	9.7	23 - 44
5	Pre-treatment	4	189 [b]	190.3	26 - 400
6	Pre-treatment	4	131 [ab]	181.5	20 - 400

Different letters shows a significant difference between ranks ($P<0.05$)

Does dominance rank affect the behaviour of the horse in the pre-treatment stocks test?

Head shaking behaviour

In the pre-treatment stocks test horses in dominance rank 1 did significantly more head shaking than horses in dominance ranks 3, 4, 5 or 6 (Table 4-3). There was no significant difference ($p>0.05$) between the number of head-shakes between horses in dominance ranks 1 and 2 or in dominance ranks 2, 3, 4, 5, and 6.

Front leg step

During the stocks test before treatment horses in dominance rank 6 were significantly different from horses in ranks 1, 2, 3, 4, and 5 (Table 4-3). There were no significant difference ($p>0.05$) between any of the other dominance ranks at this time.

Hind leg rest

During the pre-treatment stocks test horses in rank 6 did significantly less ($p<0.05$) hind leg resting than horses in ranks 1 and 2 (Table 4-3). There was no significant difference between any other dominance ranks during this stocks test.

Kicking

During the pre-treatment stocks test horses in dominance rank 1 kicked significantly ($p<0.05$) more often than horses in all other ranks (Table 4-3), but there was no significant difference in the number of kicks between any of the other 5 ranks during this stocks test.

Pawing

During the pre-treatment stocks test horses in dominance rank 2 performed significantly more ($p<0.05$) pawing than horses in dominance ranks 5 and 6, but not from horses in dominance ranks 1, 3, or 4 (Table 4-3).

Ears back

Horses in dominance rank 6 held their ears back significantly ($p<0.05$) less often than horses in ranks 1, 3, and 4 during the pre-treatment stocks test (Table 4-3).

Ear flicking

During the pre-treatment and post-treatment stocks tests, horses in rank 1 performed significantly ($p<0.05$) less ear flicks than horses in rank 4 (Table 4-3). During the 3 weeks post-treatment stocks test horses in rank 3 had significantly ($p>0.05$) more ear flicking than horses in ranks 1, 5 and 6 (Table 4-3).

Snort, Sniff or Yawn

During the pre-treatment stocks test horses in rank 2 displayed a significantly greater number of snort, sniff or yawn behaviours than horses in rank 3 (Table 4-3).

Other Behaviours

There were no significant differences ($p>0.05$) between the six different ranks within any of the three stocks tests or within any of the six ranks between any of the three stocks test for the following behaviours: tail swishing, licking and chewing, urination, head held down, head held up, head turn, defecation, tail held up, vocalisation and head rubbing behaviours (Table 4-3).

The bum push on rope behaviour was not included in this analysis because there were only two animals observed performing this behaviour and they were both in rank 5.

Table 4-3 Mean number ± standard deviation for each behaviour within each dominance rank during the pre-treatment stocks test.

Behaviour	Rank 1	Rank 2	Rank 3	Rank 4	Rank 5	Rank 6
Head Shaking (total number)	7 ± 4.8^{a}	4 ± 2.8^{ab}	2 ± 1.8^{b}	2 ± 2.6^{b}	3 ± 2.2^{b}	2 ± 1.3^{b}
Front leg step (total number)	5 ± 4.5^{a}	7 ± 1.5^{a}	1 ± 1.0^{a}	4 ± 8.0^{a}	9 ± 7.0^{a}	28 ± 48.4^{b}
Hind leg rest (bouts)	12 ± 8.3^{a}	10 ± 4.4^{a}	6 ± 2.9^{ab}	7 ± 3.3^{ab}	6 ± 5.1^{ab}	3 ± 2.2^{b}
Kicking (total number)	4 ± 3.4^{a}	1 ± 2.0^{b}	1 ± 1.5^{b}	1 ± 1.4^{b}	2 ± 1.5^{b}	0 ± 0.0^{b}
Pawing (bouts)	1 ± 0.5^{ab}	2 ± 1.7^{a}	1 ± 1.0^{ab}	1 ± 1.5^{ab}	0 ± 0.0^{b}	0 ± 0.0^{b}
Ears held back (bouts)	13 ± 4.3^{a}	9 ± 2.4^{ab}	11 ± 4.8^{a}	11 ± 3.6^{a}	7 ± 7.1^{ab}	4 ± 3.4^{b}
Ears flicking (total number)	8 ± 4.4^{a}	13 ± 9.0^{ab}	15 ± 4.8^{ab}	17 ± 9.9^{b}	10 ± 2.5^{ab}	14 ± 10.9^{ab}
Snort/sniff/yawn (bouts)	3 ± 1.7^{ab}	5 ± 1.8^{a}	2 ± 1.0^{b}	3 ± 2.0^{ab}	3 ± 2.1^{ab}	4 ± 1.7^{ab}
Tail swish (bouts)	2 ± 2.2	0 ± 0.5	0 ± 0.0	0 ± 0.0	1 ± 1.5	1 ± 1.9
Lick and chew (bouts)	12 ± 7.5	10 ± 4.4	12 ± 8.2	6 ± 2.7	11 ± 5.2	13 ± 6.1
Urine (total number)	1.00 ± 0.8	0.50 ± 0.6	0.00 ± 0.0	0.25 ± 0.5	0.75 ± 1.0	$0.50 + 0.6$
Defecation (total number)	1.0 ± 0.8	1.3 ± 0.5	1.0 ± 0.0	0.8 ± 0.5	1.5 ± 1.0	0.8 ± 0.5
Head stretched down (bouts)	5 ± 3.1	3 ± 2.9	2 ± 1.7	4 ± 1.7	2 ± 1.0	2 ± 0.8
Head held up (total number)	1 ± 1.4	2 ± 1.3	0 v 0.0	1 ± 1.0	2 ± 1.0	2 ± 1.3
Head turn (total number)	23 ± 13	23 ± 11	27 ± 13	22 ± 13	34 ± 27	34 ± 20
Vocalisation (total number)	0.5 ± 1.0	0.3 ± 0.5	0.5 ± 1.0	0.0 ± 0.0	1.3 ± 1.9	0.8 ± 1.5
Head rub (total number)	1.3 ± 1.9	1.5 ± 1.7	1.3 ± 2.5	0.3 ± 0.5	0.5 ± 1.0	0.0 ± 0.0

Letters that are different (within each behaviour) denotes a significant difference between dominance ranks for that behaviour ($P<0.05$)

Dominance effect on cortisol response during stocks test.

Before the start of the stocks test there was no difference (P>0.05) in the resting plasma cortisol concentration of horses in the different dominance ranks (Table 4-4).

Table 4-4 Mean (± s.e.) resting plasma cortisol concentrations (nmol/L) for each of the six social ranks before treatment

Dominance status	Number of horses	Mean plasma cortisol concentration (nmol/L)	Minimum (nmol/L)	Maximum (nmol/L)
1	4	193 ± 40	133	219
2	4	130 ± 15	109	144
3	4	152 ± 34	117	194
4	4	163 ± 86	88	278
5	4	174 ± 26	153	210
6	4	175 ± 43	129	227

No significant difference (P>0.05) in plasma cortisol concentration between the dominance ranks

There was no significant difference in rate of change in plasma cortisol concentration of horses in different ranks during the pre-treatment stocks test (Table 4-5).

Table 4-5 Mean (± s.e.) change in plasma cortisol concentration (nmol/L/hour) during the pre-treatment stocks test, within each dominance rank

Dominance status	Number of horses	Mean change in plasma cortisol concentration (nmol/L/hour)	Minimum change (nmol/L/hour)	Maximum change (nmol/L/hour)
1	4	8 ± 43	-50	44
2	4	46 ± 36	5	91
3	4	49 ± 13	13	65
4	4	31 ± 13	15	46
5	4	5 ± 59	-42	89
6	4	22 ± 36	-24	56

No significant difference (P>0.05) in the change in the plasma cortisol concentration during the stocks test between dominance ranks

Does the change in cortisol reflect the incidence of specific behaviours during the stocks tests?

All values for the behaviour data were pooled and spearman rank correlations were used to examine the significance of relationships between the increase in plasma cortisol concentration during the stocks tests and the incidence of certain behaviours. There were significant correlations between the cortisol and the following behaviours; head turning (r=0.3; P<0.01) (Figure 4-1), head up (r=0.26; P<0.05) (Figure 4-2), and defecation (r=0.5; P<0.0001) (Figure 4-3).

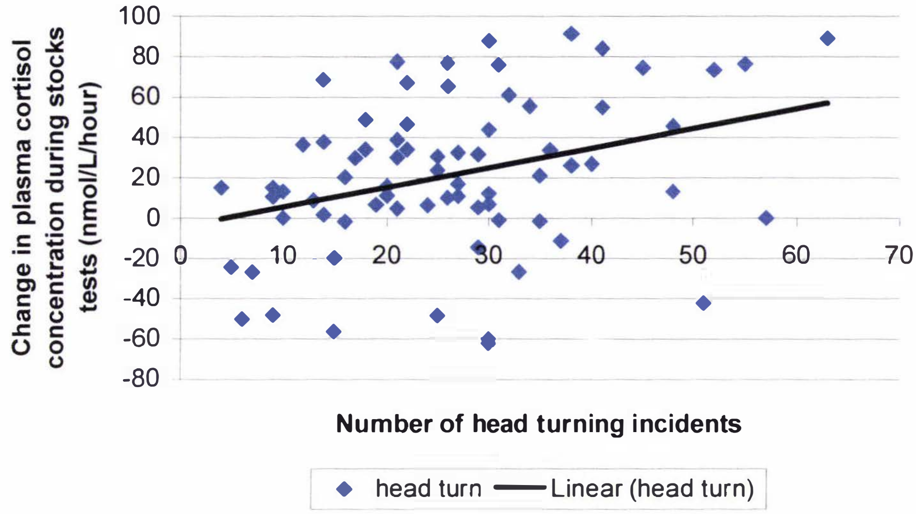

Figure 4-1 Correlation between change in plasma cortisol concentration and the number of head turning incidents observed during all three stocks tests.

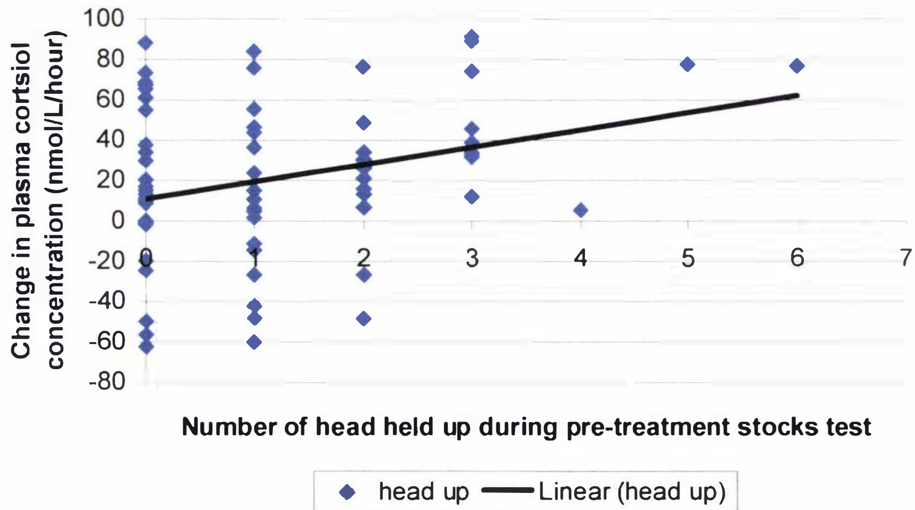

Figure 4-2 Relationship between the change in plasma cortisol concentration and the head held up behaviour during the pre-treatment stocks test.

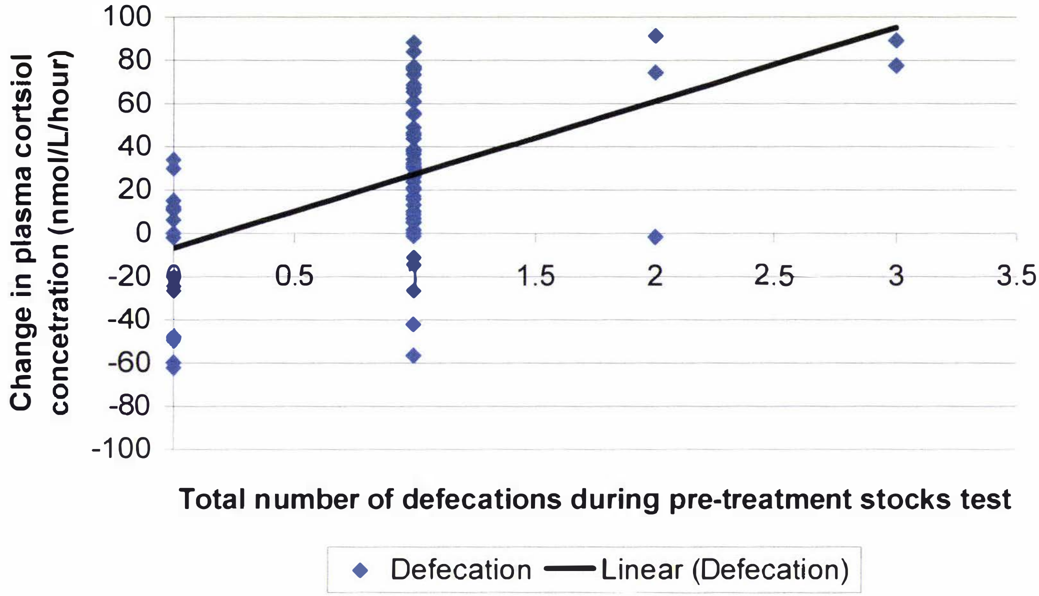

Figure 4-3 Relationship between number of defecations and change in plasma cortisol concentration during pre-treatment stocks test.

Horses that had a greater increase in plasma cortisol concentration during each of the stocks tests, generally had a greater rate of head turning and head held up high than horses that had less of an increase in plasma cortisol concentration during the stocks test. Horses that defecated more during the stocks tests generally had a greater change in plasma cortisol concentration than horses that had less defecation during the stocks tests. There were no significant correlations between change in plasma cortisol concentration and any of the other behaviours.

4.4 Discussion

4.4.1 Does dominance rank affect the ease of handling?

Dominance had an effect on the ease of handling. Horses in the lower ranks, 5 and 6, took longer to get into the stocks than horses in the more dominant ranks. The longer time to enter the stocks for the 5th and 6th ranked horses may suggest that these horses were more fearful during this handling situation, possibly due to greater reactivity than the more dominant horses. It would be interesting to subject the horses to reactivity tests, such as the umbrella test (MacKenzie and Thiboutot, 1997), to see if they are more reactive than more dominant horses. The higher ranked horses may occupy their position in the group because they are less easily frightened by novel objects or influenced less by the aggression of gaining and maintaining a dominant position than more reactive horses.

Most of the horses used in this trial have been housed at VLATU and may be habituated to the stocks, so it is not known if the handling tests the horse's reaction to the handler or its reaction to being led and restrained in the stocks. One way to see if this is a genuine reaction to the stocks would be to use naive horses and compare the ease of handling and reactivity test results to those of the VLATU horses.

4.4.2 Does dominance rank affect the behaviour of the horse during the pre-treatment stocks test?

It should be noted that some horses showed only agitation or rest behaviours while other horses showed both agitation and rest behaviours at the same time. This may mean under the conditions of the present study that some behaviours, which have been interpreted as agitation or rest, may have been misinterpreted or that horses may demonstrate both agitation and rest behaviours in response to restraint in stocks.

The occurrence of head shaking, front leg step, hind leg rest, kicking, ears held back, ear flicking and snort/sniff/yawn behaviours were different between ranks. The status of horses did not affect the incidence of the following behaviours: tail swishing, licking and chewing, urination, defecation, head held down, head held up, head turning and head rubbing behaviours during the pre-treatment stocks test. Only the behaviours observed during the pre-treatment stocks test were used to compare the difference between ranks because it was thought that after treatment there may have been too many other factors altering the occurrence between individuals.

More dominant horses showed less front leg step and ear flicking behaviours than less dominant horses and the more dominant horses had a greater incidence of resting behaviours, such as hind leg rest and snort/sniff/yawn, than lower ranked horses. This suggests that the more dominant horses were more relaxed than lower ranked horses. However the dominant horses showed more head shaking, kicking, pawing and ears held back than lower ranking horses. This suggests that the dominant horses were more agitated than the lower ranked horses, or that they displayed their agitation more openly. Weeks and Beck (1996) have described ear flattening, head shaking (tossing) and tail swishing as signs of agitation in the horse.

4.4.3 Dominance effect on cortisol response during stocks test

There was no difference between ranks in the resting plasma cortisol concentration or the change in plasma cortisol concentration during the stocks test. Therefore dominance had no apparent effect on plasma cortisol (resting or stress response) in the horse. All horses used in this trial had similar physiological states prior to testing, found being in the stocks equally stressful, and their physiological responses did not differ due to their social position. These results differ from those found in cows, where dominant cows have a lower rise in plasma cortisol concentration in response to an acute stressor (Plusquellec and Bouissou, 2001).

The lack of difference in plasma cortisol concentration between low and high ranking individuals may be due to the length of time these horses have been housed together, where the group is stable and all individuals in the group have similar resting physiological measurements. In vervet monkeys living in stable groups there was no difference in resting plasma cortisol concentration between low and high ranking individuals (McGuire *et al.*, 1986). Alternatively the lack of difference between different social ranks in the present horses could be due to either the differences between individuals of low and high rank being too small to detect or because dominance rank had no effect on the plasma cortisol concentration response to an acute stressor.

4.4.4 Does the change in cortisol concentration reflect the incidence of specific behaviours during the stocks tests

Positive relationships were fond between the overall increase in plasma cortisol concentration during the stocks tests and the following behaviours: head turning, head held up and defecation.

In the cow, a high rate of defecation is found to be associated with stress (Rushen, 2000). Therefore in the horse it may be worthwhile to further examine the relationship between increase in plasma cortisol concentration and defecation as a non-invasive measure of stress in restrained horses.

The positive correlations with head turning or head up behaviours and plasma cortisol concentration could be an indicator of restlessness and agitation. The head turning and head up behaviours could be indicative of the horse's restlessness and level of activity. The occurrence of activity in the horses while in the stocks could contribute to the greater increase in plasma cortisol (Pitman *et al.*, 1987).

4.5 Conclusions

In these horses dominance appeared to have no effect on the ease of handling. Dominance may have had an effect on the behaviours observed under restraint in stocks. There was no difference in the resting concentration or in the response to stress of plasma cortisol concentration in horses of high or low dominance rank in the present study. The change in behaviour of horses restrained in stocks may be useful as a non-invasive indicator of the onset and intensity of stress during restraint in the horse.

Chapter 5 General Discussion

"Old minds are like old horses;
you must exercise them if you wish to keep them in working order."
John Adams

General Discussion

"Natural" horsemanship is the name given to the methods of horse training that involve 'Natural' methods. Proponents claim that there are many ways of training using Natural horsemanship, however the key principles are for the horse and human to be bonded to each other and work as a partnership with natural communication. Natural horsemanship depends on the human understanding and using the natural language of the horse as a means of communication between horse and human (Roberts, 1996). Round-pen training has gained international attention due to Monty Roberts (Roberts, 1996).

One form of Natural horsemanship, Round-pen training is used to start un-handled horses or to retrain horses that are difficult to handle (Roberts, 1996). In the present study round-pen training was used on horses that were previousely handled, to determine if it improved their ease of handling and their ability to cope with the stress of restraint. It was thought that round-pen training could reduce the stress response of these horses during restraint in stocks.

In the present study it was proposed that round-pen training may help horses used for teaching, to cope with the stress of restraint. There are many issues surrounding the welfare of animals used in teaching, which include the right to use animals for research and teaching (Clark, Rager and Calpin, 1997), keeping the animal in an unnatural environment, exposure to painful stimuli, exposure to psychologically stressful stimuli and repeated exposure to chronic stressors. The horses at Massey's Veterinary Large Animal Teaching Unit (VLATU) may be exposed to stressors on a weekly basis for extended periods of time, which may cause significant implications to their welfare.

At present there have been no published studies on the effects of round-pen training on the horses behavioural or physiological response to a stressor.

The present study aimed to determine the following:

1. If round-pen training had an effect on the ease of handling of horses, or on the horses' physiological or behavioural responses to being led into and held in stocks. The behaviour of horses that had or had not been round-pen trained were

compared and a comparison made of their behaviour prior to and following round-pen training.

2. If the physiological and behavioural responses of horses during round-pen training were analysed to see if dominance status of a horse had any effect on the ease of round-pen training (time) and the behaviours seen in the round-pen, and to see if there was any correlation between the time taken to "join-up" and behaviours observed in the round-pen.
3. If dominance status had an affect on the ease of handling, and the physiological and behavioural responses of horses during the stocks test was determined.

The following conclusions of this thesis were reached:

One round-pen training session did not significantly affect the ease of handling of the horses in this experiment, however the results suggest that round-pen training could potentially improve the ease of handling in horses used in veterinary teaching; with the improvement in handling being more beneficial and noticeable in horses that are classified as difficult to handle. One session of round-pen training had no effect on the plasma cortisol and heart rate response to a stressor. Round-pen training did not alter the behaviours observed during the stocks test post-treatment in the difficult horses, but it did reduce the occurrence of agitation behaviours in the easy to handle horses.

During round-pen training the horses displayed behaviours that had been mentioned before by Roberts (1996). There was a lot of individual variation in the horses' behaviour during round-pen training. The dominance status of a horse had no effect on the time to "join-up" or the behaviours observed during round-pen training.

Certain behaviours, such as turning in and approaching the trainer, could be used as an indication of a horses willingness to form a bond with the trainer, and as such when observed could be used to predict the impending completion of training.

There was little difference in the time to enter the stocks or the behaviours seen during the stocks test, between horses in low or high ranks before round-pen training. In the stocks test head turning, head held up and defecation rate could be related to elevated plasma cortisol concentrations, and as such may be useful as indicators of the onset of stress in restrained horses.

The horses used in the present study had experience of being held in the stocks at the VLATU facility. Therefore the responses observed and the ease of handling in the stocks tests may have been affected by the horses' previous experience with the stocks. Most of the horses were regularly held in the stocks for the use in practical teaching sessions, involving procedures such as naso-gastric tube insertion, rectal palpation, and blood sampling, procedures, which may have caused some discomfort to the horses. Hence the horses may enter the stocks easily enough, but they may become anxious in anticipation of what procedures they may undergo while being held in the stocks.

The change in behaviour and physiology in these horses due to round-pen training may have been much less than if the horses had had less handling experience or if unbroken horses had been used. However getting a large group of horses that were naive to the VALTU stocks or unbroken horses was not possible. Also handling horses that had not previously been handled may cause significant safety issues for both horses and handlers. Hence the observed changes in the behavioural and physiological responses of the horses in the present study may be less significant than the effect on naïve horses.

It was decided to examine the effect of one round-pen training session only. Roberts (1996) advocates the use of one round-pen training session to form a bond between horse and trainer, then follow-up handling sessions to help establish that bond further. It is possible that if further training sessions were used that the effect of the round-pen training would be more noticeable. Therefore further round-pen training sessions for horses may have a greater effect on the physiological and behavioural responses of restrained horses.

The plasma cortisol concentration in the horses that underwent round-pen training remained elevated during the post treatment stocks test. However other studies (Alexander *et al.*, 1991; Snow and MacKenzie, 1977) have shown that plasma cortisol concentration remain elevated for at least 30 minutes following acute exercise. Therefore it would be better to carry out the post-treatment stocks test on the day following the round-pen training when plasma cortisol concentrations had returned to resting levels.

Stocks tests were carried out over four different days and this led to variation in the resting plasma cortisol concentration between trial groups. The horses were divided into four trial day groups because of time constraints and although it may not be possible to remove the effect of trial day from the experiment there are a few ways in which the effect could be minimised. It was possible to do all the testing on one day, do the treatment over several days and then do all the retesting on one day. A different stress test procedure could be used so that more horses were able to be handled at one time.

In future research it may be worthwhile to increase the number of round-pen training sessions the horses have before they are re-tested in the stocks. Further training or handling sessions would strengthen the bond between horse and human and the potential effects of round-pen training on the physiological and behavioural responses during restraint may become more distinguishable. Alternatively the same protocol as that in the present study could be used with horses that were not familiar with the VLATU facility and stocks.

In the future studies should be conducted that to compare the effect of round-pen training with other methods of training on the ease of handling, ease of training and effectiveness of the training. For example comparing the performance, or stress responses, of racehorses or sport horses that had been trained by either Natural horsemanship or conventional methods. Studies comparing different methods of horse training will be difficult because of inconsistency between trainers using the 'same' method of training, getting sufficient numbers of naive horses to train and getting horses that have had similar previous experience before training to reduce the chance of differences between individuals.

It is not known whether or not Natural horsemanship is a fashion trend or the future of horse training. Population studies over the next decade to determine the number of people using Natural horsemanship training in the different equestrian disciplines will inform us of its effectiveness and efficacy.

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