Assessment of pain in horses after surgical castration

Composition of a pain scale

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2008/2009

Contents

Summary

The objective of this study was to evaluate physiological and behavioural parameters in order to create a composite pain scale for horses after surgical castration. Based on literature several parameters were chosen and tested for their use in a composite pain scale.
Two groups of horses (3 in each group) were studied one day preoperatively and three days postoperatively after surgical castration. The horses in the control group received, according the protocol of the University of Utrecht, analgesia preoperatively. The horses in the meloxicam group received analgesia preoperatively and extra analgesia (meloxicam) during the first three days postoperatively. The physiological parameters tested were body temperature, body weight, plasma cortisol concentration, gait, heart rate variability and swelling of the scrotal area. The behavioural parameters consisted of a direct behavioural assessment and a time budget of behaviour, composed of videorecordings of the horses.
No important significant differences were found between the meloxicam group and the control group in most of the tested parameters. Almost no significant differences within a group between the base line value and any other day were seen either.
This suggests either that the horses of the control group did not experience more pain than the horses in the meloxicam group, or that none of the parameters was sensitive enough to measure the difference between the meloxicam group and the control group.
However, only six horses were tested, limiting the results and conclusions that can be drawn from this study. The parameters tested in this study were concluded to need more testing in following studies containing more animals before they can be exluded for sure.

1. Introduction

Ideas and perspectives change with the years. So has the attitude among veterinarians with regard to pain in animals. Animals became more and more important as companions; most of them are even important members of the family. Therefore adequate pain management in animals is becoming more and more desirable. Sufficient knowledge, however, often lacks. Time for some research. When does an animal experience pain and how can it be measured?

René Descartes (philosopher, physician, 1596-1650) stated 'The greatest of all the prejudices we have retained from our infancy is that of believing that the beasts think.'
His idea was that since animals have no capacity for reasoning they cannot have perception of pain.
Descartes' idea isn't generally accepted anymore and nowadays most veterinarians do not doubt that animals are able to experience pain. Nevertheless, his daring statement and his philosophy of putting all beliefs, ideas and thoughts in doubt contributed to the critical approach on which scientific research is grounded. Such a critical approach should be adopted in any scientific research project.

Definition of pain

In order to start a scientific research project with regard to pain, one should first know how to define pain.
Pain is defined as an unpleasant sensory and/or an emotional experience, which can range from mild, localized discomfort to agony, caused by actual or possible tissue damage or described in terms of such damage (Flecknell, 2000). Sometimes, however, it exists without any apparent physical cause.
The pain results from stimulation of nociceptors in the body, that send electrical signals from the place of injury to the central nervous system, eliciting motor actions that protect the body from more tissue damage. The motor action, often a withdrawal response, is initiated by transferring the signal from the peripheral sensory nerve to the dorsal horn in the spinal cord, where it is transferred to the ventral horn and from there to the motor neurons that innervate the muscles, resulting in their activation. In the spinal cord the signal is also modulated and sent from the dorsal horn to the brain (cortex/thalamus) via the spinothalamic and spinoreticular tracts, resulting in the actual realization of pain.
Modulation of pain (in the periphery as well as the spinal cord) is a complex process. The more is known about the modulation of pain, the clearer it becomes how complex this process is. Secondary messengers and inflammatory substances result in summation or inhibition of signals causing more or less pain.
Because pain is defined as a sensory and emotional experience, only animals that are conscious can experience pain. Pain without consciousness is called nociception.

'Analogy postulate'

The question comes to mind whether animals are conscious and thus can experience pain. After Descartes many other philosophers also made up their minds about this.
Jeremy Bentham, (English jurist and philosopher, 1748-1832), one of the earliest proponents of animal rights argued that the ability to suffer, not the ability to reason, must be the benchmark of how to treat other beings. He stated:

"It may one day come to be recognised that the number of the legs, the villosity of the skin, or the termination of the os sacrum are reasons equally insufficient for abandoning a sensitive being to the same fate.
What else is it that should trace the insuperable line? Is it the faculty of reason or perhaps the faculty of discourse? But a full-grown horse or dog is beyond comparison a more rational, as well as more conversable animal, than an infant of a day or a week or even a month old. But suppose they were otherwise, what would it avail? The question is not, Can they reason? nor Can they talk? but Can they suffer?"

The so-called analogy postulate is also often used. This postulate states that based on the homologous anatomy of the perception and reception organs of humans and animals we should acknowledge that animals, in analogy with humans, are able to experience pain. Verhoog en Wemelsfelder (1988) formulated it as: 'The analogy postulate implies that the presence or absence of consciousness in animals cannot directly be scientifically proven. Based on similarities between humans and animals in anatomy, physiology and behaviour however, one may assume that there are also similarities in subjective perception.' *(Free translation from Dutch.)*
The analogy postulate makes it plausible that animals are able to emotionally experience pain.

The question whether animals are conscious or not, will however probably never be answered scientifically. Therefore animals should be given the benefit of the doubt.

Recognition of pain

The notion in veterinarians that animals are able to experience pain is seen in the outcome of questionnaires by Dohoo&Dohoo (1996a, b), Capner et al. (1999), Lascelles et al. (2000) and Raekallio et al. (2003). They investigated veterinarians in their countries with regard to their attitude towards pain management in animals. They found the majority of the respondents convinced that animals experience pain in a range of circumstances.

However, the use of analgesics in veterinary medicine is still little compared to the use in man. The questionnaires of Dohoo & Dohoo (1996a, b), returned by 275 Canadian veterinarians, revealed that 84% of the dogs and 70% of the cats received analgesics postoperatively after orthopaedic surgery. After castration however percentages of 10% of the dogs and 9% of the cats receiving analgesics postoperatively were obtained.

Difficulties in assessing the level of pain the animal experiences, might contribute to these results. Raekallio et al. (2003) found in his questionnaire 40% of 411 Finnish veterinarians agreeing more or less with the statement 'it is difficult to recognize pain in animals'. In the study performed by Dohoo&Dohoo (1996a) 77% of the 275 Canadian veterinarians that were surveyed, considered their knowledge of issues related to the recognition and control of postoperative pain inadequate.

These problems in recognizing (levels of) pain cause doubt about the use of analgesics in animals.

There is, for example, considerable professional debate over the necessity to provide analgesia following castration in horses. In a survey of Price et al. (2002) of 400 veterinary practitioners in the UK that worked with horses, 70% of 93 respondents defined the pain severity of castration in horses as 'low'. In another study Price et al. (2005) found that only 36.9% of 282 veterinarians in the UK provided analgesia following castration.routinely.

No records of well-performed studies defining postoperative pain in horses following castration are known. Each veterinarian individually seems to estimate the severity of pain the procedure causes, resulting in substantial differences in the management of pain. Information from well-controlled clinical studies would therefore be very useful and could be beneficial for many animals.

Pain assessment

Effective pain management can only be achieved if one is able to accurately, reliably and objectively assess the signs of pain expressed by the animal.

Without this, prescription of analgesics is solely based on interpretation and perception of pain in the animal by the veterinarian, who bases his decision on his own experience in similar cases. The veterinarian probably also compares this with his own or with other human experiences; than it can be called anthropomorphism.

This approach may lead to unnecessary administration of analgesics or worse, inadequate analgesia. Therefore adequate assessment systems are needed that contain practical, validated parameters to indicate pain.

Over the years several pain-scoring systems have been developed for different kind of species. (REF"S). Some are based on objective measurements of physiological variables, others on behavioural assessment, or a combination of both. Furthermore, there are subjective scoring systems, using a visual analogue scale, simple descriptive systems or a numerical rating scale, and multidimensional scoring systems, for example the University of Melbourne Pain Scale (Firth et al. 1999) and the University of Glasgow Composite Pain Scale (Thomas et al. 1996, Holton et al. 2001). The University of Melbourne Pain Scale for example, includes 6 categories (i.e. physiologic data, response to palpation, activity, mental status, posture and vocalization). Each category contains descriptions of various behaviours that are assigned numeric values. The assessor decides which description approximates the dog's behaviour and adds the scores of each category. The maximum total pain score is 27 points, corresponding with severe pain (Hellebrekers, 2000). The University of Glasgow Composite Pain Scale is comparable to this system.

All systems are based on more or less reliable parameters and usually assessed under certain specific circumstances and therefore not applicable during all circumstances. There are different kinds of pain, for example systemic, musculair and neurological pain On top of this, species, breed, sexe, hormonal status and environmental circumstances may cause differences in the experience and expression of pain. Therefore one should be very critical when one uses one of these systems.

An ideal pain assessment system should be sensitive, simple and easy to apply, have well defined criteria for the circumstances in which it can be applied and be well validated in the species it is used for.

So far, little research has been done in pain assessment in horses.

As horses are prey species they have evolved to minimise the signs of pain, making it even more difficult for humans to recognize it properly. Several different parameters have been studied over the years, resulting in different results. One should keep in mind however, that each study was performed under different circumstances, measuring different types of pain.

Since there appears to be little consensus among veterinarians on the presence or absence of pain in horses after castration (Price et al. 2002) and because Green (2001) suggested that the castration of a horse is not a particularly painful procedure, it would be interesting to try to define, quantify and validate pain in horses after castration.

Objective of the study

The objective of this study was to evaluate physiological and behavioural parameters, based on recent literature, in order to create a composite pain scale in horses after surgical castration.
The second objective was to measure, up to three days postoperatively, the influence of postoperatively administered NSAIDs, compared to a placebo, on the physiological and behavioural responses of the castrated horse, assessing the selected parameters.

2. Evaluation of potential parameters for pain assessment

As far as the author is informed, no published research has been performed into postoperative pain in horses after castration. Therefore no pain scale to assess this type of pain existed yet. As castration is performed daily on hundreds of horses, development of a system to score postoperative pain after castration would be very useful.
Next, some recent literature concerning pain assessment in horses after other types of surgery and the parameters used in these studies will be discussed. One should keep in mind however that different types of pain need different assessment systems composed of different parameters. If a parameter has proven to be useful in one study, it doesn't have to be useful in the next study.

Physiological parameters

A composite pain scale ideally is composed of physiological and behavioural parameters.
Examples of physiological parameters are heart rate, body temperature, levels of stress-related hormones etc.
Price et al. (2002) did research into attitudes towards pain in horses. When equine veterinarians were asked how they recognize pain in their patients, the most frequent answers were "heart rate" and "the animal's demeanour".
Physiological parameters, like heart rate have been tested several times during the last few years and increased heart rate is often seen as an indicator of severe pain (REF's toevoegen). Indeed Pritchett et al. (2002) evaluated increased heart rate as a potential indicator of postoperative pain in horses after abdominal surgery. However, Price et al. (2003) recorded heart rate and respiration rate postoperatively in horses that had undergone arthroscopic surgery and in horses from a pain free control group, but didn't find differences between the two groups, suggesting that these parameters are less useful. Bussières et al. (2008) concluded heart rate and respiratory rate not to be indicative for the level of pain in a orthopaedic pain scale used in horses with orthopedic pain..
One should keep in mind that severe pain can cause elevated heart rate, but a lot of other factors can influence it as well, for example hydration status, sepsis, arrhythmia, anxiety and fear (Sellon, 2006). Therefore, measuring heart rate by auscultation at a fixed point in time was not chosen as a parameter in this study.
Heart rate variability was evaluated in this study because it gives an indication of the function of the autonomic nervous system, especially the balance between sympathetic and parasympathetic activity. In the past decade, HRV has increasingly been applied in veterinary and behavioural research related to pathological conditions, stress, behavioural dysfunction, training regimes, temperament and emotional states. However compared to biomedical research studies measuring HRV in animals is still very basic. In horses, HRV was investigated in several studies concerning mental stress, grass sickness and atrial fibrillation. A study using HRV to 'measure' pain was performed by Rietmann et al. (2004). Rietmann et al. compared the stress response of horses suffering from laminitis before and after treatment with NSAIDs, measuring several parameters including HRV. The HRV parameters showed some positive correlations, but it was concluded it needed more testing on a larger number of individuals. Because of this conclusion HRV was also included in this study.

In the study of Rietmann et al. (2004) assessing horses suffering from laminitis, besides HRV plasma concentrations of cortisol, adrenalin and noradrenalin were investigated as well. However, no significant changes in hormone concentrations were seen after treatment with NSAIDs.
Pritchett et al. (2002) also evaluated plasma cortisol concentrations, but as an indicator of postoperative pain in horses after exploratory celiotomy for colic. In contrast, they concluded it to be a potential indicator of postoperative pain in horses after abdominal surgery.
Raekallio et al. (1997b) found plasma cortisol concentrations only correlating incidentally with the other variables measured in his study of pain in horses after orthopaedic surgery.
Difficulties in use of cortisol concentrations in pain assessment are the circadian rhythm and the fact that it is a stress-related hormone. The highest plasma levels are found in the morning, the lowest in the evening. (Zolovick et al. 1966, Evans et al 1977, Larsson et al. 1979). As cortisol is a stress-related hormone, product of the pituitary-adrenal system, elevated levels of cortisol are not necessarily elucidated by pain only. Stress without pain, for example after transport or transfer to a novel environment can also induce high levels of cortisol. Therefore interpretation of this parameter should be done very carefully. However, if one can minimise stress without pain and standardize the circumstances for each experimental animal, cortisol could possibly still be an interesting parameter. Therefore it was included in this study.
Catecholamines (adrenaline, noradrenaline and dopamine) were not included in this study as Raekallio et al. (1997b) and Rietmann et al (2004) both concluded in their studies they were not reliable as indicators of pain

in horses. Also anaesthetics, for example detomidine, can influence plasma catecholamines. (Raekallio et al. 1991)
Another hormone evaluated, but not used in this study, is plasma ß-endorphin (an endogenous opioid primarily released from the adenohypophysis). Mc Carthy et al (1993) studied the use of plasma beta-endorphin in horses as an indicator of stress and pain under several circumstances. He concluded plasma ß-endorphin concentration to be a useful indicator of stress; however its role as an indicator of pain was less obvious. Raekallio et al. (1997b) found a correlation between subjective pain score and ß-endorphin levels and stated that further investigation may be merited.

The other physiological parameters used in this study into postoperative pain in horses after castration, are based on the clinical aspects of castration.
Body temperature was chosen because elevation (fever) can be caused by inflammation of the scrotal area, which may cause pain. Subsequently swelling of the scrotal area was also included, as swelling can give an impression of the extent of inflammation.
Gait was scored because it was assumed that pain in the scrotal area, due to castration, would cause horses to walk differently. Finally body weight was chosen, as horses were believed to eat less or stop eating when they are in pain.

Behavioural parameters

Behavioural elements can be valuable parameters for assessment of pain.
However, research after behaviour as an indicator of pain was more extensively performed in companion animals than in horses. For example, Hardie et al. (1997) demonstrated that subtle changes in behaviour and interaction with caregivers were the most reliable indicators of pain after ovariohysterectomy in dogs.
A few studies including behavioural parameters of postoperative pain in horses were performed.
Raekallio et al. (1997a) investigated postoperative pain after comparable orthopaedic surgery in horses given phenylbutazone or a placebo. Predefined postures, movements and physiological variables were measured to assess pain and calculate a total postoperative pain severity index. The pain severity index was higher in the placebo group than in the phenylbutazone group.
Besides physiological parameters, Pritchett et al. (2002), also tried to identify potential behavioural indicators of postoperative pain in horses after exploratory celiotomy for colic. A numerical rating scale of behaviour and a time budget of behaviour led to the conclusion that reduced locomotion is a potential indicator of postoperative pain in horses after abdominal surgery. They also found the horses that underwent surgery less responsive to positive stimuli, (i.e. response to open door, response to approach and response to grain) compared to the control groups, which consisted of horses which were anesthetized without surgery and horses which didn't receive any treatment.
Price et al. (2003) also used behavioural indicators in assessing pain after arthroscopic surgery. Horses that underwent surgery as well as 'pain free' controls were observed directly and by video. The video observations were used to compose activity budgets. There were significant differences in behaviour observed between the two groups, but the influence of general anaesthesia had to be considered. Therefore the usefulness of these results for pain assessment was concluded to be equivocal.
A composite orthopaedic pain scale was recently developed and evaluated by Bussières et al. (2008) in an experimental model of acute orthopaedic pain in horses caused by chemically induced synovitis. They concluded that the behavioural parameters 'posture' and 'pawing on the floor' and possibly 'head movement', 'kicking abdomen' and 'appearance' should be included in an orthopaedic composite pain scale for horses. Responsiveness to palpation of the painful area was found to be both very specific and sensitive.

Because several studies, which included behavioural elements, show interesting results, analysis of behavioural elements was included in this study as well. Direct behavioural observation was performed using a slightly modified numerical rating scale from Pritchett et al. (2002). A time budget of behaviour was composed to be able to measure time spent on certain behaviours, for example locomotion, eating and pawing. Furthermore, since horses that experienced pain were assumed to eat less, food intake was measured directly as well.

3. Materials and methods

The study protocol was approved by the Institutional Ethical Committee for Animal Experiments of Utrecht University.

3.1 Animals

Six Dutch warm blood stallions in the age of 2 to 5 years old were included in this study. This category of age was chosen to standardize the group of experimental animals. To avoid influence of character or temperament as much as possible only warm blood horses were included.
All horses were healthy, not lame and had not received any medication in two weeks prior to the castration.

3.2 Study design

Each horse was studied for five days (one day preoperatively, on the day of surgery and three days post-operatively), recording several parameters to measure the influence of postoperatively administered NSAIDs on the physiological and behavioural state of the horse after castration. The study was double blind, placebo controlled.

Therefore two groups were defined:

* Meloxicam group treated with meloxicam, 0.6 mg/ml (Metacam® oral suspension 15 mg/ml), during the first three days postoperatively
* Control group treated with a placebo, during the first three days postoperatively

On arrival at the University Equine Clinic all horses were blindly assigned to one of the two treatment groups.
On day 0, the day of arrival at the clinic, several measurements and recordings were performed to obtain baseline values for each horse, the different measurements, with their time schedule are listed in Table 1. For details about the measurements see 'Equipment and procedures'
On day 1, between 8.30 and 11.00 am., castration took place. Anaesthesia and surgery were performed according to a standardised protocol (see 'Equipment and procedures'). In the afternoon all recordings and measurements were repeated. There was no difference between the two groups at this point, since the horses had not received oral medication yet.
In the morning of day 2 all horses received oral medication (placebo or meloxicam), (depending on the group they were assigned to) by the animal caretakers. During the day measurements and recordings were performed. (Table 1)
Day 3 was identical to day 2: all horses received oral medication and measurements and recordings were performed during the day.
On day 4 all horses received oral medication for the last time. Subsequently the last blood sample for cortisol was taken.

Table 1. Time schedule for measurements

Day 0	Arrival at the clinic (around 1.30 pm. in the afternoon)	
	1.30 pm.	- body weight
	2.30 pm.	- food intake (time spent eating hay)
	3.00 pm.	- video recordings of gait and scrotal area
	4.00 pm.	- video recordings for time budget of behaviour
		- heart rate variability
		- direct behavioural assessment
	5.30 pm.	- body temperature
Day 1	Castration	
	7.30 am.	- body temperature

7.45 am.	- cortisol (blood sample)
8.30 – 11.00 am.	- surgery (castration)
1.00 pm.	- body weight
3.00 pm.	- video recordings of gait and scrotal area
4.00 pm.	- video recordings for time budget of behaviour
	- heart rate variability
	- direct behavioural assessment
5.30 pm.	- body temperature

Day 2	1 day postoperatively

7.00 am.	- oral medication
7.30 am.	- body temperature
7.45 am.	- cortisol (blood sample)
12.00 pm.	- video recordings for time budget of behaviour
	- heart rate variability
	- direct behavioural assessment
1.00 pm.	- food intake (time spent eating hay)
1.30 pm.	- body weight
3.00 pm.	- video recordings of gait and scrotal area
4.00 pm.	- video recordings for time budget of behaviour
	- heart rate variability
	- direct behavioural assessment
5.30 pm.	- body temperature

Day 3	2 days postoperatively

7.00 am.	- oral medication
7.30 am.	- body temperature
7.45 am.	- cortisol (blood sample)
12.00 pm.	- video recordings for time budget of behaviour
	- heart rate variability
	- direct behavioural assessment
1.00 pm.	- food intake (time spent eating hay)
1.30 pm.	- body weight
3.00 pm.	- video recordings of gait and scrotal area
4.00 pm.	- video recordings for time budget of behaviour
	- heart rate variability
	- direct behavioural assessment
5.30 pm.	- body temperature

Day 4	3 days postoperatively

7.00 am.	- oral medication
7.45 am.	- cortisol (blood sample)

- End of observations -

3.3 Equipment and procedures

3.3.1. Anaesthesia and surgery

Castration was performed on day 1 between 8.30 and 11.00 am. Food was withheld for 6 hours before surgery. All horses were anaesthetized according to protocol (see also the appendix): Following intravenous catheterisation, pre-anaesthetic medication (romifidine 80 µg/kg, nalbuphine 0.1 mg/kg and meloxicam 0.6 mg/kg was administered. Intravenous administration of ketamine (2.2 mg/kg) and midazolam (0.05 mg/kg) induced anaesthesia. Maintenance was obtained by the so-called 'triple drip', containing 50 g guaiaphenesine, 1 g ketamine and 80 mg romifidine at 1 ml/kg/hr. All horses were also anaesthetized locally with 20 ml of lidocaine (2%) intratesticularly. Oxygen (12ml/hr) was provided through the endothracheal tube.
Castration was performed with the horses placed in lateral recumbency, using a half-closed technique.
All horses were castrated in the morning, to equalize the influence of anaesthesia on the measurements in the afternoon.

3.3.2. Oral medication

On day 2, 3 and 4, the first three days after surgery, the horses received oral medication by the animal caretakers around 7.00 am. in the morning.
The horses assigned to the meloxicam group received analgesia, consisting of meloxicam (Metacam® oral suspension 15 mg/ml) in a dose of 0.6 mg/kg. The horses assigned to the control group received a placebo.
As mentioned before, the study was organized in a double blind fashion, meaning nor the researchers, nor the surgeons, students or animal caretakers knew which horse received analgesia or a placebo. …

3.3.3. Measurements and recordings

The parameters tested, were (see Chapter 2):

* Body temperature
Body temperature was measured rectally at 7.30 am. on day 1, 2 and 3. At 5.30 pm. on day 0, 1, 2, and 3, using a Microlife® Vet-Temp MT1831 thermometer.

* Body weight
Body weight was measured in kilograms on day 0 on arrival and on day 1, 2 and 3 in the afternoon, around 1.30 pm., before roughage was provided.

* Cortisol concentration in blood plasma
A jugular blood sample was collected from each horse at day 1, 2, 3 and 4.
Around 7.45 am. an 52 mm 16-G polytetrafluorethylene catheter (Intraflon 2®, Vygon Nederland BV.) was placed in the jugular vein. After ten minutes a blood sample was taken without flushing the catheter and stored in an EDTA blood tube. In between the placement of the catheter and the blood sample being taken the horse had to be as calm as possible to avoid any rise in cortisol resulting from venepuncture. Ten minutes, between placement of the catheter and the blood sample being taken, were put in the protocol to avoid measuring an elevated level of cortisol level due to the procedure of the placement of the intravenous catheter itself. The blood sample was immediately centrifuged and the serum was transferred to an Eppendorf tube. The serum was stored in the freezer at a temperature of -20 degrees Celsius until the assay was performed. The serum cortisol concentrations were analysed using a solid-phase radioimmunoasssay using a Coat-A-Count® Cortisol kit (Diagnostic Products Corporation®, Los Angeles, USA).

* Gait
Gait was filmed on day 0, 1, 2 and 3 around 3.00 pm. using a Canon MVX 200i digital video camcorder. The horse was filmed two times walking by: one time from the left to the right side, one time from the right to the left side. Afterwards the fragments were coded and mixed. After the study was finished an independed surgeon working at the clinic scored the gait in each video fragment using a simple descriptive scale: 0 – normal gait, 1 – quite stiff gait, 2 – very stiff gait.
This surgeon was blinded for the animals and treatments.

* Heart Rate Variability (HRV)
At the same time as the recordings for the time-budget of behaviour, recordings of heart rate and heart rate variability were made. For this a Polar Horse Trainer (S810i, Polar Electro Europe BV) was used, consisting of a watch receiver and a transmitter with electrodes. The transmitter was attached to an elastic girth and connected to the girth on the back of the horse. The electrodes were wetted with transmission gel for better contact. Since all horses were used to wear a elastic girth, the polar equipment didn't interfere with the horse's behaviour. Filming while recording HRV had the advantage of being able to see what happened if major changes in HRV recordings occurred. The horses were habituated to wear the equipment for 5 minutes. Recordings lasted for 20 minutes.
Afterwards the data were transferred from the transmitter to the computer and analysed using the Polar Equine® SW4.03 software. Some error corrections were made, but only measurements with an error percentage of < 10% over 20 minutes were used to calculate the HRV parameters. Mean heart rate, RMSSD, pNN50, LF, HF and the LF/HF ratio were analysed using the Polar software.

* Scrotal area
The scrotal area of each horse was filmed on day 0, 1, 2 and 3 at 2.30 pm. using a Canon MVX 200i digital video camcorder. The scrotal area of the horse was filmed, without filming the rest of the horse's body. A flashlight was used to light the area if necessary. The video recordings lasted for about 30 seconds. All fragments were coded and mixed. After the study was finished a surgeon, who was blinded for the animals and treatments, scored the degree of swelling of the scrotal area. A simple descriptive scale was used: 0 – no swelling, 1 - little swelling, 2 – much swelling

* Food intake
Food intake was measured on day 0, 2 and 3 around 1.00 pm. After roughage was provided the horse was observed for five minutes. The fraction of time spent on eating was measured in seconds using a stopwatch. On day 1, the day of surgery, this measurement wasn't performed, as the horse wasn't allowed to eat for some time after anaesthesia.

* Time budget of behaviour
A time budget of behaviour was made for each horse. Therefore a video camera was installed in a corner, above the box. Each horse was recorded for 20 minutes: on day 0 and 1 at 4.00 pm, on day 2 and 3 at 12.00 pm. and 4.00 pm. During the recordings the door of the stable (2 boxes) was closed and the window in it covered, so disturbance from outside was prevented as much as possible. Disturbing sounds or change in environmental circumstances during the recordings were noted and taken into account in the analysing process later. The recordings were analysed using a video recorder and a computer with the software application The Observer® 5.0 (Noldus, Information Technology). An ethogram was composed (based on literature (McDonnell, 2003) and earlier ethograms made by colleague students) and used to score all behavioural actions (See Appendix 2). The ethogram consisted of several behavioural elements divided in so-called 'states' and 'events'. Behavioural elements that lasted for some time, for example eating, were defined as 'state'. Behavioural elements that only last a few seconds and from which only frequency could be calculated, were defined as 'event'.
For each 20-minute video recording the amount of time spent in each state and the total number of exhibited events was calculated. The data were transferred to Microsoft® Excel (2002) and put in tables and diagrams so horses in both groups could be compared.

* Direct behavioural assessment
Direct behavioural assessment was performed directly after the recordings for the time budget of behaviour: on day 0 and 1 around 4.30 pm., on day 2 and 3 around 12.30 pm. and 4.30 pm.
A numerical rating scale was used to score the horse's behaviour in realtime. The scale that was used (see Appendix 2) was modified from the numerical rating scale of behaviour used in Pritchett et al., 2003 and contained elements like posture, position in the stable and response to potential triggers, like people entering the stable and food offering. Each element was scored from 0 to 3, with a score of zero points corresponding to normal behaviour, expected to be displayed in the absence of pain. A score of three points corresponded to the maximum modification of behaviour expected in the presence of pain.
In each assessment a maximum score of 27 points could be achieved. (Nine categories or elements scored with a numerical rating scale of 0 to 3). For each category the scores were compared between the meloxicam group and the control group, as well as the total scores of each assessment.
The direct behavioural assessment was performed according to the following protocol:

The observer is standing approximately one metre in front of the stable, attracts the horse's attention, observes it for a few minutes and scores the elements a – g (i.e. head position, ear position, look, nostrils, position in the stable, spontaneous locomotion and exploratory behaviour, see Appendix 2). Then, he/she calls the horse again, walks to the door of the stable with a hand of concentrates, opens it and feeds the horse. Meanwhile he/she observes the horse's reaction and afterwards scores the elements h and i (i.e. response to opening the door and response to approach with a hand of concentrates). It was important to take into account that the horse should not be sleeping or lying when direct behavioural assessment was performed.

3.4 Statistical analysis

Although the number of animals in both groups was very small, statistical analysis of the data was performed. Differences between the means of the meloxicam group and the control group (all measurements) were analysed using the Mann-Whitney U test. The Mann-Whitney U test was also performed to test the differences within groups between different points in time.
Differences between data were considered significant when $P < 0.05$.
The statistical analysis was performed using SPSS® 16.0.

4. Results

Based on mean number of patients presented for castration in previous years, the goal was to assess 25 horses. However, due to unknown reasons, most likely "bad luck", there were only 6 horses presented during this study for which all inclusive criteria were met.

All results are summarized in Table 2, 3 and 4.

Table 2. Data and Mann-Whitney U test of physiological parameters in the control group and meloxicam group.

Parameter	Baseline, Day 0, A	Day 1, M	Day 1, A	Day 2, M	Day 2, A	Day 3, M	Day 3, A	Day 4, M
Body temperature (C)								
C group	37,8 ± 0,1	37,4 ± 0,4*	37,0 ± 0,5	37,4 ± 0,5	38,03 ± 0,3	37,7 ± 0,3	37,9 ± 0,2	
M group	38,2 ± 0,4	37,5 ± 0	38,1 ± 0,6	Could not be obtained	38,05 ± 0,77	Could not be obtained	38,1 ± 0,4	
P-value	NS	NS	P = 0.083		NS		NS	
Body weight (kg)								
C group	595 ± 97		579 ± 92		577 ± 97		582 ± 101	
M group	471 ± 7		456 ± 6*		461 ± 12		469 ± 3	
P-value	*P = 0.05*		*P = 0.046*		P = 0.077		*P = 0.05*	
Body weight (difference in weight (kg) compared to baseline)								
C group			-17 ± 10		-18 ± 7		-14 ± 6	
M group			-15 ± 3		-10 ± 6		-2 ± 6	
P-value			NS		NS		NS	
Cortisol (nmol/L)								
C group		82 ± 4		93 ± 3		81 ± 18		63 ± 12
M group		127 ± 99		165 ± 67		135 ± 66		101 ± 45
P-value		NS		P = 0.083		NS		NS
Cortisol (difference in level compared to baseline)								
C group				14 ± 7.1		-1,5 ± 13		-20 ± 16
M group				38 ± 118		8 ± 41		-26 ± 64
P-value				NS		NS		NS
Gait (score 0-2)								
C group	0		1		0		0	
M group	0		0		0		0	
P-value	NS		NS		NS		NS	
Scrotal area (0-2)								
C group	0		0		1		1	
M group	0		1		1		1*	
P-value	NS		NS		NS		NS	

* Significant difference between this value and the baseline value (day 0)
A= afternoon, M= morning, C group = control group, M group = meloxicam group, NS = non significant
Values are expressed as mean ± S.D.
Values for gait and scrotal area are expressed as median.

Table 3. Data and Mann-Whitney U test of heart rate variability parameters and direct behavioural assessment of the control group and meloxicam group.

Parameter		Baseline, Day 0, A	Day 1, M	Day 1, A	Day 2, M	Day 2, A	Day 3, M	Day 3, A	Day 4, M
Heart rate variability:									
Mean heart rate									
	C group	32 ± 3.5		33 ± 4.5	40 ± 6	36 ± 4.0	35 ± 4.6	33 ± 3.1	
	M group	37 ± 7		36 ± 4.7	37 ± 2.5	40 ± 3.5	34 ± 1.5	35 ± 1.5	
	P - value	NS		NS	NS	NS	NS	NS	
RMSSD									
	C group	111 ± 21		124 ± 48	114 ± 86	121 ± 125	99.3 ± 84	102 ± 93	
	M group	161 ± 77		360 ± 321	120 ± 34	162 ± 77	126 ± 70	197 ± 134	
	P - value	NS		NS	NS	NS	NS	NS	
pNN50									
	C group	30 ± 7.8		28,5 ± 0.3	25,5 ± 14	22 ± 22	21 ± 17	23,8 ± 21	
	M group	25,6 ± 9.4		34,7 ± 9.3	22 ± 2.8	30,1 ± 7.2	26,1 ± 12	30,1 ± 12	
	P - value	NS		NS	NS	NS	NS	NS	
LF									
	C group	5470 ± 3104		7435 ± 4344	5197 ± 3175	5872 ± 7229	4778 ± 4533	5122 ± 5155	
	M group	8642 ± 3750		18988 ± 19403	3870 ± 1564	6329 ± 3289	3445 ± 3904	5233 ± 1487	
	P - value	NS		NS	NS	NS	NS	NS	
HF									
	C group	1946 ± 21		2916 ± 2129	4069 ± 4876	4376 ± 6001	2646 ± 3462	2949 ± 3989	
	M group	6258 ± 4842		47579 ± 67842	3537 ± 2045	8352 ± 8801	4524 ± 3769	12600 ± 14594	
	P - value	NS		P = 0.083	NS	NS	NS	NS	
LF/HF ratio									
	C group	280 ± 157		274 ± 51	287 ± 266	352 ± 317	475 ± 449	652 ± 707	
	M group	217 ± 155		99± 101	119 ± 45	171 ± 207	134 ± 129	226 ± 319	
	P - value	NS		NS	NS	NS	NS	NS	
Direct behavioural assessment									
Assessment score (max. 27)									
	C group	1,3 ± 2,3		1,7 ± 2,1	0,67 ± 0,6	1 ± 1,7	2,3 ± 2,1	2 ± 2	
	M group	2,3 ± 4,0		1,3 ± 1,5	3,3 ± 4,2	4,7 ± 3,5	1,7 ± 1,5	2,3 ± 3,2	
	P-value	NS		NS	NS	NS	NS	NS	

* Significant difference between this value and the baseline value (day 0)
A= afternoon, M= morning, C group = control group, M group = meloxicam group, NS = non significant
Values are expressed as mean ± S.D..

Table 4. Data and Mann-Whitney U test of behavioural elements from the time budget of behaviour of the control group and meloxicam group.

Time budget of behaviour		Baseline, Day 0, A	Day 1, A	Day 2, M	Day 2, A	Day 3, M	Day 3, A
Duration							
Eating while standing still							
	C group	214 ±101,8	697±316,0	169±216,9	659±373,5	284±285,5	265±404,5
	M group	156±188,2	423±514,7	266±211,3	389±61,3	53±55,2	240±203,4
	P-value	NS	NS	NS	NS	NS	NS
Eating while walking							
	C group	10±5,9	40±13,5	21±22,5	6±6,8	31±32,5	18±19,9
	M group	25±29,2	4±4,2	17±7	13±4,1	3±5,6	25±23,1
	P-value	NS	*P = 0,05*	NS	NS	NS	NS
Stand still, alert							
	C group	722±154,3	321±335,3	680±391,1	234±151,1	594±381,0	598±508,3
	M group	550±279,0	633±440,6	619±366,2	312±246,4	502±353,2	371±508,3
	P-value	NS	NS	NS	NS	NS	NS
Stand still, at rest							
	C group	8±14,2	25,7±44,6	112±194,3	216±301,9	176±240,8	271±331,1
	M group	437±400,9	56±96,1	144±148,5	432±298,9	495±470,2	509±467,6
	P-value	NS	NS	NS	NS	NS	NS
A few steps							
	C group	101±83,4	71±40,2	*171*±66.6	29±22	73±49,0	29±27,9
	M group	26,4±10,5	54±60	*68*±13,4	24±14,9	59±71,1	45±25,8
	P-value	NS	NS	*P = 0,05*	NS	NS	NS
Frequency							
Lift a leg							
	C group	0±0	0,67±1,15	0±0	1,33±2,3	0±0	1,67±1,53
	M group	0,33±0,58	0,33±0,58	0,33±0,58	2,33±3,21	1,33±2,3	1±1,73
	P-value	NS	NS	NS	NS	NS	NS
Pawing							
	C group	0±0	0±0	0,33±0,58	0±0	0±0	0±0
	M group	0±0	1,33±1,53	0±0	2±2	1,66±2,89	2,33±2,08
	P-value	NS	NS	NS	NS	NS	NS
Head shaking							
	C group	3±1,73	0,67±1,15	6±1,73	2±1,73	3,33±1,15	1,33±1,53
	M group	2±1,73	8,7±5,5	2,33±2.5	1±0	6±5,29	1,67±0,58
	P-value	NS	*P = 0,046*	NS	NS	NS	NS
Self grooming							
	C group	3,33±5,58	4,7±6,4	1,67±1,15	1,33±1,53	2±2,65	1,67±2,08
	M group	7,67±11,6	0,67±1,15	0,33±0,58	3±4,36	8,67±5,54	2,67±4,61
	P-value	NS	NS	,P = 0,099	NS	P = 0,077	NS
Penis prolaps							
	C group	0,67±1,15	0±0	0±0	0±0	0±0	0±0
	M group	0±0	0,33±0,58	0±0	0±0	0±0	0,33±0,58
	P-value	NS	NS	NS	NS	NS	NS

* Significant difference between this value and the baseline value (day 0)
A= afternoon, M= morning, C group = control group, M group = meloxicam group, NS = non significant
Values are expressed as mean ± S.D.

* Body temperature

Body temperature was planned to be measured twice daily in each horse. However, due to lack of staff and cooperation of some horses, not all values could be obtained. The body temperature of horse no. 4 (meloxicam group) could not be measured at any point in time. Values for body temperature in a horse normally vary between 37.4 and 38.0 ^{0}C. Except for horse no. 6, all horses measured had a baseline value on day 0 within this reference. None of the horses developed severe fever during the period of observation.

No significant differences were found between the meloxicam group and the control group at any point in time. The difference in body temperature between the two groups in the afternoon of day 1 was not found to be significant since P = 0.083, Z = -1.732. Within the control group the body temperature of the afternoon of day 1 differed not significantly from the values of day 0 (P = 0.083, Z = -1.732), however, the differences

between day 1 and 2 and day 1 and 3 in the control group were significant (both P = 0.05, Z = -1.964). In the meloxicam group no significant differences were seen between any different points in time.

* Body weight

The body weight of the control group was significantly higher at all measurements, including the baseline and except for day 2, compared to the meloxicam group.

All horses showed a decrease in body weight between day 0, pre-operatively, and day 1, postoperatively. The decrease varied between 8 and 27 kg with a mean of 15.5 kg.

However, only in the meloxicam group the difference between the measurements of day 0 and 1 was significant (P = 0.046, Z = -1.993).

In this group the difference in body weight between day 1 and 3 was also significant.

The relative increase or decrease of body weight compared to the baseline measurement was analysed between both groups as well. The initial significant difference in body weight between the groups was thereby excluded.

* Cortisol concentration

Horse no. 1 was excluded from measurement of cortisol because the collection of the blood sample was very stressful for the horse, since he didn't cooperate. Therefore no more samples were taken after day 1 as cortisol levels were expected to be high and not representative for this research project.

Except for horse no. 6 all horses had a higher cortisol level on day 2, postoperatively, than on day 1, preoperatively. However, neither in the meloxicam group, nor in the control group this difference was significant.

In all horses (except for horse no. 6) the level of cortisol was lower on day 3 compared to day 2, but these differences were not significant in both groups either.

No significant difference was seen between the two groups at any point in time. There was a trend on day 2 (P = 0.083, Z = -1.732); the meloxicam group tended to have higher cortisol values compared to the control group.

For cortisol the relative increase or decrease of the level compared to the baseline measurement was analysed between both groups as well, excluding initial significant differences in plasma cortisol concentration between the groups.

* Gait

On day 0 all horses were scored a normal gait. On day 1, after surgery, three out of six horses were scored a quite stiff gait (two in the control group, one in the meloxicam group).

On day 2 and 3 no difference was seen between the meloxicam group and the control group: both groups contained one horse that was scored a quite stiff gait, and two horses that were scored a normal gait.

Three horses kept walking normally during the whole study period: one of the control group, two of the meloxicam group. None of the horses developed a very stiff gait (score 2). (Fig. 1)

Differences between the meloxicam group and the control group were not significant at any point in time. Differences within the groups between different points in time were not significant either.

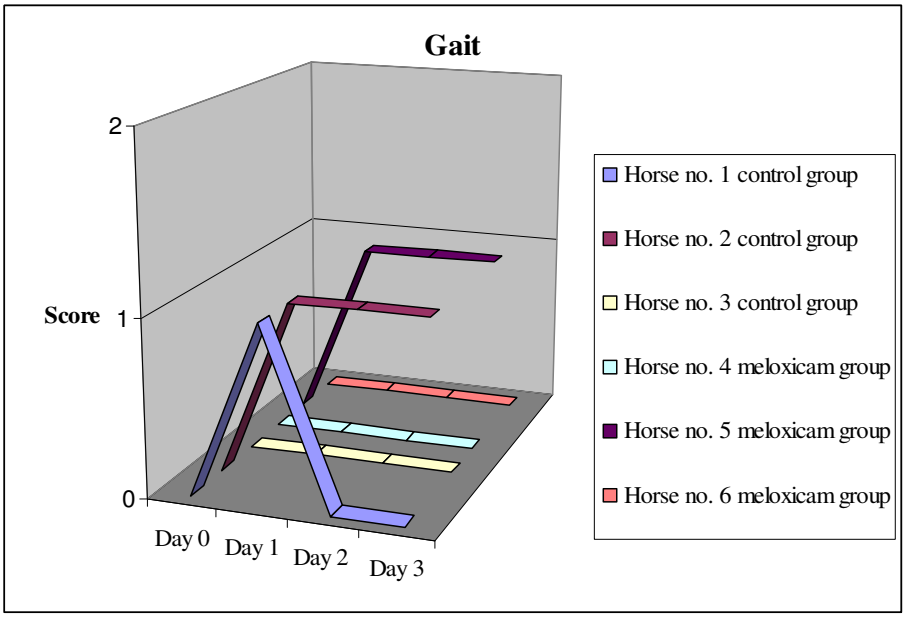

Fig. 1. Development of gait score of all individual horses in the control group and meloxicam group. Gait was scored preoperatively (day 0) and postoperatively (day 1, 2 and 3) after surgical castration. A numerical rating scale of 0 to 2 points was used with 0 points corresponding to normal gait, 1 point to 'quite stiff'gait and 2 points to 'very stiff gait'.

* Heart rate variability

None of the parameters of heart rate variability, which were analysed (i.e. mean heart rate, RMSSD, LF, HF and the LF/HF ratio), showed significant differences between the control group and the meloxicam group.
In the afternoon of day 1 the p-value for difference between the two groups in HF was 0.083, Z = -1.732.

* Scrotal area

On day 0, preoperatively, the surgeon scored one horse to have little swelling.
All horses developed little swelling some time after surgery. However, serious swelling (score 2) was never recorded.
Postoperatively on day 1, two horses of the meloxicam group were scored as having some swelling, while none of the horses of the control group had swelling of the scrotal area (Fig. 2).
On day 2, two horses of the control group were scored as having little swelling in the scrotal area, as well as two horses from the meloxicam group. So no difference was seen between the two groups. On day 3 five horses were scored as having little swelling of the scrotal area. This group consisted of all three meloxicam-treated horses and two horses from the placebo-treated group. The horses in the meloxicam group developed little swelling one day sooner compared to the horses in the control group.
None of the differences between the groups was significant.
Within the control group none of the scores differed significantly from the scores on other days. In the meloxicam group the score of swelling of the scrotal area differed significantly between day 0 and 3 (P = 0.025, Z=-2.236), since all horses in this group scored zero points on day 0 and 1 point on day 3.

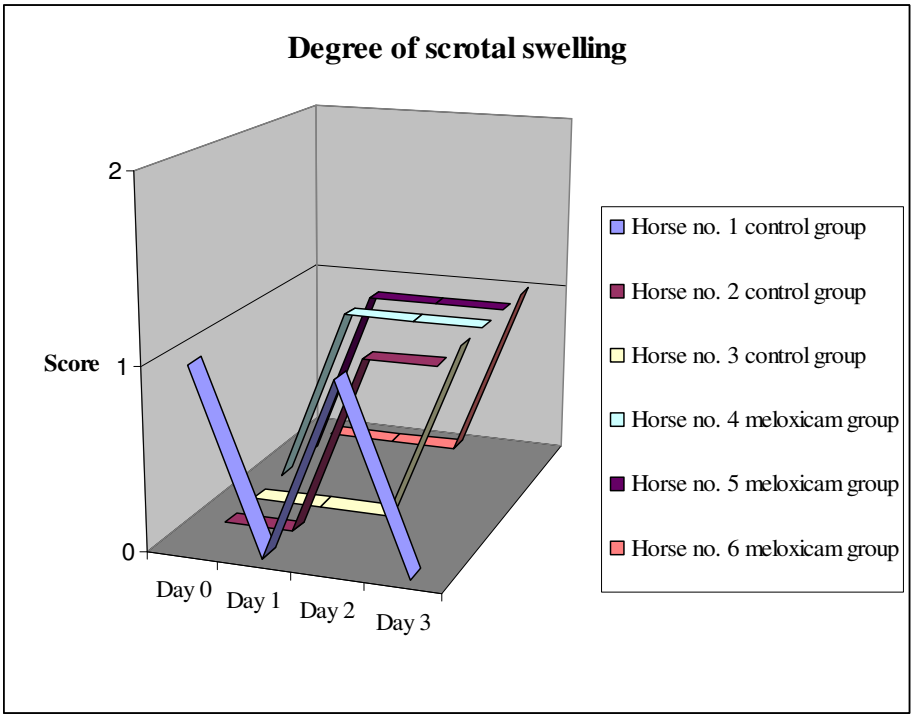

Fig. 1. Development of score of scrotal swelling in all individual horses in the control group and meloxicam group.
Scrotal swelling was scored preoperatively (day 0) and postoperatively (day 1, 2 and 3) after surgical castration. A numerical rating scale of 0 to 2 points was used with 0 points corresponding to 'no swelling', 1 point to 'little swelling' and 2 points to 'much swelling'.

* Food intake

All six horses spent all 5 minutes, in which they were observed, eating. Therefore no difference was seen between the horses in both groups.

* Time budget of behaviour

For each 20-minute video recording the time spent in each state and the frequency of each event was analysed. The groups were compared as well as the measurements within the groups.

Duration of certain behaviours

No significant differences between the two groups were seen in 'nibbling on the polar', 'urinating', 'chafing' or 'drinking'.
For all horses most of the time was either spent on standing still (alert or at rest) or on eating (while standing still or while walking).
After castration both groups did not show a significant decrease in time spent on eating. The part of time spent on 'eating while walking' compared to the time spent on eating in total didn't show a decrease in both groups either. No significant difference was seen between the two groups in time spent on 'eating while standing still' and total time spent on eating (i.e. 'eating while walking' plus 'eating while standing still')
However, significant difference between the meloxicam group and the control group was seen in the time spent on 'eating while walking' on day 1 after castration ($P = 0.05$, $Z = -1.964$) (Fig. 3). The meloxicam group spent less time in this state compared to the control group.

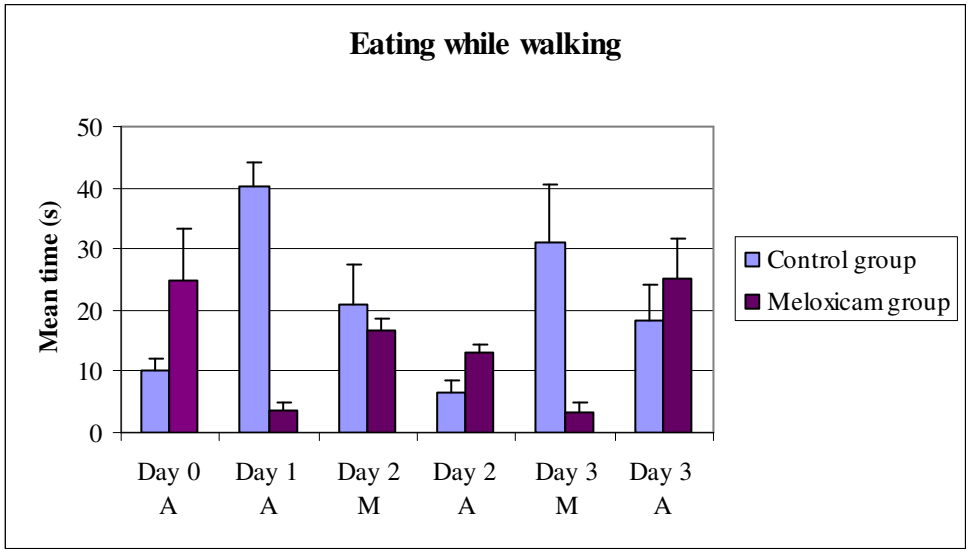

Fig. 3. Mean time (± S.E.M.) spent on 'eating while walking' for the control group and the meloxicam group, measured by real time video recordings, before (day 0) and after (day 1, 2 and 3) surgical castration.
A = Afternoon, M = Morning.
The difference between the groups in the afternoon of day 1 was statistically significant (p = 0.05, Z = -1.964).

When one compared the amount of time standing still (including eating while standing still) to the amount of time walking ('a few steps' plus 'eating while walking') all horses spent at least 900 seconds (out of 1200) on standing still; thus much more than on walking. This was seen before castration was performed as well as after.
Significant difference between the control group and the meloxicam group was also seen in the morning of day 2 (P = 0.05, Z = -1.964). The control group spent more time on 'a few steps' compared to the meloxicam group (Fig. 4)
No significant differences were seen between the two groups in time spent on 'eating while standing still', 'standing still alert' and 'standing still at rest'.

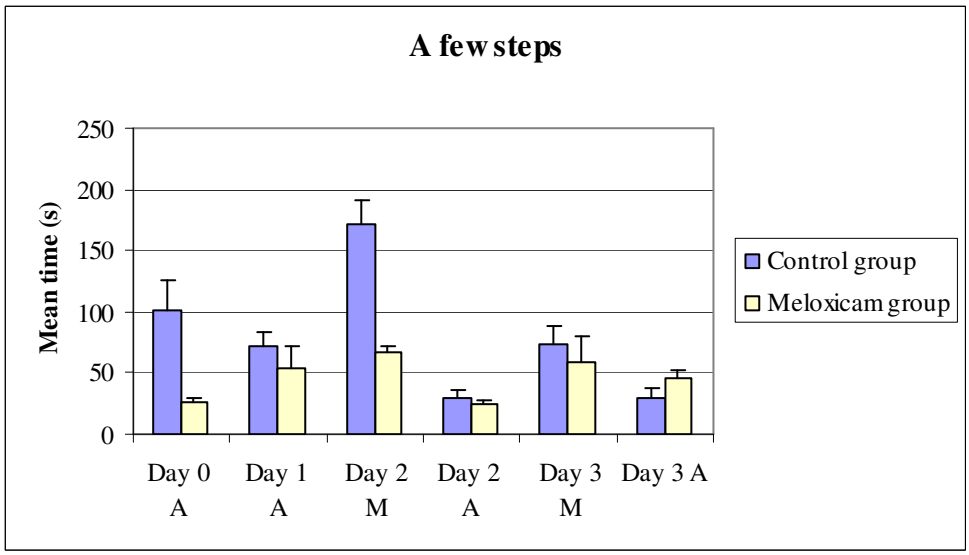

Fig. 4 Mean time (± S.E.M.) spent on 'a few steps' for the control group and the meloxicam group, measured by realtime video recordings, before (day 0) and after (day 1, 2 and 3) surgical castration.,
A = Afternoon, M = Morning.
The difference between the control group and the meloxicam group in the morning of day 2 was statistically significant (p = 0.05, Z = -1.964).

Frequency of certain behaviours
The number of times 'headshaking' was significantly different between the two groups in the afternoon of day 1 (P = 0.046, Z = -1.993). The horses in the meloxicam group exhibited this event more often.

The differences between the meloxicam group and the control group in 'self grooming' were not significant, but in the morning of day 3, the meloxicam group spent more time on 'self grooming' compared to the control group ($P = 0.077$, $Z = -1.771$).
'Chewing' was not analysed because the scoring of this behavioural event was not consistent between the observations.

* Direct behavioural assessment
In the afternoon of day 0, the baseline measurement, all horses were expected to score zero points on each category of the direct behavioural assessment, since no castration was performed yet and the horses were not expected to experience pain. However, only four out of six horses had a total score of zero points. Horse no. 3 from the control group and horse no. 5 from the meloxicam group scored respectively four and seven points in total in this first assessment.
One horse of the control group (horse no. 1) scored zero points in all categories during all assessments, except in the afternoon of day 3 when two points in 'head position' were scored.
In the category 'head position' all horses scored zero points in the afternoon on day 0. In the other assessments several horses scored two points (head at the level of withers), but no difference was seen between the meloxicam group and the control group. None of the horses scored three points (head below withers) at any assessment.
For 'location in the stable' one horse (horse no. 3) from the control group and one horse from the meloxicam group (horse no. 4) scored more than zero points after castration. But no difference was seen between the meloxicam group and the control group as all other horses scored zero points during all assessments.
In the category 'spontaneous locomotion' horse no. 5 (meloxicam group) scored three points for spontaneous locomotion in the assessment of day 0, afternoon, meaning he didn't move at all during direct observation in the baseline measurement. The control group as well as the meloxicam group scored several times more than zero points before and after castration. So no difference between the two groups was seen. No difference was seen between the two groups in the category 'exploratory behaviour' either.
For 'response to open door' all horses belonging to the meloxicam group scored more than zero points in at least one assessment, while only one horse from the control group did. The two other horses from the control group scored zero points for this category at all assessments.
All horses in the control group responded well to approach with food: all three horses scored zero points at all assessments. The meloxicam group contained two horses that scored more than zero points. Horse no. 4 scored one point in the morning of day 2. Horse no. 6 scored one point in the afternoon of day 2.

At none of the different points in time a significant difference was seen between the assessment score of the control group and the meloxicam group.
In both groups, there was no significant difference between the assessment score on day 0 and the assessment scores on the other days either.

5. Discussion

In this study an attempt was made to compose a pain scale for assessing pain in horses after castration.
Several parameters were tested for their use.
Manteca et al. (1993) concluded the combination of physiological and behavioral parameters to be more useful in assessing individual animal differences in response to environmental change and challenge compared to physiological measures alone. Therefore, this study included both physiological and behavioural parameters.
The statistical analysis of the data was not optimal because the sample size of both the control group and meloxicam group was very small (n = 3).
Subsequently the tested parameters will be discussed and evaluated for their use.

* Body temperature
None of the studies mentioned in chapter 2 included body temperature as a parameter. It was included in this study since body temperature is a reflection of the degree of inflammation in the body. However, castration normally only causes inflammation in the scrotal area and such a local inflammation should not cause a rise in body temperature. In this study none of the horses developed fever, meaning that none of the horses had an inflammation throughout the whole body.
No significant differences in body temperature were found between the meloxicam group and the control group at any point in time.
Since there was no difference in treatment between the two groups at that point yet, no explanation was found for why the control group did have significant difference in body temperature between day 0 (before castration) and day 1 (after castration) while the meloxicam group did not.
In conclusion, the horses in the meloxicam group seemed to have had a more stable body temperature than the horses in the control group. However, this was not due to the meloxicam, since differences in body temperature between the two groups were already high (although not significant) in the afternoon of day 1, after castration, when there was no actual difference in treatment between the two groups yet, as no meloxicam had been administered. The significant differences within the control group can be explained as the horses in the control group managed to normalise their body temperature back to values within normal range, without developing fever. The horses in the meloxicam group did not show this decrease in body temperature on day 1 and did not develop fever as well, resulting in a stable body temperature measured, without significant differences between different points in time.
Since the sample size of both groups was very small, the physiological variation of body temperature in the horse had much influence on the mean and standard deviation of the group. This might have contributed to the fact that no significant differences of importance were found. Therefore it is worthwhile to study this parameter in another study with a higher number of animals, before it is excluded as an indicator of pain.
Also it is important to measure body temperature since fever is a sign of severe inflammation, which can cause pain. The presence of severe inflammation however might not necessarily indicate the presence of pain.

* Body weight
The significant differences in body weight between the groups, found on al measurements except for day 2, are probably coincidence. Since the difference was already significant at the baseline measurement, no conclusions can be drawn from these results. However, the relative differences in body weight between both groups were not significant either.
Loss of body weight is seen in animals after periods of stress and pain. Reluctance to eat or increased expenditure can be the cause of this.
However, in this study, only the meloxicam group showed a significant difference in body weight between the baseline value and the value on day 1. This decrease in body weight was probably due to the deprivation of food prior to surgery. All horses started eating again after surgery as soon as food was offered.
Therefore this parameter doesn't seem to be suited for pain assessment in horses after castration. It is probably of more value in cases of severe chronic pain.

* Cortisol concentration
In four out of five horses the level of cortisol was higher on postoperative day 2, compared to preoperative day 1. The cortisol level decreased on day 3 compared to day 2. This suggests that cortisol in plasma is elevated due to surgery and decreases soon after. However the differences in cortisol levels between consecutive days were not significant for both groups and no significant difference was seen between the meloxicam group and the control group at any point in time. Furthermore, the trend on day 2 in which the meloxicam group tended to have a higher cortisol value could not be explained.
The small number of animals in which cortisol could be measured might have contributed to these results.

As cortisol is a stress related hormone, product of the hypothalamic-pituitary-adrenal axis, its concentration rises in situation of stress. Pain can be a cause of stress and subsequently can cause a rise in cortisol level. However, a rise of the cortisol concentration doesn't have to be caused by pain. Stress without pain, for example transport or a novel environment (for example to/in a veterinary clinic), stimulates the pituitary-adrenal system and results in an increased production of cortisol. Other factors that influence the cortisol level, like the circadian rhythm (Zolovick et al. 1966, Evans et al 1977, Larsson et al. 1979), have to be taken in account as well. In this study this factor was diminished by sampling the horses at a fixed point in time.
When the outcomes of this study and several other studies, testing plasma cortisols levels as an indicator of pain (Pritchett(2002), Raekallio (1997b), Rietmann (2004)) are evaluated, cortisol is found to be an inconsistent parameter. Even if the rise in cortisol had been significant, one should have been very careful to interpret these results. The inconsistency of the results of cortisol as a parameter to assess pain, makes it worthwhile to investigate it again in a study containing more animals to definitely exclude it (or include it).
However, because of the costs, the duration of the results and the invasiveness it is concluded not to be a usefull parameter in a pain scale developed for pain assessment under clinical circumstances.

* Gait
Gait was measured as a parameter for the composite pain scale because it was thought that walking causes pain due to movement of the scrotal area.
Statistically, differences between the meloxicam group and the control group were not significant. Differences within the groups between different points in time were also not significant.
However difference (although not significant) was seen between the two groups as three horses kept walking normal during the period of observation: one horse of the control group and two horses of the meloxicam group. This can be coincidence, but could also be ascribed to the anti-inflammatory effect of meloxicam. On the other hand, one horse of the control group didn't develop an abnormal gait. This could mean that not all horses develop an abnormal gait due to pain caused by movement of the scrotal area.
Another explanation would be that the method used in this study to score gait was not sensitive enough to differentiate between the two groups. The video recordings were quite short. To prevent this next time, the observation could be improved next time by performing the assessment of gait in real life by an equine veterinary specialist. Also the period of observation of the horse should be lengthened (for example up to one week after castration), but in a clinical environment this is not always able to be realised..
More important however is to make the circumstances for all horses equal. In this study some horses did walk in the treadmill postoperatively, while other horses, that were not used to this, did not.
As walking is thought to have a positive effect on the swelling of the scrotal area this difference has much influence on the results of the parameters gait and scrotal area, especially when the number of animals in both groups is as low as in this study.
Since circumstances were not optimal in this study, gait could still be a good parameter to indicate pain and should be included in a next study.

* Heart Rate Variability (HRV)
No significant differences in HRV parameters were obtained between the control group and the meloxicam group.
A trend in HF was found in the afternoon of day 1, as the horses in the meloxicam group had higher values compared to the control group. However, at that moment no difference in treatment between the two groups existed yet.
An explanation for the lack of significant results may be that heart rate variability is not a good parameter to assess pain. Another explanation might be that the horses in the control group did not experience more pain compared to the horses in the meloxicam group.
No conclusions can be drawn from these results. In accordance with Rietmann (2004) it was concluded that further studies, including more horses should be performed to assess the usefulness of these parameters.

* Scrotal area
The swelling of the scrotal area was chosen as a parameter because that's the area where the castration took place and where pain, due to damage of the tissue and inflammation, originated.
Meloxicam has an analgesic effect and also an anti-inflammatory effect. Therefore it has direct influence on the scrotal area and when observing this area one would expect to see difference in swelling between the two groups. However, significant difference in degree of swelling between the groups was not seen.
All horses developed 'little swelling' at some time and none of the horses developed 'much swelling'. The significant difference within the meloxicam group between the baseline score and the score on day 3 (which was not the case in the control group) was not expected since meloxicam works as an anti-inflammatory drug and reduces the classical signs of inflammation: 'rubor', 'calor', 'dolor' and 'tumor'.

An explanation for these results can be that a score ranging from 0 to 2 is not very sensitive. Also the video recordings were not that clear sometimes. This could also explain why one horse was scored 'little swelling' already preoperatively on day 0. Scoring in real life by an equine veterinary specialist and up to one week after surgery would improve sensitivity. In a next study one could also consider to measure the other symptoms of inflammation, for example the temperature of the skin in the scrotal area (calor), the redness (rubor) and production of wound fluid. However, this is less practical and not suitable for a composite pain scale which is used in clinical circumstances. Reaction on palpation of the scrotal area would be very useful. However one should be very careful and only horses that are used to be touched in the scrotal area are suited.
As mentioned above, some horses walked in the treadmill, while others did not. As this is thought to diminish swelling of the scrotal area, this probably has had much influence on the results since the number of animals was very low. Therefore drawing conclusions from these results seems not right and it would be interesting to test this parameter again in a next study containing more animals.

* Food intake
No difference was seen between the two groups in food intake as all horses were eating all 5 minutes in which they were being observed.
Not eating can be caused by not feeling well or by reluctance to walk, when walking after castration causes pain due to movement of the scrotal area. Following the results of this study one could say that the (pain of) castration didn't cause reluctance to walk and the appetite wasn't influenced by it. The measurement could be made more sensitive by feeding all horses exactly the same amount of food and measuring how much is left after some time.
When food was offered during this study, it was always nearby and easy to reach for the horses. Therefore, not much effort had to be put in by them to gain food. In a next study one could consider putting the food further away to see if they are prepared to put more effort in it to reach it.
However, in clinical circumstances, in which this project was performed this is less practical.

* Time budget of behaviour
<u>Duration of certain behaviours</u>
In the time budget of behaviour one would have expected to see a difference between the meloxicam group and the control group. If the control group experiences pain after castration and the meloxicam does not, then one would expect for example that the time spent on walking ('a few steps') in the control group is less after castration, compared to before castration.
Furthermore, the control group was expected to spend more time on standing still and less on walking, compared to the meloxicam group. A reduced appetite, resulting in less time spent on eating was also expected.
However, when the groups were compared, no significant differences were seen between the two groups, except for 'a few steps' and 'eating while walking'.
The time spent on 'eating while walking' was significantly less on day 1 in the meloxicam group compared to the control group. Since there was no difference in treatment between the two groups at that moment yet, this result was not valuable.
Difference was seen between the two groups in the morning of day 2. The horses in the control group spent more time in this state compared to the meloxicam group. Since it was expected to be the other way around no clear explanation can be given for this result.

<u>Frequencies of certain behaviours</u>
No significant differences were seen between the control group and the meloxicam group in any of the events except for the number of head shaking in the afternoon of day 1.
This last finding can not be explained clearly since no difference between the two groups in treatment existed yet.

The obtained results of the time budget of behaviour suggest that this parameter was not sensitive enough to discover differences between the two groups. Another possibility is that the horses in the control group did not experience more pain than the horses in the meloxicam group, or overall behaviour of the animals were not influenced by it.
The time budget of behaviour for each horse was measured six times for twenty minutes. This is only a brief period. Video recordings that last a few hours would for example be more sensitive because factors that influence the behaviour (for example the presence of a mare in the neighbourhood or the presence of food in the stable during the recordings) then take a smaller part of the time budget. Therefore recordings that take longer would make this parameter more sensitive.

No obvious conclusions were drawn from these results, but a time budget of behaviour still seems a good parameter to 'measure' pain. However, in a composite pain scale, used in clinical circumstances, this parameter is not an option as analysing the video recordings takes a lot of time.

* Direct behavioural assessment

No significant differences were seen between the control group and the meloxicam group at any point in time. No significant difference was seen between the assessment score on day 0 and the assessment scores on the other days, for both groups either, nor in the scores of consecutive days.

This suggest that pain from castration doesn't influence the horse's behaviour, or that the horses in both groups did not experience pain, or that the direct behavioural assessment is not sensitive enough to measure differences between the two groups.

It was curious that two out of six horses scored quite a few points already in the baseline assessment on day 0. This might be caused by the problem that horses, which are standing at rest or are asleep, show the same behavioural elements that are thought to also be representative for pain. Not walking, head on withers and ears to the back are all examples of this. Dullness due to pain or not being well can therefore easily be confused with dullness after sleeping, resulting in a high assessment score.

The stimuli 'opening the door of the stable and 'approach with food'seemed very powerful. All horses were curious when someone entered the stable and soon all horses knew the observer had food with him/her, extracting them from everything else (for example pain).

Pritchett et al. (2002) found some interesting results with direct behavioural assessment in his study. In this study however, it did not prove it's usefulness. However it is worthwhile to test this parameter again in another study. Then, it is recommended to perform the direct behavioural assessment after some activity of the horse to prevent high scores from the horse being sleepy.

Performance of a research project in a clinical setting has some disadvantages. Researchers are dependent on circumstances in the clinic. Although arrangements are made to optimalize the circumstances, some things are not foreseeable.

Therefore circumstances were not exactly equal in all horses in this study. For example the presence of a horse in the stable next door can influence the behaviour of the horse remarkably.

Not all horses walked in the treadmill after castration and not all horses were in the same stable, meaning some horses had a smaller surface than others.

Furthermore, one of the stables used was near to the central hall, meaning there was usually more noise than in the other stable that was used.

Not all horses received food at exactly the same time, so during the time budget of behaviour some horses still had food left, while others didn't. These differences in circumstances can cause big differences, even significant ones, between the groups, which are not due to pain. When the number of experimental animals would have been higher, differences in circumstances would have been less important. However, in a group of only 6 animals, different circumstances are of great influence on the results. Therefore it is very difficult to interpret these results and draw conclusions from them.

Therefore the composition of a pain scale was not performed as none of the parameters tested in this study could be excluded or proven to be a good parameter in the assessment of postoperative pain after castration.

Since the effect of a quite simple and frequently performed procedure, like castration, still can not be measured further research into this aspect is of important value.

6. Conclusion

The objective of this study was to evaluate physiological and behavioural parameters in order to create a composite pain scale for horses after surgical castration.
Based on literature several parameters were chosen and tested for their use in a composite pain scale. No important significant differences were seen between the meloxicam group and the control group in most of the tested parameters. Almost no significant differences within a group between the base line value and any other day were seen either.
This suggests either that the horses of the control group did not experience more pain than the horses in the meloxicam group, or that none of the parameters was sensitive enough to measure the difference between the meloxicam group and the control group.
However, only six horses were tested, limiting the results and conclusions that can be drawn from this study.
The parameters food intake and body weight seemed less useful in a scale to assess pain after castration. The other parameters are worth to be tested in a next study. However none of the parameters can be excluded for sure based on this study. Therefore no composite pain scale was composed to measure pain after castration.
Following studies, containing the parameters tested in this study and other parameters, are necessary to develop a composite pain scale for horses after castration.

7. Acknowledgements

The author would like to thank:
- All staff members of the Department of Equine Sciences, Faculty of Veterinary Medicine, University of Utrecht for their kind help in this study.
- The supervisors Drs. J.P.A.M. van Loon and Dr. M. van Dierendonck for their advice, support and patience during this research project.
- My friends Aafke en Janne and fellow students Elise, Susanne and Aukje for their help with the practical work.

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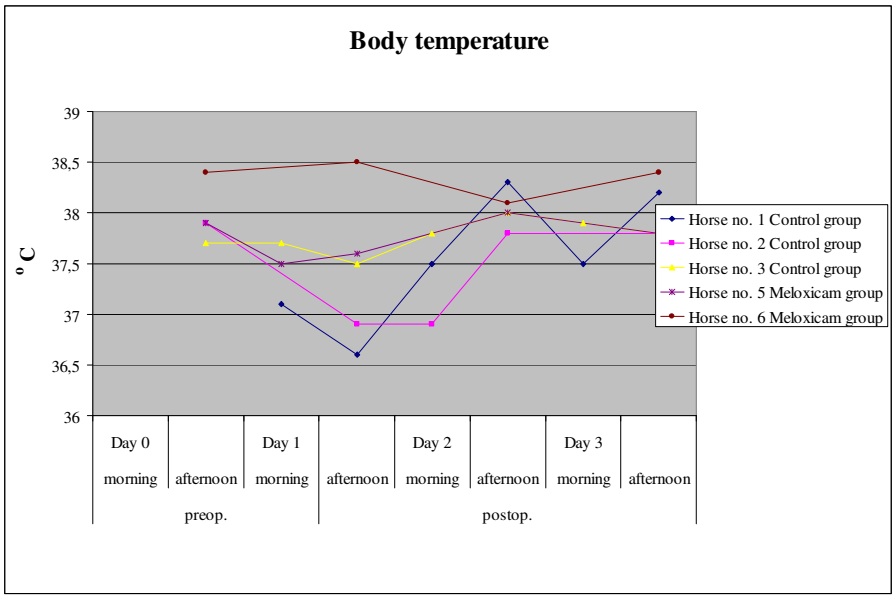
Appendix 1

1.1 Body temperature

	Day 0 afternoon	Day 1 morning	Day 1 afternoon	Day 2 morning	Day 2 afternoon	Day 3 morning	Day 3 afternoon
Horse no. 1 Control group		37,1	36,6	37,5	38,3	37,5	38,2
Horse no. 2 Control group	37,9		36,9	36,9	37,8		37,8
Horse no. 3 Control group	37,7	37,7	37,5	37,8	38	37,9	37,8
Horse no. 4 Meloxicam group	*	*	*	*	*	*	*
Horse no. 5 Meloxicam group	37,9	37,5	37,6		38		37,8
Horse no. 6 Meloxicam group	38,4		38,5		38,1		38,4

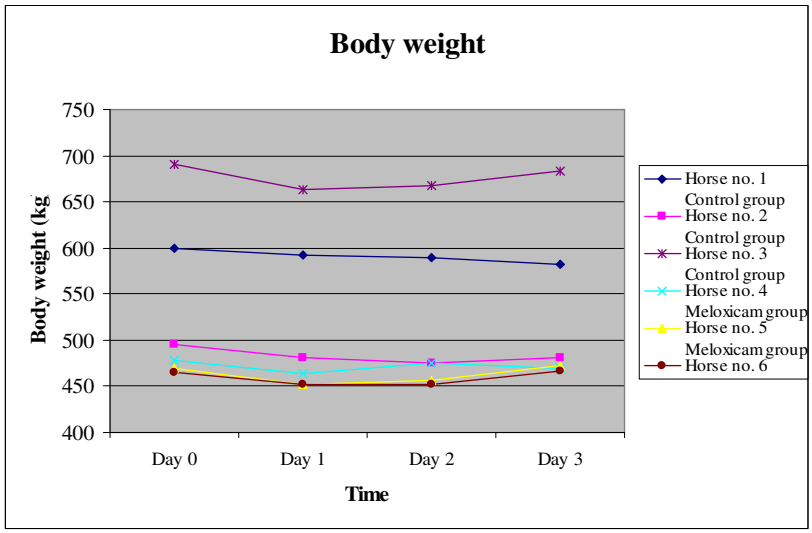
* The body temperature values of horse no. 4 were not obtained due to non cooperation of this horse.



1.2 Body weight

	Day 0	Day 1	Day 2	Day 3
Horse no. 1 Control group	600	592	590	582
Horse no. 2 Control group	496	481	475	481
Horse no. 3 Control group	690	663	667	683
Horse no. 4 Meloxicam group	478	463	475	469
Horse no. 5 Meloxicam group	470	452	456	472
Horse no. 6 Meloxicam group	465	452	452	466

Values are in kg

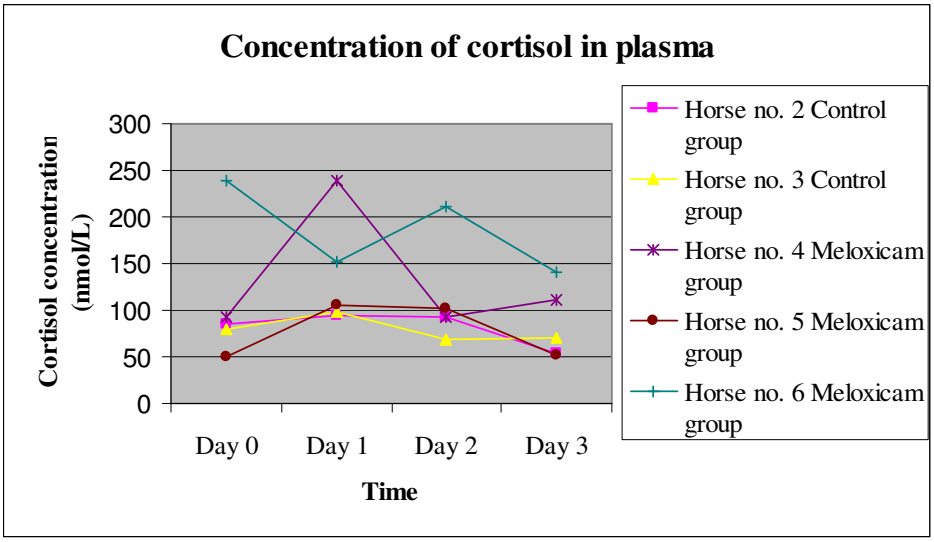


1.3 Plasma cortisol concentration

	Day 0	Day 1	Day 2	Day 3
Horse no. 1 Control group	*	|*	*	*
Horse no. 2 Control group	85	94	93	54
Horse no. 3 Control group	79	98	68	71
Horse no. 4 Meloxicam group	93	238	93	112
Horse no. 5 Meloxicam group	50	106	102	52
Horse no. 6 Meloxicam group	239	151	211	140

Values are in nmol/L

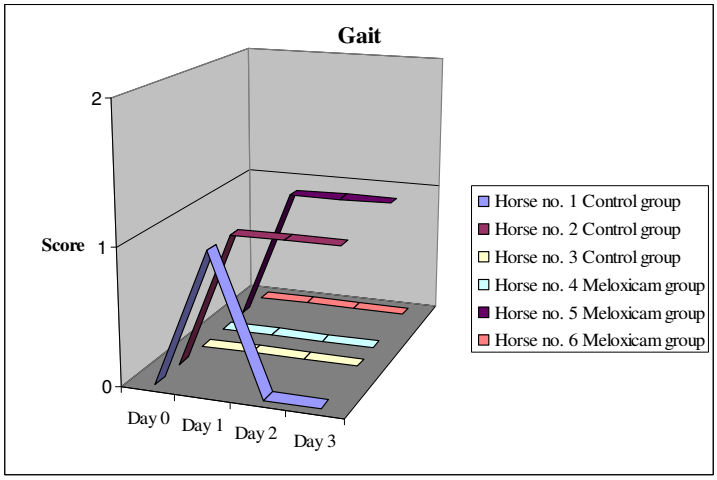
* The values of cortisol of horse no. 1 were not obtained, due to noncooperation of this horse.



1.4 Gait

	Day 0	Day 1	Day 2	Day 3
Horse no. 1 Control group	0	1	0	0
Horse no. 2 Control group	0	1	1	1
Horse no. 3 Control group	0	0	0	0
Horse no. 4 Meloxicam group	0	0	0	0
Horse no. 5 Meloxicam group	0	1	1	1
Hose no. 6 Meloxicam group	0	0	0	0

0 = normal gait
1 = quite stiff gait
2 = very stiff gait



1.5 Heart Rate Variability

Mean heart rate						
Group	Day 0, A	Day 1, A	Day 2, M	Day 2, A	Day 3, M	Day 3, A
Horse no. 1 Control group	29	29	34	35	31	30
Horse no. 2 Control group	36	38	46	40	40	36
Horse no. 3 Control group	32	33	40	32	34	34
Mean	32,33333	33,33333	40	35,66667	35	33,33333
SD	3,511885	4,50925	6	4,041452	4,582576	3,05505
SEM	2,027588	2,603417	3,464102	2,333333	2,645751	1,763834
Horse no. 4 Meloxicam group	34	31	37	36	35	35
Horse no. 5 Meloxicam group	45	40	39	42	32	34
Horse no. 6 Meloxicam group	32	38	34	42	34	37
Mean	37	36,33333	36,66667	40	33,66667	35,33333
SD	7	4,725816	2,516611	3,464102	1,527525	1,527525
SEM	4,041452	2,728451	1,452966	2	0,881917	0,881917

A = Afternoon, M = Morning

RMSSD						
Group	RMSD0A	RMSD1A	RMSD2M	RMSD2A	RMSD3M	RMSD3A
Horse no. 1 Control group						
Horse no. 2 Control group	96,2	89,4	53,3	32,8	40	36,1
Horse no. 3 Control group	126,2	157,7	174,9	209,7	158,5	167,8
Mean	111,2	123,55	114,1	121,25	99,25	101,95
SD	21,2132	48,29539	85,98418	125,0872	83,79215	93,12596
SEM	15	34,15	60,8	88,45	59,25	65,85
Horse no. 4 Meloxicam group	218,7	727,7	89,8	131,9	144,3	339,4
Horse no. 5 Meloxicam group	73,2	138,9	114,2	104,7	48,7	74,2
Horse no. 6 Meloxicam group	191,3	212,8	156,9	249,1	186,2	178,3
Mean	161,0667	359,8	120,3	161,9	126,4	197,3
SD	77,3182	320,7462	33,96336	76,73226	70,47602	133,617
SEM	44,63968	185,1829	19,60876	44,30139	40,68935	77,14383

A = Afternoon, M = Morning

pNN50						
Group	pNND0A	pNND1A	pNND2M	pNND2A	pNND3M	pNND3A
Horse no. 1 Control group						
Horse no. 2 Control group	24,5	28,3	15,6	6,5	8,9	8,7
Horse no. 3 Control group	35,5	28,7	35,4	37,5	33	38,8
Mean	30	28,5	25,5	22	20,95	23,75
SD	7,778175	0,282843	14,00071	21,92031	17,04127	21,28391
SEM	5,5	0,2	9,9	15,5	12,05	15,05
Horse no. 4 Meloxicam group	34,6	42,4	18,9	27,1	34,7	39,8
Horse no. 5 Meloxicam group	15,8	24,4	24,4	24,9	12,4	17,3
Horse no. 6 Meloxicam group	26,3	37,4	22,7	38,3	31,2	33,2
Mean	25,56667	34,73333	22	30,1	26,1	30,1
SD	9,421429	9,291573	2,816026	7,186098	11,99291	11,5659
SEM	5,439465	5,364492	1,625833	4,148895	6,924112	6,677574

A = Afternoon, M = Morning

LF						
Group	LF0A	LF1A	LF2M	LF2A	LF3M	LF3A
Horse no. 1 Control group						
Horse no. 2 Control group	3275,03	4363,38	2952,04	760,18	1572,18	1476,3
Horse no. 3 Control group	7665,8	10506,1	7442	10983,93	7983,29	8767,24
Mean	5470,415	7434,74	5197,02	5872,055	4777,735	5121,77
SD	3104,743	4343,559	3174,881	7229,283	4533,339	5155,473
SEM	2195,385	3071,36	2244,98	5111,875	3205,555	3645,47
Horse no. 4 Meloxicam group	10030,58	41290,81	2065,22	2898,19	1329,05	6852,44
Horse no. 5 Meloxicam group	4396,4	9682,03	4839,18	9454,87	1056,07	3929,58
Horse no. 6 Meloxicam group	11499,33	5990,33	4705,71	6634,57	7950,63	4918,46
Mean	8642,103	18987,72	3870,037	6329,21	3445,25	5233,493
SD	3749,507	19403,04	1564,441	3288,989	3904,16	1486,678
SEM	2164,779	11202,35	903,2305	1898,899	2254,068	858,3341

A = Afternoon, M = Morning

HF						
Group	HF0A	HF1A	HF2M	HF2A	HF3M	HF3A
Horse no. 1 Control group						
Horse no. 2 Control group	1931,03	1410,02	621,77	132,01	198,41	128,23
Horse no. 3 Control group	1960,38	4421,4	7517,01	8619,04	5094,56	5768,83
Mean	1945,705	2915,71	4069,39	4375,525	2646,485	2948,53
SD	20,75358	2129,367	4875,671	6001,236	3462,101	3988,507
SEM	14,675	1505,69	3447,62	4243,515	2448,075	2820,3
Horse no. 4 Meloxicam group	10741,02	125781,7	1910,16	4299,17	5434,41	28868,92
Horse no. 5 Meloxicam group	1123,24	4496,85	2866,8	2307,95	382,89	662,13
Horse no. 6 Meloxicam group	6909,26	12459,26	5832,87	18449,49	7754,81	8269,26
Mean	6257,84	47579,26	3536,61	8352,203	4524,037	12600,1
SD	4841,868	67842,19	2045,336	8801,002	3769,335	14593,59
SEM	2795,454	39168,71	1180,875	5081,261	2176,227	8425,614

A = Afternoon, M = Morning

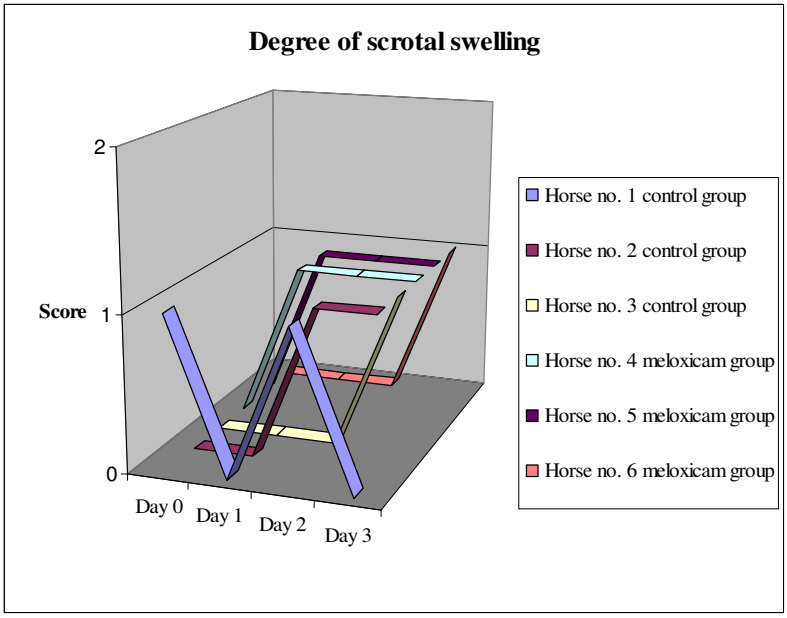
LF/HF ratio						
Group	Ratio0A	Ratio1A	Ratio2M	Ratio2A	Ratio3M	Ratio3A
Horse no. 1 Control group						
Horse no. 2 Control group	169,6	309,5	474,8	575,9	792,4	1151,4
Horse no. 3 Control group	391,1	237,7	99,1	127,5	156,8	152
Mean	280,35	273,6	286,95	351,7	474,6	651,7
SD	156,6242	50,77027	265,66	317,0667	449,4371	706,6825
SEM	110,75	35,9	187,85	224,2	317,8	499,7
Horse no. 4 Meloxicam group	93,4	32,9	108,2	67,5	24,5	23,8
Horse no. 5 Meloxicam group	391,5	215,4	168,9	409,7	275,9	593,5
Horse no. 6 Meloxicam group	166,5	48,1	80,7	36	102,6	59,5
Mean	217,1333	98,8	119,2667	171,0667	134,3333	225,6
SD	155,3664	101,2642	45,12941	207,2618	128,6691	319,1104
SEM	89,70081	58,46489	26,05548	119,6627	74,28715	184,2385

A = Afternoon, M = Morning

1.6 Scrotal area

	Day 0	Day 1	Day 2	Day 3
Horse no. 1 control group	1	0	1	0
Horse no. 2 control group	0	0	1	1
Horse no. 3 control group	0	0	0	1
Horse no. 4 meloxicam group	0	1	1	1
Horse no. 5 meloxicam group	0	1	1	1
Horse no. 6 meloxicam group	0	0	0	1

0 = no swelling
1 = little swelling
2 = much swelling



1.7 Time budget of behaviour
Duration of behaviours

Day 0, Afternoon

	Stand still, alert	Stand still, at rest	Lean against stable	Stand still, head not visible	A few paces	Chafing	Eating while standing still	Eating while walking	Drinking	Urinating	Knibbling on polar	Someone in stable
Horse no.1 Placebo	745,29	0	0	54,09	115,74	0	99,07	5,16	0	0	59,34	128,56
Horse no. 2 Placebo	863,56	24,53	0	0	11,29	0	292,81	8,65	0	0	0	0
Horse no. 3 Placebo	557,65	0	0	199,41	176,09	0	249,98	16,84	0	0	0	0
Sum	2166,5	24,53	0	303,12	303,12	0	641,86	30,65	0	0	59,34	128,56
Mean	722,1667	8,176667	0	84,5	101,04	0	213,9533	10,21667	0	0	19,78	42,85333
SD	154,2603	14,1624	0	103,1245	83,37762	0	101,7705	5,995534	0	0	34,25996	74,22415
SEM	89,06224	8,176667		59,53896	48,13809		58,75723	3,461523			19,78	42,85333
Horse no. 4 Metacam®	230,71	806,83	0	11,46	32,44	0	101,77	17,62	0	0	0	0
Horse no. 5 Metacam®	746,62	11,08	0	0	14,26	0	364,75	57,1	0	0	7,1	0
Horse no. 6 Metacam®	672,92	493,72	0	0	32,5	0	0	0	0	0	0	0
Sum	1650,25	1311,63	0	11,46	79,2	0	466,52	74,72	0	0	7,1	0
Mean	550,0833	437,21	0	3,82	26,4	0	155,5067	24,90667	0	0	2,366667	0
SD	279,0294	400,8735	0	6,616434	10,51359	0	188,2189	29,23909	0	0	4,099187	0
SEM	161,0977	231,4444		3,82	6,070025		108,6682	16,88119			2,366667	

Day 1, Afternoon

	Stand still, alert	Stand still, at rest	Lean against stable	Stand still, head not visible	A few paces	Chafing	Eating while standing still	Eating while walking	Drinking	Urinating	Knibbling on polar	Someone in stable
Horse no. 1 Placebo	84,25	0	0	56,39	72,97	3,33	949,24	31,88	2,63	0	0	0
Horse no. 2 Placebo	704,78	77,17	0	0	30,43	2,32	342,78	32,69	0	9,62	0	0
Horse no. 3 Placebo	174,19	0	0	0	110,76	6,13	800,25	55,73	34,22	20,56	0	0
Sum	963,22	77,17	0	56,39	214,16	11,78	2092,27	120,3	36,85	30,18	0	0
Mean	321,0733	25,72333	0	18,79667	71,38667	3,926667	697,4233	40,1	12,28333	10,06	0	0
SD	335,3288	44,55412	0	32,55678	40,1884	1,973837	316,0355	13,54203	19,04317	10,28706	0	0
SEM	193,6022	25,72333		18,79667	23,20278	1,139595	182,4632	7,818497				
Horse no. 4 Meloxicam	131,14	0	0	9,92	28,9	12,45	1009,64	8,31	0	0	0	0
Horse no. 5 Meloxicam	813,17	166,49	0	0	11,04	0	209,84	0	0	0	0	0
Horse no. 6 Meloxicam	955,25	0	0	23,54	122,71	48,54	48,44	2,61	0	0	0	0
Sum	1899,56	166,49	0	33,46	162,65	60,99	1267,92	10,92	0	0	0	0
Mean	633,1867	55,49667	0	11,15333	54,21667	20,33	422,64	3,64	0	0	0	0
SD	440,5506	96,12305	0	11,81836	59,98539	25,21118	514,7225	4,249671	0	0	0	0
SEM					34,63258			2,453548				

Day 2, Morning

	Stand still, alert	Stand still, at rest	Lean against stable	Stand still, head not visible	A few paces	Chafing	Eating while standing still	Eating while walking	Drinking	Urinating	Knibbling on polar	Someone in stable
Horse no. 1 Placebo	234,58	336,55	0	49,47	100,76	0	418,23	46,8	0	13,69	0	0
Horse no. 2 Placebo	966,05	0	0	0	180,43	0	27,09	7,81	0	19,87	0	0
Horse no. 3 Placebo	840,13	0	0	24,75	233,14	0	60,09	7,96	33,2	0	0	0
Sum	2040,76	336,55	0	74,22	514,33	0	505,41	62,57	33,2	33,56	0	0
Mean	680,2533	112,1833	0	24,74	171,4433	0	168,47	20,85667	11,06667	11,18667	0	0
SD	391,0659	194,3072	0	24,735	66,64598	0	216,9269	22,46771	19,16803	10,16879	0	0
SEM					38,47807		125,2428	12,97174				
Horse no. 4 Meloxicam	197	296,64	0	107,99	76,13	0	509,96	8,67	3,64	0	0	0
Horse no. 5 Meloxicam	857,6	0	0	113	74,34	0	134,94	20,96	0	0	0	0
Horse no. 6 Meloxicam	801,29	135,09	0	31,67	52,1	5,61	153,77	20,54	0	0	0	0
Sum	1855,89	431,73	0	252,66	202,57	5,61	798,67	50,17	3,64	0	0	0
Mean	618,63	143,91	0	84,22	67,52333	1,87	266,2233	16,72333	1,213333	0	0	0
SD	366,2262	148,5166	0	45,57852	13,38695	3,238935	211,292	6,977552	2,101555	0	0	0
SEM					7,728959		121,9895	4,028492				

Day 2, Afternoon

	Stand still, alert	Stand still, at rest	Lean against stable	Stand still, head not visible	A few paces	Chafing	Eating while standing still	Eating while walking	Drinking	Urinating	Knibbling on polar	Someone in stable
Horse no. 1 Placebo	168,35	87,17	0	0	31,3	0	875,91	5,62	30,17	0	0	0
Horse no. 2 Placebo	406,24	561,03	0	0	6,05	0	227,95	0	0	0	0	0
Horse no. 3 Placebo	125,91	0	0	65,88	49,82	1,83	873,86	13,55	69,73	0	0	0
Sum	700,5	648,2	0	65,88	87,17	1,83	1977,72	19,17	99,9	0	0	0
Mean	233,5	216,0667	0	21,96	29,05667	0,61	659,24	6,39	33,3	0	0	0
SD	151,0947	301,9096	0	38,03584	21,97106	1,056551	373,5095	6,807738	34,97021	0	0	0
SEM					12,685			3,93045				
Horse no. 4 Metacam®	29,24	769,12	0	33,44	22,41	0	338,51	8,51	0	0	0	0
Horse no. 5 Metacam®	479,95	200,38	0	0	10,2	0	457,18	15,01	37,86	0	0	0
Horse no. 6 Metacam®	427,04	325,28	0	0	39,84	0	371,08	16,06	20,87	0	0	0
Sum	936,23	1294,78	0	33,44	72,45	0	1166,77	39,58	58,73	0	0	0
Mean	312,0767	431,5933	0	11,14667	24,15	0	388,9233	13,19333	19,57667	0	0	0
SD	246,3682	298,9033	0	19,30659	14,89641	0	61,3142	4,089723	18,96311	0	0	0
SEM					8,600448			2,361203				

Day 3, Morning

	Stand still, alert	Stand still, at rest	Lean against stable	Stand still, head not visible	A few paces	Chafing	Eating while standing still	Eating while walking	Drinking	Urinating	Knibbling on polar	Someone in stable
Horse no. 1 Placebo	329,11	0	4,34	107,39	116,27	0	578,77	64,93	1,55	0	0	0
Horse no. 2 Placebo	1030,36	77,72	0	0	84	0	8,76	0	0	0	0	0
Horse no. 3 Placebo	421,3	450,48	0	15,8	19,92	0	264,32	28,63	0	0	0	0
Sum	1780,77	528,2	4,34	123,19	220,19	0	851,85	93,56	1,55	0	0	0
Mean	593,59	176,0667	1,446667	41,06333	73,39667	0	283,95	31,18667	0,516667	0	0	0
SD	381,0522	240,8051	2,5057	57,98129	49,04237	0	285,5116	32,54042	0,894893	0	0	0
SEM					28,31462			18,78722				
Horse no. 4 Meloxicam	97,34	942,87	0	11,4	39,45	0	110,23	0	0	0	0	0
Horse no. 5 Meloxicam	747,1	5,27	0	251,39	138,07	0	49,22	9,63	0	0	0	0
Horse no. 6 Meloxicam	662,26	537,24	0	0	0	1,22	0	0	0	0	0	0
Sum	1506,7	1485,38	0	262,79	177,52	1,22	159,45	9,63	0	0	0	0
Mean	502,2333	495,1267	0	87,59667	59,17333	0,406667	53,15	3,21	0	0	0	0
SD	353,2045	470,2165	0	141,9637	71,11673	0,704367	55,21999	5,559883	0	0	0	0
SEM					41,05926			3,21				

Day 3, Afternoon

	Stand still, alert	Stand still, at rest	Lean against stable	Stand still, head not visible	A few paces	Chafing	Eating while standing still	Eating while walking	Drinking	Urinating	Knibbling on polar	Someone in stable
Horse no.1 Placebo	146,6	174,14	0	10,24	55,77	0	731,86	39,6	41,68	0	0	0
Horse no. 2 Placebo	1148,68	0	0	0	31,91	0,83	17,87	0	0	3,3	0	0
Horse no. 3 Placebo	499,15	640,31	0	0	0	0	45,54	15,59	0	0	0	0
Sum	1794,43	814,45	0	10,24	87,68	0,83	795,27	55,19	41,68	3,3	0	0
Mean	598,1433	271,4833	0	3,413333	29,22667	0,276667	265,09	18,39667	13,89333	1,1	0	0
SD	508,3216	331,068	0	5,912067	27,98166	0,479201	404,4714	19,94863	24,06396	1,905256	0	0
SEM					16,15522			11,51735				
Horse no. 4 Meloxicam	243,19	919,31	0	0	20,44	0	16,91	0	0	0	0	0
Horse no. 5 Meloxicam	675,27	0	0	6,11	72,03	0	415,55	29,78	0	0	0	0
Horse no. 6 Meloxicam	193,84	608,13	0	22,3	43,56	0	286,17	45,5	0	0	0	0
Sum	1112,3	1527,44	0	28,41	136,03	0	718,63	75,28	0	0	0	0
Mean	370,7667	509,1467	0	9,47	45,34333	0	239,5433	25,09333	0	0	0	0
SD	264,8595	467,5799	0	11,52344	25,84119	0	203,3691	23,10922	0	0	0	0
SEM					14,91942			13,34212				

Frequency of behaviours

Day 0		Lift a leg	Pawing	Chewing	Defecating	Head shaking	Flehming	Neighing	Autogrooming	Nosing	Licking	Yawning	Extension of penis
	Horse no.1												
Afternoon	Control group	0	0	16	0	2	2	1	10	29	3	0	0
	Horse no. 2												
	Control group	0	0	9	0	2	2	0	0	17	2	0	0
	Horse no. 3												
	Control group	0	0	54	1	5	0	4	0	6	0	2	2
	Sum	0	0	79	1	9	4	5	10	52	5	2	2
	Mean	0	0	26,33333	0,333333	3	1,333333	1,666667	3,333333	17,33333	1,666667	0,666667	0,666667
	SD	0	0	24,21432	0,57735	1,732051	1,154701	2,081666	5,773503	11,50362	1,527525	1,154701	1,154701
	Horse no. 4												
	Meloxicam												
	group	0	0	8	1	3	0	1	0	1	2	2	0
	Horse no. 5												
	Meloxicam												
	group	0	0	23	0	3	0	1	21	11	0	0	0
	Horse no. 6												
	Meloxicam												
	group	1	0	8	0	0	0	1	2	8	0	0	0
	Sum	1	0	39	1	6	0	3	23	20	2	2	0
	Mean	0,333333	0	13	0,333333	2	0	1	7,666667	6,666667	0,666667	0,666667	0
	SD	0,57735	0	8,660254	0,57735	1,732051	0	0	11,59023	5,131601	1,154701	1,154701	0
			NS										

Day 1		Lift a leg	Pawing	Chewing	Defecating	Head shaking	Flehming	Neighing	Autogrooming	Nosing	Licking	Yawning	Extension of penis
	Horse no.1												
Afternoon	Control group	0	0	45	0	0	0	0	0	6	0	0	0
	Horse no. 2												
	Control group	0	0	10	0	0	0	0	2	4	0	0	0
	Horse no. 3												
	Control group	2	0	20	1	2	0	1	12	2	0	0	0
	Sum	2	0	75	1	2	0	1	14	12	0	0	0
	Mean	0,666667	0	25	0,333333	0,666667	0	0,333333	4,666667	4	0	0	0
	SD	1,154701	0	18,02776	0,57735	1,154701	0	0,57735	6,429101	2	0	0	0
	Horse no. 4 Meloxicam group	1	1	3	0	5	0	0	0	11	2	0	0
	Horse no. 5 Meloxicam group	0	3	6	0	15	0	0	2	4	0	3	0
	Horse no. 6 Meloxicam group	0	0	2	0	6	0	1	0	14	0	0	1
	Sum	1	4	11	0	26	0	1	2	29	2	3	1
	Mean	0,333333	1,333333	3,666667	0	8,666667	0	0,333333	0,666667	9,666667	0,666667	1	0,333333
	SD	0,57735	1,527525	2,081666	0	5,507571	0	0,57735	1,154701	5,131601	1,154701	1,732051	0,57735

Day 2		Lift a leg	Pawing	Chewing	Defecating	Head shaking	Flehming	Neighing	Autogrooming	Nosing	Licking	Yawning	Extension of penis
	Horse no.1												
Morning	Control group	0	1	31	1	5	0	1	1	7	0	0	0
	Horse no. 2												
	Control group	0	0	20	1	8	1	0	3	12	10	0	0
	Horse no. 3												
	Control group	0	0	21	1	5	0	3	1	2	0	0	0
	Sum	0	1	72	3	18	1	4	5	21	10	0	0
	Mean	0	0,333333	24	1	6	0,333333	1,333333	1,666667	7	3,333333	0	0
	SD	0	0,57735	6,082763	0	1,732051	0,57735	1,527525	1,154701	5	5,773503	0	0
	Horse no. 4												
	Meloxicam group	0	0	8	1	0	0	0	0	6	3	0	0
	Horse no. 5												
	Meloxicam group	0	0	16	0	5	1	4	0	7	0	0	0
	Horse no. 6												
	Meloxicam group	1	0	42	0	2	0	1	1	18	0	0	0
	Sum	1	0	66	1	7	1	5	1	31	3	0	0
	Mean	0,333333	0	22	0,333333	2,333333	0,333333	1,666667	0,333333	10,33333	1	0	0
	SD	0,57735	0	17,77639	0,57735	2,516611	0,57735	2,081666	0,57735	6,658328	1,732051	0	0
			NS										

Day 2		Lift a leg	Pawing	Chewing	Defecating	Head shaking	Flehming	Neighing	Autogrooming	Nosing	Licking	Yawning	Extension of penis
	Horse no.1												
Afternoon	Control group	4	0	34	0	4	0	0	1	5	0	0	0
	Horse no. 2												
	Control group	0	0	14	0	1	0	0	0	6	0	0	0
	Horse no. 3												
	Control group	0	0	32	1	1	0	0	3	5	0	0	0
	Sum	4	0	80	1	6	0	0	4	16	0	0	0
	Mean	1,333333	0	26,66667	0,333333	2	0	0	1,333333	5,333333	0	0	0
	SD	2,309401	0	11,01514	0,57735	1,732051	0	0	1,527525	0,57735	0	0	0
	Horse no. 4												
	Meloxicam group	1	2	16	0	1	0	0	1	2	0	0	0
	Horse no. 5												
	Meloxicam group	0	0	11	1	1	0	0	0	0	0	0	0
	Horse no. 6												
	Meloxicam group	6	4	70	0	1	0	0	8	9	0	0	0
	Sum	7	6	97	1	3	0	0	9	11	0	0	0
	Mean	2,333333	2	32,33333	0,333333	1	0	0	3	3,666667	0	0	0
	SD	3,21455	2	32,71595	0,57735	0	0	0	4,358899	4,725816	0	0	0
			NS										

Day 3		Lift a leg	Pawing	Chewing	Defecating	Head shaking	Flehming	Neighing	Autogrooming	Nosing	Licking	Yawning	Extension of penis
	Horse no.1												
Morning	Control group	0	0	42	0	2	0	1	5	4	2	0	1
	Horse no. 2												
	Control group	0	0	13	1	4	0	0	0	9	0	0	0
	Horse no. 3												
	Control group	0	0	9	0	4	0	1	1	2	0	0	1
	Sum	0	0	64	1	10	0	2	6	15	2	0	2
	Mean	0	0	21,33333	0,333333	3,333333	0	0,666667	2	5	0,666667	0	0,666667
	SD	0	0	18,00926	0,57735	1,154701	0	0,57735	2,645751	3,605551	1,154701	0	0,57735
	Horse no. 4												
	Meloxicam group	0	0	6	0	0	0	0	6	5	2	0	0
	Horse no. 5												
	Meloxicam group	0	0	12	1	10	0	2	5	14	2	0	0
	Horse no. 6												
	Meloxicam group	4	5	19	0	8	0	0	15	11	0	1	0
	Sum	4	5	37	1	18	0	2	26	30	4	1	0
	Mean	1,333333	1,666667	12,33333	0,333333	6	0	0,666667	8,666667	10	1,333333	0,333333	0
	SD	2,309401	2,886751	6,506407	0,57735	5,291503	0	1,154701	5,507571	4,582576	1,154701	0,57735	0

Day 3		Lift a leg	Pawing	Chewing	Defecating	Head shaking	Flehming	Neighing	Autogrooming	Nosing	Licking	Yawning	Extension of penis
	Horse no.1												
Afternoon	Control group	2	0	42	1	1	1	0	0	13	5	1	0
	Horse no. 2												
	Control group	3	0	15	0	0	0	0	1	8	1	0	0
	Horse no. 3												
	Control group	0	0	20	0	3	0	0	4	1	0	2	0
	Sum	5	0	77	1	4	1	0	5	22	6	3	0
	Mean	1,666667	0	25,66667	0,333333	1,333333	0,333333	0	1,666667	7,333333	2	1	0
	SD	1,527525	0	14,36431	0,57735	1,527525	0,57735	0	2,081666	6,027714	2,645751	1	0
	Horse no. 4												
	Meloxicam group	0	0	0	0	2	0	0	0	3	0	0	0
	Horse no. 5												
	Meloxicam group	0	3	12	1	2	3	3	0	9	0	4	1
	Horse no. 6												
	Meloxicam group	3	4	15	1	1	0	3	8	4	0	0	0
	Sum	3	7	27	2	5	3	6	8	16	0	4	1
	Mean	1	2,333333	9	0,666667	1,666667	1	2	2,666667	5,333333	0	1,333333	0,333333
	SD	1,732051	2,081666	7,937254	0,57735	0,57735	1,732051	1,732051	4,618802	3,21455	0	2,309401	0,57735

1.8 Direct behavioural assessment

Total Assessment score)							
	Day 0	Day 1	Day 2	Day 2	Day 3	Day 3	
	Afternoon	Afternoon	Morning	Afternoon	Morning	Afternoon	Total
Horse no. 1 control group	0	0	0	0	0	2	2
Horse no. 2 control group	0	1	1	3	3	0	8
Horse no. 3 control group	4	4	1	0	4	4	17
Horse no. 4 meloxicam group	0	3	8	8	2	1	22
Horse no. 5 meloxicam group	7	1	0	1	0	0	9
Horse no. 6 meloxicam group	0	0	2	5	3	6	16

		Head position	Ear position	Look	Nostrils	Location in stable	Spontaneous locomotion	Exploratory behaviour	Response to open door	Response to approach with food
Day 0 A	C - group	0	0	0	0	0	1	2	1	0
	M - group	0	0	0	0	0	3	3	1	0
Day 1 A	C - group	0	0	0	0	1	2	2	0	0
	M - group	2	0	0	0	0	2	0	0	0
Day 2 M	C - group	0	0	0	0	0	2	0	0	0
	M - group	2	0	0	0	2	1	3	1	1
Day 2 A	C - group	2	0	0	0	0	1	0	0	0
	M - group	2	0	0	0	2	5	0	1	1
Day 3 M	C - group	2	2	0	0	0	1	2	0	0
	M - group	4	0	0	0	0	1	0	0	0
Day 3 A	C - group	2	0	0	0	0	1	2	1	0
	M - group	2	0	0	0	0	2	2	1	0

Appendix 2

Anaesthetic protocol

All horses were anaesthetized according the following protocol:

Premedication:	Romifidine	80 mcg/kg iv
	Nalbuphine	0.1 mg/kg iv
	Meloxicam	0.6 mg/kg iv (Metacam®)
Induction:	Ketamine	2.2 mg/kg iv
	Midazolam	0.05 mg/kg iv
Maintenance:	Triple drip	1 ml/kg/hour (50 g Guaiphenesine, 1 g Ketamine, 80 mg Romifidine),
	Lidocaine 2%	20 ml, intratesticularly
	Oxygen	12 L/min, through endotracheal tube

The horses were blindly assigned to a group at arrival. The first three days postoperatively the control group received a placebo. The meloxicam group received extra analgesia.

Control group :	Placebo, first three days postoperatively
Meloxicam group :	Meloxicam p.o. (0.6 mg/kg) (Metacam® oral suspension 15 mg/ml), first three days postoperatively

Invulformulier onderzoek naar pijn na castratie

Patientnr.:
Naam Eigenaar:
Naam Paard:
Geb. datum:
Tijdstip castratie:
Nr.:

Tijdstip	Handeling	Dag 0 aankomst*	Dag 1 castratie*	Dag 2*	Dag 3*	Dag 4*
7.00	Medicatie oraal					
7.30-7.45	Cortisol		A	B	C	D
8.00	Temperatuur					
11.00/12.00**	Gedragsvideo			C1	D1	
	HRV			C1	D1	
	NRS			C1	D1	
13.00-15.00	Voerobservatie					
14.30-15.00	Video zwelling	A	B	C	D	
	Video gang	A	B	C	D	
	Gewicht					
15.00/16.00**	Gedragsvideo	A	B	C2	D2	
	HRV	A	B	C2	D2	
	NRS	A	B	C2	D2	
17.00	Temperatuur					

Indien de handeling verricht is deze a.u.b. afkruisen of indien van toepassing de gemeten waarde invullen.
* A.u.b. datum invullen
** Afhankelijk van tijdstip van castratie
Ong. 8.30 -> 11.00 en 15.00 uur
Ong. 10.30 -> 12.00 en 16.00 uur

Opmerkingen / Bijzonderheden

NRS score

Patientnr.:
Naam eigenaar:
Naam paard:
Geb. datum:
Tijdstip castratie:
Nr.:

		Score		
Gedragskenmerk	**0**	**1**	**2**	**3**
a. Positie van hoofd	Boven schofthoogte		Op schofthoogte	Continu onder schofthoogte
b. Stand van oren	Beide oren naar voren, reactie op omgeving		Beide oren naar de zijkant	Oren naar achteren, nauwelijks beweging
c. Blik	Ogen open, reactie op omgeving		Ogen open, reageert nauwelijks op omgeving	Gefixeerde blik, oogwit zichtbaar of halfgeloken ogen.
d. Neusgaten	Niet verwijd			Sterk verwijde neusgaten
e. Positie in de stal	Bij de voorkant, de omgeving bekijkend	In het midden, ogen gericht op de voorkant	In het midden/ achterin, ogen gericht op zijkant van stal	In het midden/achterin, ogen gericht op achterkant van de stal
f. Spontane locomotie	Loopt vrij door de stal	Af en toe een paar stappen		Geen locomotie
g. Exploratiegedrag	Actief bezig om aan stalmuren of zichzelf te ruiken/ of is op zoek naar eten		Heel af en toe aanwezig. Het grootste deel van de tijd vertoont het dier dit gedrag niet.	Geheel niet aanwezig, staat 'niets' te doen.
h. Respons op openen van deur	Loopt naar de staldeur	Kijkt naar de staldeur	Reageert nauwelijks	Geen respons
i. Respons op benadering met wat krachtvoer in de hand	Komt naar waarnemer toe, oren naar voren gericht	Kijkt naar waarnemer, oren naar voren	Loopt weg van waarnemer	Geen beweging, oren naar achteren

Opmerkingen/bijzonderheden

Protocol

De waarnemer komt aanlopen, blijft op 1 meter afstand voor de staldeur staan, roept het dier en scoort gedurende 5 minuten de onderdelen a t/m g. Daarna loopt hij/zij naar de staldeur toe, roept het paard nogmaals bij de naam en opent de staldeur, waar hij/zij even blijft staan om de respons af te wachten. Vervolgens benadert de waarnemer het paard met wat krachtvoer in de hand en bekijkt weer de respons.
Let op! Het paard mag niet liggen of slapen tijdens het scoren.

Ethogram

Nr.	Name	Description	Code	Properties
	Eten	Voer of stro in mond nemen, kauwen of fourageren	ET	State
	Drinken	Water uit waterbak drinken	DR	State
	Mesten		ME	Event
	Urineren		UR	State
	Liggen lateraal	Liggen in zijligging	LZ	State
	Liggen sternaal	Liggen in sternale ligging (buik)	LB	State
	Rollen	Rollen over zijde	RO	State
	Stilstaan attent	Paard staat min. 2 sec. stil met opgericht hoofd en levendig orenspel	SA	State
	Stilstaan rust	Paard staat min. 2 sec. op rust of vierkant, lage hoofd/halshouding	SR	State
	Leunen tegen stalwand	Leunen met zijkant of achterhand tegen wand van stal of tralies	LS	State
	Staan, hoofd buiten beeld	Staan met hoofd buiten beeld van camera	BB	State
	Enkele passen	Min. 2, max. 4 passen met voorbenen voorwaarts of zijwaarts	EP	State
	Lopen	Meer dan 4 passen met voorbenen voorwaarts of zijwaarts	LO	State
	Rondlopen	Loopt min. ¾ rondje door de box	RL	State
	Achteruitlopen	Min. 2 passen met voorbenen achterwaarts	AL	State
	Bokken	Beide achterbenen los van de grond	BO	Event
	Steigeren	Beide voorbenen los van grond	SY	Event
	Poging tot steigeren	Omhoogkomen, maar niet beide voorbenen komen van de grond	PS	Event
	Schuren	Met hoofd of lichaam schuren tegen wand van stal of tralies	SC	State
	Stampen	Optillen van voor of achterbeen en met kracht weer neerzetten	ST	Event
	Schrapen	Schrapen met voorbeen over vloer of langs muur	SS	State
	Schrapen	Schrapen met voorbeen over vloer of langs muur	SE	Event
	Slaan achterbeen		AC	Event
	Slaan voorbeen		VO	Event

	Hoofdschudden	Hoofd snel bewegen van links naar rechts of van boven naar beneden	HS	Event
	Flehmen	Hals gestrekt en bovenlip omhoog gekruld	FL	Event
	Hinniken		HI	Event
	Autogrooming	Met mond knabbelen / bijten aan eigen lichaam. Incl. happen naar polar	AG	Event
	Snuffelen	Snuffelen aan stalwanden, tralies of bodembekking	SN	Event
	Kribbebijten	Bijten aan voerbakrand of andere objecten	KB	Event
	Likken	Likken aan stalmuur, tralies of andere objecten	LI	Event
	Geeuwen		GE	Event
	Optillen been		OP	Event
	Kauwen		KA	Event
	Eten stilstaan	Stilstaand fourageren	ES	State
	Eten lopen	Meer dan 2 passen kauwend of fouragerend	EL	State
	Polar trekken	Trekken aan polar met mond	PT	State
	Begin observatie (bordje start)		WI	State
	Einde observatie		EO	State
	Overig State		OS	State
	Overig Event		OE	Event

Betreft: Medewerking onderzoek naar pijn na castratie

Utrecht, februari 2008

Geachte heer/mevrouw,

Middels deze brief willen wij u toestemming vragen voor deelname van uw paard aan een onderzoek naar indicatoren voor pijn bij paarden na castratie.
Dit onderzoek is in januari 2008 op de Faculteit der Diergeneeskunde vanuit de afdeling Anesthesiologie gestart en heeft tot doel een duidelijker beeld te krijgen van de manier waarop paarden de pijn uiten die ze, ondanks pijnstilling, mogelijk nog ervaren na castratie.

Waarom dit onderzoek?
Onderzoek bij paarden op het gebied van pijn is tot op heden slechts op beperkte schaal verricht. Er zijn wel ideeën en beschrijvingen van manieren waarop pijn door paarden geuit wordt, maar deze zijn niet zonder meer in elke situatie van toepassing.
Toepassing van (extra) pijnstilling is dan ook vaak gebaseerd op factoren waarvan de interpretatie subjectief is en welke mogelijk niet in elke situatie even relevant zijn.
In dit onderzoek zal geprobeerd worden in kaart te brengen welke factoren in het geval van castratie van belang zijn als goede indicator voor pijn. Verder zal geprobeerd worden deze indicatoren zo goed mogelijk meetbaar te maken.
Indien er duidelijke resultaten uit dit onderzoek komen, kunnen deze gebruikt worden om patiënten in de toekomst een beter aansluitende pijnbestrijding te bieden.
Daarnaast kunnen de resultaten van dit onderzoek gebruikt worden om het huidige beleid van de Universiteitskliniek voor Paarden op het gebied van pijnstilling na castratie te evalueren.

Onderzoeksopzet
In dit onderzoek worden zowel fysiologische parameters, zoals stresshormoon en lichaamstemperatuur, als gedragsparameters bekeken om een zo breed mogelijk beeld te krijgen. De deelnemende paarden worden willekeurig ingedeeld in twee groepen, te weten een testgroep en een controlegroep. Alle deelnemende dieren worden gecastreerd volgens de methode die gebruikelijk is in de Universiteitskliniek voor Paarden en ontvangen daarbij anesthesie en pijnstilling volgens protocol, zoals ieder paard dat voor castratie wordt aangeboden.
Het verschil tussen de testgroep en de controlegroep bevindt zich in het feit dat de dieren in de testgroep gedurende drie dagen na de operatie nog extra pijnstilling krijgen in de vorm van Metacam® suspensie voor oraal gebruik. De dieren in de controlegroep krijgen geen Metacam®, maar een placebo, zodat het onderzoek dubbelblind wordt uitgevoerd. De gang van zaken zoals in de controlegroep, dus geen extra pijnstilling, is de gebruikelijke gang van zaken in de Universiteitskliniek voor Paarden.

Dieren die zich in de controlegroep bevinden, maar naar beoordeling van de behandelend dierenarts toch extra pijnstilling of andere medicamenten nodig hebben, zullen deze uiteraard ontvangen.

Indien u toestemming geeft voor dit onderzoek zullen bij uw paard gedurende 4 dagen, (de dag van aankomst t/m twee dagen na de operatie) de volgende handelingen worden verricht:

- 2 x per dag lichaamstemperatuur meten
- 2 x per dag gedurende 30 minuten registratie van hartactie in rust
- 2 x per dag gedurende 30 minuten videoregistratie van het paard in de box
- 1 x per dag gewichtsbepaling
- 1 x per dag gedurende enkele minuten videoregistratie van gang van paard
- 1 x per dag gedurende een minuut videoregistratie van de liesstreek van het paard
- 1 x per dag bloedafname voor cortisolbepaling
- 1 x per dag registratie van voeropname

De handelingen die worden verricht zijn zo gekozen dat de belasting voor het paard zo klein mogelijk is en interfereren niet met voedertijden en andere belangrijke zaken, zoals bijvoorbeeld de gang naar de stapmolen. Het gehele onderzoeksprotocol is goedgekeurd door de Dierexperimentencommissie (DEC) van de Universiteit Utrecht.

Alle gegevens die gebruikt worden voor het onderzoek zullen bewaard en verwerkt worden met inachtneming van de geldende privacywetgeving. Dit houdt in dat uw naam, of die van het paard, nergens in het onderzoeksverslag genoemd zal worden of op een andere manier herkenbaar zal worden gemaakt.

Mocht u nog vragen hebben over het onderzoek of t.z.t. belangstelling hebben voor de resultaten, dan staan wij u graag te woord. U kunt daarvoor contact op nemen met Drs. J.P.A.M. van Loon, via het telefoonnummer 030-2531111.

Wij hopen op uw medewerking.

Hopende u hiermee voldoende geïnformeerd te hebben verblijven wij,

Met vriendelijke groet,

Drs. J.P.A.M. van Loon
SIO Veterinaire Anesthesiologie

Drs. M.K.Schaafsma
6e jaars student Diergeneeskunde

Akkoordverklaring

Hierbij verklaart ondergetekende toestemming te geven aan de Faculteit der Diergeneeskunde, Universiteitskliniek voor Paarden, afdeling anesthesiologie voor deelname van zijn/haar paard in het onderzoek naar pijn na castratie met inbegrip van alle bijbehorende handelingen, zoals vermeld in bijgaande brief.

Naam: _____

Adres: _____

Woonplaats: _____

Naam van paard: _____

Ras: _____

Geboortedatum van paard: _____

Datum: _____

Plaats: _____