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Pain evaluation and control after routine interventions in cattle

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TESE DE DOUTORAMENTO EM CIÊNCIAS VETERINÁRIAS

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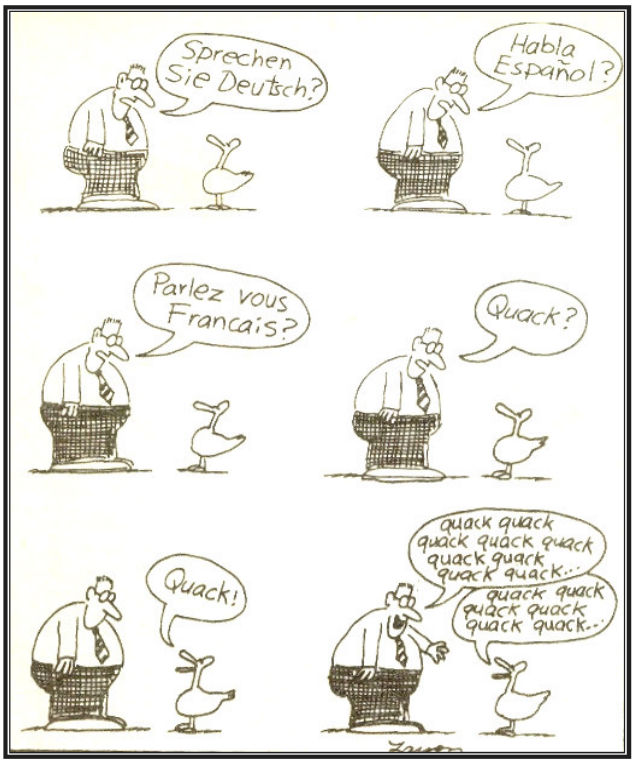
To my parents, Pamela and Francis

Understanding animals (The Far Side, by Garry Larson)



"The most crucial limitation to the moral philosophy approach to animal welfare is the fact that what matters to the animal is not what we think or feel but what we do"

(In: Animal Welfare: A Cool Eye Towards Eden. J. Webster, 2005)

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ABSTRACT:

Pain evaluation and control after routine interventions in cattle.

Disbudding and castration are two routine interventions in cattle practice. Both can cause severe pain and cause poor welfare. Through plasma cortisol levels and behaviour evaluation we measured pain caused by different disbudding and castration methods. We also studied the efficacy of several anaesthesia and analgesia protocols.

The main conclusions are:

- Cortisol together with behaviour assessment is very useful in detecting calves in pain.

- Certain behaviours are only shown by very young calves.

- Vocalization should not be used as a sign of pain in calves.

- Scoop disbudding causes long term pain and local anaesthesia is not efficient.

- Hot-iron disbudding causes severe pain during the procedure but does not differ from paste disbudding in the next hours. Local anaesthesia plus analgesia does reduce pain cause by these methods.

- Xylazine causes an increase in cortisol even if pain is not induced.

- Pain caused by clamp-castration lasts for at least 48 hours and is only controlled by long acting analgesics.

- Surgical castration causes intense pain but shorter if two incisions are made instead of just one.

Keywords: cattle; pain assessement; pain management; analgesia; cortisol; behaviour; castration; dehorning.

RESUMO:

Avaliação e controlo da dor causada por intervenções de rotina em bovinos.

A descorna e a castração de bovinos jovens são duas intervenções de rotina nas explorações. Ambas intervenções têm o potencial de causar dor e, portanto, de afectar gravemente o bem-estar animal. Através da medição do cortisol plasmático e avaliação do comportamento medimos a dor causada por diversos métodos de descorna e castração. Testámos ainda diversos protocolos de anestesia e analgesia.

Principais conclusões:

- O cortisol associado à observação do comportamento é eficaz na detecção de vitelos em dor.

- Certos comportamentos de dor apenas são exibidos por animais muito novos.

- A vocalização não é um sinal útil na identificação da dor em vitelos.

- A descorna por amputação causa dor prolongada e a anestesia local não é eficaz.

- O procedimento de descorna por ferro causa dor elevada, mas nas horas seguintes a dor não difere da descorna com pasta. A anestesia local associada a um analgésico controla a dor nestes dois métodos.

- A xilazina causa elevação de cortisol mesmo quando não há dor.

- Dor causada pela castração por esmagamento dura pelo menos 48 horas e só é controlada por analgésicos com acção prolongada.

- Castração cirúrgica causa dor intensa mas menos prolongada quando feita através de duas incisões do que através de uma incisão.

Palavras-chave: Bovino, avaliação de dor; controlo de dor; analgesia; cortisol; comportamento; castração; descorna.

PUBLICATIONS:

The chapters on this thesis are based on the following papers published in national and international peer- reviewed journals.

1. Stilwell G, Lima MS, Broom DM (2008) The effects of two non-steroidal anti-inflammatory drugs and of epidural injection on plasma cortisol and behaviour of calves castrated using a Burdizzo clamp. American Journal Veterinary Research. 69: 744-750.
2. Stilwell G (2008) O efeito de anestesia regional associada ou não a um AINE sobre o cortisol e comportamento de vitelas descornadas por amputação com alicate. Veterinary Medicine. 10: 60-68.
3. Stilwell G, Lima MS, Broom DM (2008) Comparing plasma cortisol and behaviour of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia. Livestock Science. 119: 63–69.
4. Stilwell G, Campos Carvalho R, Lima MS, Broom DM (2008) The effect of duration of manual restraint during blood sampling on plasma cortisol levels in calves. Animal Welfare. 17: 383-385.
5. Stilwell G, Campos de Carvalho R, Lima MS., Broom DM (2009) Effect of caustic paste disbudding, using local anaesthesia with and without analgesia, on behaviour and cortisol of calves. Applied Animal Behaviour Science. 116: 35-44.
6. Stilwell G, Lima MS, Broom DM (2009) Comparing the effect of three different disbudding methods on behaviour and plasma cortisol of calves. Revista Portuguesa Ciências Veterinárias. 102 (563-564): 281-288.

7. Effect of hot-iron disbudding on behaviour and plasma cortisol of calves sedated with xylazine. Accepted in 2009 as Research Paper for publication in Research in Veterinary Science.
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The results presented on this thesis were also used in the following publications:

Books:

Stilwell G (2006) Manual da Dor em Bovinos. Published by Pfizer – Saúde Animal.

Proceedings:

- Stilwell G, Lima MS, Nunes T, Capitão E (2004) Effect of three different methods of dehorning on plasma cortisol levels and behaviour of calves. In Proceedings XXIII World Buiatric Congress. 11-16 July. Quebec, Canada. Pp: 665 (3339).

- Stilwell G, Lima MS, Nunes T, Capitão E (2004) Evaluation of the effect of local anaesthesia and local anaesthesia associated with analgesia on the levels of cortisol after hot-iron, chemical or scoop dehorning. In Proceedings XXIII World Buiatrics Congress. 11-16 July. Quebec, Canada. Pp: 173 (3342).

- Stilwell G, Capitão E, Nunes T, Lima MS (2004) Efeito de anestesia e anestesia associada a analgesia na prevenção de dor após descorna. In Proceedings: II Encontro de Epidemiologia e Medicina Preventiva. Outubro. Coimbra, Portugal.

- Stilwell G (2005) Avaliação e controlo da dor em animais de produção. In Proceedings: Congresso da Sociedade Portuguesa de Ciências Veterinárias. October. Santarém, Portugal. Pp: 89.

- Stilwell G, Lima MS, Pinto F (2006) Effect of handling on bovine plasma cortisol. In Proceedings I International Congress on Conservation and Welfare. 23-31 May. Lisbon, Portugal. Pp: 35-36

- Stilwell G, Lima MS, Broom DM (2006) Comparing the efficacy of two non-steroidal anti-inflammatory drugs in reducing pain after castration of calves. In: Proceedings XXIV World Buiatrics Congress. 15-19 October. Nice, France. Pp:

- Stilwell G, Lima MS, Broom DM (2007) Avaliação e controlo da dor provocada por intervenções de rotina em explorações de bovinos. In Proceedings: Jornadas do Ensino Pós-Graduado da FMV – Simpósio do CIISA. 14-15 Junho. FMV, Lisbon. Pp: 62

- Stilwell G, Campos de Carvalho R, Lima MS, Broom DM (2008). Effect of caustic paste disbudding on cortisol and behaviour of dairy calves treated with regional anaesthetic. In Proceedings "XXV World Buiatrics Congress". 6-11 July. Budapeste, Hungary. Pp: 263

- Stilwell G, Campos de Carvalho R, Lima MS, Broom DM (2008). Plasma cortisol and behaviour of dairy calves after regional anaesthesia with lidocaine has subsided. In Proceedings "XXV World Buiatrics Congress". 6-11 July. Budapeste, Hungary. Pp: 263.

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6.2. Evaluating and controlling pain after paste disbudding.

Study 5: Effect of caustic paste disbudding, using local anaesthesia with and without analgesia, on behaviour and cortisol of calves. Published in: Applied Animal Behaviour Science.

Study 6: Comparing plasma cortisol and behaviour of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia. Published in: Livestock Science.

6.3. Evaluating and controlling pain after hot-iron disbudding.

Study 7: Effect of hot-iron disbudding, using regional anaesthesia with and without analgesia, on cortisol and behaviour of calves. (Submitted).

Study 8: Effect of hot-iron disbudding on behaviour and plasma cortisol of calves sedated with xylazine. Research in Veterinary Science. IN PRESS.

6.4. Comparing disbudding methods.

The effect of three methods of disbudding on behaviour and plasma cortisol of calves. Published in: Revista Portuguesa de Ciências Veterinárias.

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7.1. General discussion

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LIST OF ABBREVIATIONS

ACTH – Adrenocorticotrophic Hormone.

ATP – Adenosine Tri-Phosphate.

BRSV – Bovine Respiratory Syncitial Virus.

BSE – Bovine Spongiform Encephalopathy.

BVD – Bovine Viral Diarrhoea.

BWC – White Blood Cells.

CBG – Corticosteroid-Binding Globulin.

CNS – Central Nervous System.

COX-1 – Cyclo-oxygenase 1.

COX-2 – Cyclo-oxygenase 2.

CRH – Corticotrophic Releasing Hormone.

GABA – Gamma-Amino-Butyric Acid.

HPA – Hypothalamo-Pituitary-Adrenal (axis).

IASP – International Association for the Study of Pain.

IBR – Infectious Bovine Rhinotracheitis.

IL-6 – Interleukin-6.

LA – Local Anaesthesia.

MHC – Major Histocompatibility Complex.

NSAID – Non-Steroid-Anti-Inflammatory Drugs.

PI_3 – Parainfluenza 3 (virus).

RA – Regional Anaesthesia.

VP – Vasopressin.

WDR – Wide-Dynamic-Range (neurones).

CHAPTER 1 – Introduction.

1.1. The Project.

Several routine husbandry procedures, usually termed mutilations, may affect severely the welfare of farm animals. These procedures generally influence welfare by causing short or long term pain which, most of the times, is underestimated by stockpersons and practitioners. Therefore it is essential to evaluate how deep and prolonged is the distress caused by painful routine interventions performed under field conditions. Additionally, studying how distress can be accurately assessed in farm animals and how pain can be minimized, is needed to ensure good welfare.

There are three main reasons for studying pain after castration and disbudding. The first is a practical reason: both are painful procedures consistently performed at farm level and so easily provide enough numbers to do a thorough and comprehensive study of the different pain management protocols. The second is an ethical reason: these procedures are routinely performed at the farms and, therefore, would take place in any case. This means that no animal has to be submitted to additional suffering. Finally a utilitarian motive: by showing how painful these interventions are and how pain can be controlled there is an increase in human concern hopefully leading to the implementation of adequate guidelines and codes of practice for these procedures thus improving the welfare of millions of calves worldwide.

When designing and conceiving a study on farm animal pain two main concerns should be addressed:

1) To replicate, as accurately as possible, the procedures used in the field so as to reproduce the real behavioural and physiological changes caused by the interventions. This implies doing the experiments on the farms (on-farm assessment) and not in a more controlled laboratory environment (e.g. university stables). This also justifies choosing the regular stockpersons/veterinarians to perform the disbudding and castration. It is important to emphasise the significance of the "field conditions" because many published studies limit the real situations animals go through after the procedures by restricting movements and interactions. It is true that laboratory conditions do reduce the effect of restraining, novelty, insects, weather etc… on the behaviour and physiological parameters measured. However, this also means that

daily activities and interactions with other animals, that can exacerbate a state of hyperalgesia that may follow the procedures, are very much reduced. In contrast, in field conditions we may get cortisol increases that correspond to renewed moments of pain (Breazile, 1988). This is an important welfare issue.

2) To evaluate the beneficial effects of treatments that would be expected to be used in day-to-day conditions of a commercial farm. Factors like treatment cost, drug restrictions, possible meat residues, manpower and restraining conditions available, the unlikely daily presence of a veterinarian, the need to move animals from paddocks etc… should all be taken in account. To these important factors it should be added the limitations caused by legal restrains on the use of some products (i.e. opioids) and other legislation that impacts on the ability of veterinarians to prevent or alleviate pain in food producing animals. Consequently some of the analgesic protocols may not be the perfect ones but the feasible ones.

Finally some words on the outline of the thesis. All of the studies included in this thesis have been published, are "in press" or have been submitted to peer-reviewed journals. The studies are presented in the same way they were published/submitted except for the Introduction sections that were compiled, edited and extended along the first chapter of the thesis. This reduces duplication and allows for a more comprehensive reading. The same was done with each paper's Reference list.

1.2. Pain.

The International Association for the Study of Pain's (IASP) definition for pain is that it is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey, 1979). However, for the purpose of our studies, and because we prefer not to use the expression "described in terms" we suggest the definition presented by Broom and Fraser (2007): "pain is (…) an aversive sensation and feeling associated with actual or potential tissue damage" or the one proposed by Molony and Kent (1997) in which pain is defined as

"an aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of its tissues".

1.2.1. Pain mechanisms in mammals.

Animals depend on their ability to respond to challenges coming from the environment and other animals. The only way available to receive and convey this information to the central nervous system (CNS) is through sensory organs distributed all over the body. Neurons have evolved specialized properties that allow them to receive information, process it and transmit it to other cells. The stimuli translated into nerve impulses are, to name just a few, light, pressure, chemicals, temperature, vibration, sound waves etc… Sensory reception begins in receptor cells, that are specialized to respond to particular kinds of stimuli, and transmitted through a corresponding nerve fibre (afferent neurons) to the central nervous system to be processed. Table 1.1 describes the cutaneous receptors and corresponding fibres that are usually associated with pain transmission.

1.2.1.1. Nociception.

Nociception is the unconscious afferent activity produced in the peripheral and central nervous system by stimuli that have the potential to damage tissues. Although many authors assert that nociception should not be confused with pain, which is a conscious experience, others say it is the first and basic part of the pain mechanism. For presentation purposes we will address nociception physiology separately from the emotional element of pain that will be discussed later.

Nociception mechanism depends essentially on two stages (Fig.1.1.):

Transduction, in which the noxious stimuli (mechanical, temperature or chemical) is translated into electrical activity (Raja *et al*, 1999). This occurs at the sensory endings of special nerve fibres termed "nociceptors" which are presented and described in Table 1.1.

Transmission, which is the propagation of the electrical impulses throughout the sensory nervous system to the CNS. Glutamate is the predominant excitatory neurotransmitter in all nociceptors (Julius and Basbaum, 2001)

Fibre	Structure	Function	Stimuli	Caractheristics
Aβ	Myelinated	Touch, pressure. Very rapid transmission (35-75 m/s)	Mechanical	Large diameter (6-12 μm). Easily blocked by local anaesthetics.
A δ	Thick, thinly myelinated	Mediate "first pain": - acute, sharp and immediate pain - rapid transmission (5-30 m/s) - responsible for "withdrawal reflex"	Mechanical, thermal	Easily blocked by local anaesthetics.
C	Thin and unmyelinated	Transmits "second pain" or "deep pain" - delayed, diffuse and dull pain - slow transmission (0.5 – 2 m/s) - intensifies first pain	Polymodal: mechanical thermal, chemical	Hypersensitive in the presence of certain substances: Substance P, H+, K+, serotonin, histamine, prostaglandins.. Less easily blocked by local anaesthetics.
Silent	Various	- are not activated by initial noxious stimuli - they are "awaken" when tissues are already damaged. - important role in hyperalgesia and allodynia	Inflammation	Activated by "inflammatio bradykinin, prostaglandins, cytokines, H+, K+ ... Difficult to control with local anaesthesia.

Table 1.1. – Types of nerve fibres responsible for transmission of noxious stimuli (adapted from Stilwell, Manual da Dor, 2006).

When discussing nociception we also have to address the concept of "modulation" which is an important component of pain and by which transmission of pain impulses through the spinal cord are inhibited by descending reflexes that originate in the noradrenergic neurons of the mesencephalic periductal gray matter, and the pontine locus ceruleus (Hayes *et al*, 1978). This is the way by which an organism avoids being overridden by pain allowing, for example, a wounded gazelle to escape the cheetah, or a horse with a broken leg to get to the finish line.

When a noxious stimuli is induced it causes a "first pain", also termed "physiologic pain", that serves a protective biological function by acting as a warning of on–going (or potential) tissue damage. This is an almost instant transmitted sensation that travels through thinly myelinated Aδ fibres (Table 1.1.). It instigates defensive activity, like the "withdrawal reflex" or the "fight or flight behaviour". However, it should be mentioned that even short but repeated bursts of acute pain can induce long-term neuronal sensitisation (Clark *et al*, 1997; Carr and Goudes, 1999) as we will see further down.

The “second pain” or “deep pain”, resulting from C fibres activation (thin, unmyelinated and slow conducting fibres, see Table1.1.), is interpreted by the SNC as a dull, diffuse, aching or throbbing sensation and is sometimes called clinic, chronic or pathologic pain (George, 2003). It causes discomfort and may have the role of prompting the animal to rest and so recover from injury. Chronic pain management is complex but perhaps more important because it affects the welfare of animals in a more intense and prolonged manner.

A third kind of pain, called neurophatic pain, results from injuries to the nerve fibre and is important in animal welfare because it might be the cause of enduring pain after mutilations (e.g. tail docking) (Eicher *et al*, 2006) or Downer Cow Syndrome (Stilwell, personal observations). There is no known effective way to treat or minimize this neurophatic pain and so the only sensible thing to do is prevent it as much as possible (Woolf and Mannion, 1999).

Finally, visceral pain is unique in that there are no first (fast) and second (slow) components; instead, pain is often poorly localized, deep and dull (Gebhart, 1996). It is usually triggered by other kind of stimuli, namely stretching, compression or ischemia.

Tactile nerve fibres (Aβ fibres) detect innocuous stimuli applied to skin, muscle and joints and thus do not contribute to pain. However, some of these fibres are connected with interneurons linked to descending noxious stimuli inhibitory paths and so stimulation of large Aβ fibres can reduce pain, as occurs when you activate them by rubbing the area near a wound. This may explain why animals tend to lick or scratch painful spot’s surrounding area.

The idea of different ascending and descending nerve fibres interacting and influencing the nociceptor transmission led to the theory called “the gate control theory of pain” (Melzack and Wall, 1965).

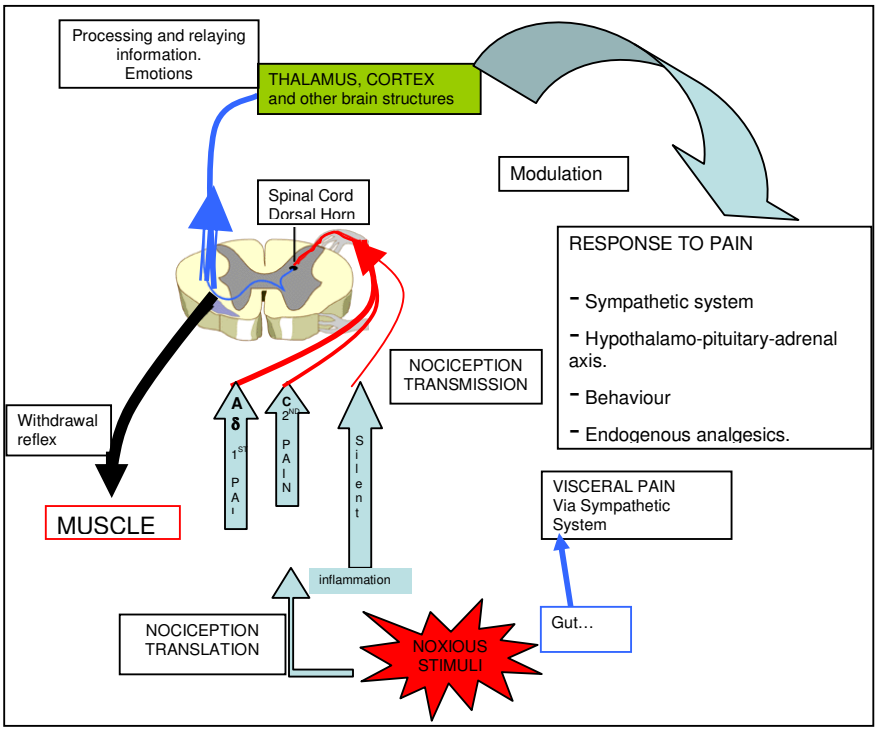

Fig 1.1. - Schematic description of pain system in mammals (adapted from Manual da Dor, Stilwell, 2006)

This theory describes how the perception of pain is not only and directly a result of activation of nociceptors, but is instead modulated by interaction between different neurons, both pain-transmitting and non-pain-transmitting (like a gate opening or closing to the passage of stimuli). In other words, the theory states that activation, at the spine level or even in the brain, of nerves that do not usually transmit pain signals can interfere with signals from pain fibres and inhibit or modulate an animal's experience of pain.

All nociceptors have their axons synapse in the dorsal horn of the spinal cord, where the neurons of laminae I, II and V are most involved in the perception of pain (except on the head in which nociceptive stimuli are transmitted by the trigeminal system). It is here, by way of interneurons connecting to the ipsilateral ventral horn, that the reflex arch is produced allowing for rapid muscle contraction and body withdrawal from the stimuli source reducing further damage. The signals then travel through a spinothalamic tract or a spinoreticular tract of the spinal cord to several structures of the brain, namely the mesencephalon, thalamus (considered the 'central switching station' of the brain: translating, processing and relaying information), reticular formation (important relation to consciousness), hypothalamus (regulating sympathetic and pituitary activity), limbic system (emotions) and cortex (consciousness).

1.2.1.2. Chronic pain.

Pain resulting from inflammation is dealt with in more detail because it is probably the most common and problematic pain in cattle medicine. It is very frequent in the field (lameness, mastitis, surgery, downer-cows etc…), causes a great deal of suffering, reduces welfare and is much more difficult to manage than acute pain.

After tissue is damaged (trauma, surgery, stretching, infection etc…) there is usually an inflammatory reaction. There are vascular components, fibroblastic components and tissue cell components activated: blood vessels carry circulating precursors that are released into the area of injury and are activated by enzymes; mast cells release histamines and other substances; macrophages activate fibroblasts, which in turn release interleukin and Tumor Necrosis Factor (TNF); cycoloxygenase activates prostaglandin and leukotrienes etc… Initial pain is exacerbated when nociceptor terminals become exposed to these products of tissue damage and inflammation, referred to collectively as the "inflammatory soup" (Julius and Basbaum, 2001)

Some of the main components of the "inflammatory soup" include peptides (bradykinin), lipids (prostaglandins), neurotransmitters (Substance P, serotonin and ATP) and neurotrophins. The acidic nature of the inflammatory soup is also important in nerve sensitization. Each of these factors sensitizes (lower the threshold) or excites the terminals of the nociceptor by interacting with cell-surface receptors expressed by these neurons. Additionally other type of nociceptors, the silent or sleeping nociceptors become very responsive when sensitized by the inflammatory soup. This nociceptor hyper excitability leads to a condition, called "primary sensitization" or "primary hyperalgesia", in which almost any stimulus is felt as pain. That is to say that the animal is in constant pain or will feel pain when simply touched or moved. Local vasodilatation, plasma extravasation and extension of the inflammatory soup results in a further amplification of the inflammatory response by reducing other nerve endings threshold to stimuli, even innocuous ones, giving place to pain even without any tissue damage. This state is called secondary peripheral hyperalgesia (Anderson and Muir, 2005).

Repetitive noxious stimulation of unmyelinated C–fibbers can result in prolonged discharge of dorsal horn cells because of increase release of glutamate, Substance P and other neurotrophic agents. Activation of peripheral nociceptors also results in a use-dependent neuronal plasticity in the spinal cord that modifies the subsequent performance of the nociceptive pathway by exaggerating or prolonging the response to

noxious inputs (hyperalgesia) or enabling normally innocuous inputs to activate it (allodynia) (Ji and Wilson, 2001). This phenomenon is termed "wind–up" or "central sensitization" and could be summarized as a progressive increase in the magnitude of C-fibre evoked responses of dorsal horn neurons produced by repetitive activation of C-fibres (Li *et al*, 1999; Hellyer *et al*, 2007). Windup occurs only if stimulation of the nerve or tissue is sufficiently intense to activate C-fibres and delivered at frequencies greater than 0.3 Hz (Hellyer *et al*, 2007). Assuming that prolonged noxious stimulus produces greater sensitivity to subsequent stimuli we can consider that this is what probably happens in cows with chronic lameness that lead to hyperalgesia, emaciation, drop in production, sub-fertility, increased susceptibility to other disease etc… It is even conceivable that the hypersensitivity status can prolong pain even when the primary hoof lesion is treated. It should also be added that hypersensitive animals respond poorly to analgesic therapy, especially when treatment is initiated after the onset of the painful stimulus (Coderre *et al*, 1990; Ley *et al*, 1995)

Wide-dynamic-range (WDR) neurons are cells on the dorsal horn of the spinal cord that respond to both noxious and non-noxious stimuli. This means that pain is no longer easily localized or limited to the injured site. In fact, WDR neurons receive information from visceral structures and this could explain the phenomenon called "referred pain" in which noxious stimuli originating in viscera are perceived as originating in a somatic region (afferents fibres converging on the same WDR neurons). Hypersensitivity may also result from a very high imbalance between excitatory stimuli and modulating pathways, with a reduction in glicin, GABA and opioid inhibitory activity (Li *et al*, 1999). Much less is known about pain-induced sensitization of the supraspinal components of the CNS.

Because of all the mechanisms already described, leading to primary, secondary and central sensitization, allodynia and hyperalgesia are considered common features of inflammatory pain (Julius and Basbaum, 2001; Fitzpatrick *et al*, 2004, Anderson and Muir, 2005).

How soon after an injury is inflicted is hypersensitivity established? This is an important question when addressing human caused trauma to animals because its justification may depend on the severity of pain and its duration. For example, acceptance of some mutilations would be different if we could be sure that pain lasted 1 minute and not 1 hour. Car and Goudes (1999) suggest that "*even brief intervals of acute pain can induce long-term neuronal remodelling and sensitisation (plasticity),*

chronic pain, and lasting psychological distress". Some preclinical studies show that neuronal expression of new genes—the basis for neuronal sensitisation and remodelling—occurs within 20 min of injury. These observations indicate that the biological and psychological foundation for long-term persistent pain is in place within hours of injury (Basbaum, 1999; Carr and Goudes, 1999). So an individual's long-term responses after transient injury (e.g. castration or surgery) may be determined by pain processes that occur within the first moments.

Summarizing, we can say that tissue damage and subsequent inflammation will increase:

- sensitivity – reducing pain threshold (and tolerance?) and activating silent and non-nociceptive fibres.

- area – pain is extended to sound tissues (secondary peripheral hypersensitivity and central hyperalgesia)

- duration – pain endures, sometimes beyond healing.

1.2.2. Pain in animals – an emotional experience.

The Interagency Research Animal Committee (IRAC) in 1985 advised that "unless the contrary is established, investigators should consider that procedures that cause pain or distress in human beings may cause pain or distress in other animals." In 1965 a report on animal welfare called the Brambell Report stated something along the lines of "*…although it is justifiable to think that there are differences in human and animal suffering, it is also justifiable to think that animals have the same ability to feel pain as humans*". Berridge (2003) defends that although differences between humans and non-human animals may be found in CNS physiology, these can not be very significant in quantity, quality or degree. What these statements really imply is that not only the pain mechanisms are similar, but so is the distress caused by pain.

The "Analogy Principle" holds that the similarity in anatomy (pain system), physiology (pain perception), and behaviour (expression of pain) between humans and higher animals makes it reasonable to assume that the sensation and effects of pain are analogous in both (Federation of Veterinarians of Europe – FVE, 2001. Report on pig castration). Although it is indisputable that there are differences in the brain structure

and function (for example, neocortex size) between different species, this seems to be irrelevant to the existence of perceptual consciousness (Baars, 2001).

Arguments in favour of the separation of humans from other animals, in relation to pain perception, claim that objective evidence of consciousness tells us little about subjective experience of conscious pain. For this reason, some scientists have suggested that animals are incapable of experiencing pain. A prominent example is psychologist Bermond (2001) who tries to distinguish between ''*the registration of pain as a stimulus, which does not induce feelings of suffering, and the experience of pain as an emotion, which does induce suffering*''. Bermond (2001) noted that for humans the unpleasant emotional experience of pain requires the involvement of the prefrontal cerebral cortex. For example, Damásio (1995) describes the case of a human patient, whose cortex was damaged in an accident, who was able to perceive pain as a stimulus although being indifferent to it. The man used to refer to its multiple injuries: "It still hurts, but I don't mind".

Given that only humans and the great apes show a well-developed prefrontal cortex, Bermond (2001) concluded that although many species may recognize and respond to pain as a stimulus, only humans and the anthropoid apes have the capacity to suffer as a result. Other species would be like the patient in Damásios' example: able to respond to pain stimuli, but exempt from any associated emotional experience.

So the big question remains – are humans different from other mammals in the way they perceive or appraise noxious stimuli?

First let's settle the definition of some concepts that will be used. We should clearly distinguish "intelligence" from "consciousness". Baars (2001) suggests that the first is limited to the capacity to solve problems and the latter to "*wakeful alertness and conscious perception, including the perception of pain and pleasure*". Pain is a complex experience dependent not only on the severity of the insult and the degree of tissue and nerve damage but also on previous experience, genotype, social position, other stresses, presence of other animals, food deprivation etc (Koolhaas *et al*, 1999). It is also related to the animal's ability to evoke responses that help it cope with the situation (e.g been able to scratch its head after hot-iron disbudding or being fed after castration…).

António Damásio (1995) said that "*pain and pleasure are the levers the organism needs for instinctive and learnt mechanisms to act efficiently*"[a] (p 266). Other authors

[a] "a dor e o prazer são as alavancas de que o organismo necessita para que as estratégias instintivas e adquiridas actuem com eficácia" (sic, António Damásio, 1995)

also claim that nociception is a component of the fundamental and integrated behavioural and physiological response toward aversive and stressful situations facilitating the coordinated expression of adaptive defensive behavioural responses (Bolles and Fanselow, 1980; Kavaliers, 1988; Rodgers and Randall, 1988; Broom 2001b). What this means is that pain perception is essential for survival. Of all animals and not just humans!

Recent advances in neuroimaging technology have reinforced the concept that the realization of pain in humans is a multi-faceted process that involves the parallel integration of sensory, emotional and perceptual noxious information by multiple brain structures (Rainville, 2002). Damásio (1995) suggests that pain should be divided in two components: the somatic awareness (discussed above) and the cortex appraisal (sometimes called motivational-affective or emotional component). Motivational-affective processing involves the ascending reticular formation for behavioural and cortical arousal. It also involves thalamic input to the forebrain and the limbic system for perceptions such as discomfort, fear, anxiety and depression. The motivational-affective neural networks also have strong inputs to the limbic system, hypothalamus and the autonomic nervous system for reflex activation of the cardiovascular, pulmonary and pituitary-adrenal systems. Responses activated by these systems feed back to the forebrain and enhance perceptions derived via motivational-affective inputs (Damásio, 1995)

But is this second component present, or even necessary, in animals? Would it not be better to feel noxious stimuli, but not be unhappy about it, as Bermond (2001) suggests? If the somatic awareness is enough to cause withdrawal behaviour why is the emotional component necessary? Probably the same reasons Damásio (1995) states for humans can be used for animals – pain puts us on guard for next time, it helps us avoid repeating the same mistakes or go through the same situations and it means we can teach others about the dangers.

It has long been established that there are no differences in the design and functioning of the nociceptor system (same transduction, transmission and modulation mechanism) (Rogan and LeDoux, 1996) nor in the inflammatory process. Also, in all mammals, the anatomy, neurochemistry and electrical activity of the brain in alert states show striking similarities (Baars, 2001). So the only differences between humans and animals could be at the second component level – the appraisal and processing of the

noxious sensations to which Bermond (2001) suggests a well developed cortex is essential.

Every practitioner knows that a cow will try to kick when given a simple injection – transduction and transmission are both there. Also it is obvious to anybody dealing with animals that an individual to whom pain has been provoked in the past is more fearful or aggressive, depending on its temperament. Mellor *et al* (2005) state that tissue damage, which may occur through trauma or disease, usually lead to what is described as "pain-induced distress" of animals. Also, several studies have shown that ruminants and other animals can remember and discriminate places and people responsible for aversive treatments (Fell and Shutt, 1989; Munksgaard *et al*, 1997; Pajor *et al*, 2003). Even milk yield is affected by the cows' ability to remember aversive treatment carried out by people that are in the milking parlour (Rushen *et al*, 1999). These studies indicate that at least the memory of pain is long lasting and that behavioural changes in response to pain are very similar to humans.

Another way of assessing if an animal does "care" about pain is to study what it will do to avoid or minimise the origin of the stimuli (Rushen, 2005). Some experiments show that animals are prepared to sacrifice something in order to alleviate moderate to severe pain. One of the most convincing proofs that animals do suffer from pain is the experiment in which severely lame chickens choose a less palatable feed because it includes an analgesic (Danbury el al, 2000). Similarly, Colpaert *et al* (2001) showed that rats would self medicate with pain killers to relieve pain in arthritic joints even if this meant drinking less palatable water.

By the same way of thinking it would be hard to explain why animals have modulating mechanisms to reduce the possibility of being overridden by extreme pain (e.g. endogenous analgesics), unless pain could lead to severe and prolonged deleterious effects. Why would these be necessary if pain had no unfavourable mental effect?

Severe pain in cattle produces behavioural, autonomic, neuroendocrine, and immunologic responses that can result in self-mutilation, immune incompetence and a poor quality of life potentially leading to gradual deterioration and death (Anderson and Muir, 2005). Cattle exposed to chronic pain will show evident signs of suffering that can be demonstrated by isolation seeking, indifference to other and the environment, apathy, increased fearfulness, aggressive behaviour towards herdmates, signs of frustration or anxiety etc… So, if all the signs of an equal capacity to feel pain and to set up an emotional response are present in humans as well as in other animals, it seems entirely

plausible that even species lacking a well-developed prefrontal cortex may still experience harmful stimuli as unpleasant, probably through other brain structures (Weary *et al*, 2006).

Therefore, although the idea that the lack of animal consciousness is still deeply embedded in human thinking, it seems that the burden of proof for the absence of subjectivity/emotions in mammals should be placed on the sceptics (Baars, 2001; Broom and Fraser, 2007).

The (old?) idea that humans and animals perceive pain in a different way (more physical and less mental) is twofold:

- animals have learnt to respond to pain in different ways. For example, it is not that being stabbed by a sword does not hurt a bull, but rather that the animal will continue to charge against the bullfighter because that is the way he deals with menace and pain.

- humans have difficulty in assessing pain in animals. A more insensitive on-looker would say that the bull was enjoying the fight.

In conclusion, the following similarities with humans suggest that many other animals have similar subjective experiences to humans (adapted from Bateson, 1991).

1) Possession of receptors sensitive to noxious stimuli, located in functionally useful positions on or in the body.

2) Possession of brain structures analogous to the human cerebral cortex.

3) Possession of nervous pathways connecting nociceptive receptors to higher brain structures.

4) Possession of receptors for opioid substances found in the central nervous system, especially the brain.

5) Analgesics modify response to noxious stimuli and are even chosen by the animal when the experience is unavoidable.

6) Responds to noxious stimuli by avoiding them or minimizing damage to the body.

8) Response to noxious stimuli persists and the animal learns how to associate neutral events with noxious stimuli.

1.3. Assessing pain

We have seen that humans and animals have common anatomical and physiological features but that has given rise to an important question: Why is animal pain so often ignored?

A first answer to this question would be that our ability to assess pain in farm animals is still very limited. However, the fact that pain is not fully recognized does not mean that it does not exist. This is particularly true for ruminants in which concealment of vulnerability and weakness appears to be adaptive (Broom, 2001a; Dobromylskyj, 2005; Weary *et al*, 2006). Therefore, the signs of pain in these species are without a doubt, very subtle. And, if identifying and grading acute pain presents such difficulty, trying to evaluate the degree of long term pain is much harder, although perhaps more important for welfare (Stafford, 2007).

Linzey (2006) suggests four common appraisal shortcomings to explain why humans have some difficulty in recognizing pain in animals:

1. Misdescription – not being able to correctly describe signs of pain in animals or using words that apply to humans but not necessarily to animals.
2. Misrepresentation – humans relate certain behavioural features (e.g crying, bellowing, complaining etc…) to the ability to feel pain. The absence of evidence of these features would imply that feelings and emotions are also missing. The famous statement "I think, therefore I exist" is a common misrepresentation.
3. Misdirection – physical pain is admitted but not emotional suffering. If anthropomorphic reasoning is excluded from pain assessment then suffering is not evident.
4. Misperception – animals are seen as instruments with no intrinsic value. Even if animals feel pain that is not morally relevant.

When assessing pain, care should be taken to analyse potential conflict of interests because studies have shown that animals will endure pain if a superior interest is at stake. For example (Cabanac and Johnston, 1983) showed that rats would tolerate some stress in exchange for food reward and lame chickens showed fewer signs of pain when

put in a new cage or together with a strange animal or when being fed (Gentle, 2001). Likewise, cattle will probably reduce the signs of pain in the case of fear, which may occur in the presence of humans, other animals or aversive surroundings (Munksgaard *et al*, 1997; Pajor *et al*, 2003) or in the case of the anticipation of a benefit (Gentle, 2001).

Another problem in assessing pain is to scale the different changes found. By daily monitoring, practitioners and farmers can usually estimate pain duration but have difficulty in estimating pain intensity (Meyer, 2004). For example, is a high cortisol level more important in pain evaluation than an increased heart rate? Or is a calf's apathetic attitude less significant than bellowing or rubbing its head after disbudding? Is freezing a sign of less pain than struggling? These are difficult questions that need further investigation so that anthropomorphic assumption does not confuse the issue too much (Stilwell, 2005). Because the matter is so unclear, Molony and Kent (1997) suggest giving animals the benefit of doubt by overestimating the intensity of pain, so as to avoid missing out animals in pain, even at the cost of treating some that are not.

Pain is essentially a subjective experience and so it is very difficult to directly assess it, even in humans. For example, quantitative assessment of pain done by subjective scaling methods is often open to substantial disagreement among observers (Broom and Johnson, 2000). This means that, although using only indirect evidences may give indications of the mental state or suffering of a particular animal, the use of objective measures should be sought wherever possible (Grandin and Deesing, 1998). There is also a widespread agreement that if a measure of animal welfare is to be valid it should, as far as possible, make use of different methods (Sandøe *et al*, 2003). By norm, several different indices (physiological and behavioural) ought to be used simultaneously to assess pain and their evaluation is greatly improved by training and experience. These measurable evidences are of three sorts: productivity, behavioural and physiological. We will present behaviour and cortisol in more detail because these were the ones used in our studies.

1.3.1. Behaviour

Behaviour changes are useful tools for the recognition and evaluation of pain and stress in animals (Bateson, 1991; Broom and Johnson, 2000; Mellor *et al*, 2005; Rushen, 2005). We suggest that behavioural assessment is probably the best and more reliable way to recognize pain in cattle, providing that the observer has a good knowledge of

natural behaviour. Molony and Kent (1997) suggest grouping behaviour changes accordingly to their final purpose:

1) those, immediate and automatic, that protect parts or the whole animal (e.g. withdrawal reflexes);

2) those that minimize pain and assist healing (e.g. gait changes, lying or standing still);

3) those that enable the animal to avoid recurrence of the experience, which result from learning;

4) those that are designed to elicit help or to stop another animal (including humans) from inflicting more pain (e.g. communication by vocalisation, posture, menace behaviour etc…).

Although ruminants' signs of pain are not very easy-to-read, there is some general behaviour that is usually considered significant. For example: total or partial anorexia, dullness, depression, gait changes, increased respiratory rate, open mouth breathing, grunting, leaning or nose pressing, teeth grinding, reduced grooming behaviour, stretching hind limbs, aggressive behaviour or freezing (Roberts, 1997; Van Reenen *et al*, 2005; Dobromylskyj, 2005). Kicking the abdomen, rolling and posture changes are signs of abdominal pain in cattle, although never as extreme as in horses. Careful examination and a good knowledge of natural behaviour are needed because most of these signs are subtle and not very specific.

Some behaviours used to describe animal pain are well known and relatively easy to interpret. However, some changes are less evident and can even be puzzling. For example, Eicher *et al* (2000) found that heifers to which a tail rubber ring was applied ate more during the week until the tail end fell. Similarly, Mellor and Murray (1989) found that lambs increased activity and sometimes eating after tail-docking and castration. Could this be an secondary effect of endogenous opioids released?

In contrast, reduced activity is understandable after painful procedures – resting may help recovery and reduce pain recurrence due to movement. Inactivity is described as an abnormal behaviour by Wiepkema *et al* (1983) and Broom and Fraser (2007). Morton and Griffiths (1985) refer general lethargy as a sign of pain in experimental animals. This behaviour has been shown in lambs after castration and described "as the time during which it was difficult to elicit any evidence of conscious awareness" (Molony *et al*, 1993; Molony and Kent, 1997) and is also seen in cattle and wild

ruminants when young animals are left alone by their dams. All these data suggest that inert lying is closely related to high levels of distress.

Some authors consider vocalisation as being "*the more reliable and least invasive methods of assessing acute distress in cattle*" (Watts and Stookey, 2000) although they state that "*vocalisation data should be interpreted as statistical properties rather than indicating the condition of any individual animal*". However, other authors did not find this behaviour to be significant when painful procedures (branding) were used in adult cattle (Lay, 1992a). The Stunning and Killing report published by Animal Health and Welfare Council (DEFRA, UK) state that "*absence of vocalisation does not guarantee absence of pain or suffering*". However, although vocalisation cannot be considered an important sign of pain in cattle, the intensity and type of call may be helpful in recognizing different states of mood or emotion, including pain (White *et al*, 1995; Manteuffel *et al*, 2004).

Certain disturbed behaviours (e.g. vocalisation, changes in posture, changes in locomotor activity, head shaking, stamping, kicking, licking, scratching or rubbing, transitions…) have been used as indicators of pain-related distress after disbudding and castration in sheep and cattle (Robertson *et al*, 1994; Morisse *et al*, 1995; Molony and Kent, 1997; Graf and Senn, 1999; Grøndahl -Nielsen *et al*, 1999; Ting *et al,* 2003a; Mellor *et al*, 2005; Vickers *et al*, 2005; Stafford, 2007). Doherty *et al* (2007) were the only ones to assess "inactivity behaviour" immediately after hot-iron disbudding. Some studies have looked at behaviour that may indicate the intensity of pain during a procedure, like, for example, hot-iron disbudding: backing, raising front legs, vocalisation and going down on the hind-legs during the procedure (Grøndahl-Nielsen *et al*, 1999; Doherty *et al*, 2007).

There are essentially two methods for assessing behaviour after a painful procedure: the method that subjectively scores pain (e.g. Visual Analogue Scoring) and recording the frequency of certain behaviours. Subjective measures are perhaps less reliable although recording the incidence of pain-related behaviour has been shown to undervalue some situations. For example, one study tried to assess the value of specific behaviours, but found that no one behaviour or combination of behaviours was better than the overall gait score in identifying cows with sole ulcers (Flower and Weary, 2006). By comparing these two evaluation methods, Stilwell (2007b) found that both gave very similar results when pain was severe after paste-disbudding but the objective behaviour recording method allowed for a more accurately identification of animals still

in pain after a few hours. Ultimately the value of the two approaches will depend upon the type of pain experienced, the quality of the measures, and in the case of the subjective methods, the experience of the observer. (Weary *et al*, 2006).

Measuring just one type of behaviour can lead to incorrect conclusions (Broom and Johnson, 2000) because animals react differently to stress and pain. These differences are linked to age, sex, breed, previous experiences, temperament, body condition, nutritional status, other concurrent diseases or disorders and type/duration of stressors (Van Borell, 1995). For example, some cows will panic and flee when an injection is given while others will freeze. Another example is when difficult calving is taking place – most cows will lie down quietly while pushing but a few will vocalize loudly. Also physical or other type of constraints may confuse interpretation e.g. joint injuries may prevent normal movement of the joint leading to stiffness in gait that may not be associated with pain. By using an ethogram that records several behaviours it will be less likely that pain will be underestimated.

Some drugs will cause changes in behaviour and this may help in pain assessment but it may also confuse the issue. For example, if one behaviour is no longer performed by an animal after receiving an analgesic it is natural to assume that that behaviour was a response to pain. But some drugs may change or hide behaviour leading to erroneous conclusions, not because they suppress pain but because they suppress the response to pain (this is particularly true for sedatives that have no effect on nociception).

1.3.2. Cortisol

The cells of the *zona fasciculate* of the adrenal cortex secrete glucocorticoids that are primarily involved in carbohydrate metabolism, inflammation control and sodium balance. Cortisol is the most important glucocorticoid hormone secreted by mammals and is considered as one of the few hormones essential for life (Ruckebusch *et al*, 1991). Without cortisol, animals are much less able to cope with extreme adversity (Shulkin, 1999). Mortality is higher in those animals that are not able to increase cortisol concentrations (Broom and Kirkden, 2004; Muir, 2008).

The release of cortisol is controlled by the Corticotrophic Releasing Hormone (CRH) which is released from the hypothalamus into the portal veins of the pituitary gland. CRH and vasopressin (VP) stimulate the secretion of Adrenocorticotrophic Hormone (ACTH) from the frontal lobe of the pituitary into the bloodstream (Ruckebusch *et al*, 1991; Terlouw *et al*, 1997). In cattle and pigs, CRH is the more

potent, whereas VP is the more potent in sheep (Minton, 1994). The ACTH stimulates receptors in the adrenal cortex to release glucocorticoids. The system is known as the Hypothalamic-Pituitary-Adrenal axis and will be identified in this thesis from now on as HPA axis.

The production of CRH is controlled by neuroendocrine mechanisms (e.g. cytokines and nervous signals to the hypothalamus, namely stress, lactation, anxiety, hunger etc…). By a negative-feedback, control levels of cortisol in plasma reduce the release of CRH by the hypothalamus and ACTH by the adenohypophysis. Additional feed-back includes inhibitory effect of ACTH, β-endorphins and CRH on hypothalamic receptors (Johnson *et al*, 1992). The physiologic cortisol release is pulsatile and follows a circadian cycle that is affected by sleep and activity patterns – levels in animals and humans usually decrease at evening and night and increase after a period of rest (highest in the morning) (Ruckebusch *et al*, 1991; Greco and Stabenfeldt, 2002).

In blood about 70% of cortisol is bound to a special protein called corticosteroid-binding globulin (CBG), about 20% is bound to albumin and only 10% is free (Ruckebusch *et al*, 1991). This binding to CBG is reversible and produces an important reservoir of potential free cortisol. Cortisol appears in saliva and milk and is an indicator of blood levels of cortisol. Cortisol that is filtered in the kidney is almost all reabsorbed so that only about 15% is lost (the same occurs in the gut).

One of the most important functions of glucocorticoids is the stimulation of gluconeogenesis in the liver, which involves the conversion of aminoacids to carbohydrates. On the other hand, protein synthesis is inhibited by glucocorticoids. Only cardiac and brain tissues are spared from the effect of protein catabolism (Greco and Stabenfeldt, 2002).

Another important aspect of glucocorticoids activity is the inhibition of inflammation, including the prevention of capillary dilatation, extravasion of fluid into tissue spaces, leukocyte migration and connective tissue synthesis (Sapolsky *et al*, 2000; Greco and Stabenfeldt, 2002). Inflammation is reduced after cortisol release through the inhibition of mediators such as prostaglandins, tromboxanes and leukotrienes. Although it also has some inhibitory effect on Cyclo-oxygenase 2 (COX-2) production (Coyne *et al*, 1992), the way cortisol controls inflammation mediators' release is different from the non-steroid-anti-inflammatory drugs – it primarily prevents the activation of phospholipase A_2 and thus reduce arachidonic acid metabolism. As will be explained further on, cortisol may be essential in the restraining of the (too) powerful reactions

that follow an aggression (Sapolsky *et al*, 2000). This effect might be important in the control of inflammation after painful procedures such as surgery.

Finally, another effect of cortisol, especially if frequently or continuously released, is immunodepression (Munck *et al*, 1984; Minton, 1994; Sapolsky *et al*, 2000). This is an important feature when looking at animal welfare, as will be discussed further on. Among other consequences, glucocorticoids decreases T cell proliferation and natural killer cell cytotoxicity (Jain *et al*, 1991), inhibits antigen presentation and expression of major histocompatibility complex (MHC) class II proteins, reduces activation and proliferation of T and B cells (memory cells being much less sensitive than naïve cells), shifts responses from Th_1 cells to Th_2 cells and impairs the production of Interleukin 2 by T-lymphocytes, reducing the organism capability to fight bacteria, viruses, fungi and parasites (Ruckebusch *et al*, 1991; Sapolsky *et al*, 2000). Nevertheless it should be said that there is evidence that physiologic amounts of cortisol are necessary for the development and maintenance of normal immunity (Jefferies, 1991) and reproduction (Van Borell *et al*, 2007).

Various hormones (e.g., ACTH, glucocorticoids, catecholamines, prolactin, etc) are involved in the stress response of animals (for review see Matteri *et al*, 2000). As we have seen it is well established that glucocorticoid production, following the activation of the HPA axis, is a fundamental part of an emergency response (Broom and Zanella 2004) intended to defend the organism against stressful conditions (Möstl and Palme, 2002)**.** These are the reasons why cortisol is sometimes termed 'the stress hormone'. In 1984, Munck *et al*, (1984) stated that "*Almost any kind of threat to homeostasis or stress will cause plasma glucocorticoid levels to rise*." and went on to suggest that "*stress-induced increases in glucocorticoid levels protect not against the source of stress itself but rather against the body's normal reactions to stress, preventing those reactions from overshooting and themselves threatening homeostasis*". So, cortisol is expected to control and detoxify mediators released during stress-induced activation of primary defence mechanisms because these mediators would themselves lead to tissue damage if left unchecked. This is termed the "regulatory activity" of glucocorticoids. Another equally important role of glucocorticoids is the "preparative activity" by which the organism gets prepared for a subsequent stressor (Sapolsky *et al*, 2000).

Cortisol is widely used to quantify response magnitude and duration to acutely painful procedures and these seem to correspond to the predicted noxiousness of the experience (Chase *et al*, 1995; Fisher *et al*, 1996; Fisher *et al*, 2001; Earley and Crowe,

2002; Ting *et al*, 2003ab; Mellor *et al*, 2005; Stafford and Mellor., 2005ab). That is to say that lower levels of stress correspond to less cortisol production and vice-versa.

Although the concentration of cortisol in blood/plasma is widely used as an indicator of stress, caution is advised because an increase does not occur with every type of stressor (Broom and Johnson, 2000) and because a wide variety of stressors can activate the HPA axis (Molony and Kent 1997; Broom and Johnson, 2000). Examples of these in farm animals are: weaning (Hickey *et al*, 2003), social isolation (review by Cockram, 2004), transport (Crookshank *et al* 1979; Grigor *et al* 2004), social mixing (Arthington *et al*, 2003), novelty (Van Reenen *et al* 2005); restraint and handling (Ewbank *et al*, 1992) and multiple venipuncture (Hopster *et al*, 1999). See also review by Lane, 2006 of possible stressors in cattle. Pain after injury is a known activator of the HPA axis and so measuring plasma cortisol has been extensively used to evaluate the presence and severity of painful conditions (Stott, 1981; Moberg, 2005). However, the possibility that tissue damage, even in the absence of stress, may cause similar increases is the main disadvantage of cortisol assessment to indicate the presence and intensity of pain after surgery (Mellor *et al*, 2005). So, to validate blood cortisol as a sole indicator of stress or pain caused by a particular procedure, all redundant effects should be eliminated (Cook *et al*, 2005).

Measurement of stress hormones such as cortisol present some limitations, such as their already referred lack of specificity to pain and because a ‘‘ceiling-effect’’ is possible (Wood *et al*, 1991; Molony, 1991; Molony and Kent, 1997; Mellor *et al*, 2005). There are studies indicating that cortisol levels are correlated to pain severity. For example Shutt *et al*, (1988) showed that exposing lambs to three procedures simultaneously (muesling, castration and tail docking) caused a larger increase in cortisol than just doing one procedure. However, ceiling-effect is often reached, making them unsuitable for comparison of psychological or physical challenges in the higher end of the aversion spectrum (Harbuz and Lightman, 1992). In contrast, it is important to mention that in case of repeated noxious stimuli the level and time to full response may change (Gamallo *et al*, 1983). This means that a decline in time to cortisol response may not correspond to pain absence or reduction.

Age, management and the conditions in which animals are kept may influence the cortisol response to a stressor. Cows moved to large groups (Friend *et al*, 1977) and calves kept in isolation (Dantzer and Mormede, 1983) showed higher cortisol responses than animals more adequately managed. Young animals may also have a reduced ability

to produce cortisol and this has been suggested to be a contributing factor to the high levels of morbidity and mortality that occur in calves (Broom and Kirkden, 2004)

A more difficult challenge is presented by chronic stress, such as encountered in depression or unrelenting pain, because the sensitivity to novel incoming stressors should be maintained for survival. In some long lasting stressful situations, cortisol is not a reliable measure (Broom and Johnson, 2000) because the end result manifests as either hypofunction or hyperfunction of the HPA axis with loss of appropriate negative feedback (Chrousos, 2000).

Changes in concentrations of circulating glucocorticoids in cattle, reflecting the activation of the HPA axis, are commonly detected after routine farm procedures like disbudding, tail-docking and castration (Morisse *et al*, 1995; Mollony *et al*, 1995; McMeekan *et al*, 1998; Grøndahl-Nielsen *et al*, 1999; Faulkner and Weary, 2000; Sutherland *et al*, 2002ab; Schreiner and Ruegg, 2002; Stafford *et al*, 2002; Doherty *et al*, 2007). Increases in cortisol concentrations that are evident in animals submitted to a procedure but not in animals given a nerve block or some kind of analgesia, are considered to be indicative of pain (Morton and Griffiths, 1985; Mellor *et al*, 2005).

In contrast, cortisol may show increased values in some situations in which pain is controlled by anaesthetic drugs. Muir (2008) states that ACTH, cortisol and epinephrine levels are increased during emergence from anaesthesia without surgery, suggesting that anaesthesia alone can induce a stress response in animals.

Usually cortisol is measured in blood, plasma or serum. Although most cattle are tame, human proximity and blood sampling can result in stress. This means that the time interval between restraint and blood sampling usually has an effect on plasma cortisol (Lay *et al*, 1992b). This drawback can be minimised by the use of a control group not subjected to the procedure being studied. Non-invasive measurements of cortisol (milk, saliva, urine and faeces) are possible but are not very reliable in cattle (Broom and Johnson, 2000) and do not substitute plasma cortisol in acute situations. For the main disadvantages see Lane (2006).

One of the most used cortisol measuring method is solid-phased radioimmunoassay (RIA). There are commercial kits validated for cattle (e.g. Coat-A-Count Cortisol In-vitro Diagnostic Test Kit, Diagnostic Products Corporation 5700 W. 96th St., Los Angeles CA USA).

1.3.3. Other measures.

The release of catecholamines depends on the Automatic Nervous System stimulation. Sympathetic and Parasympathetic systems have opposing effects and cause the release of adrenaline/noradrenaline and acetylcholine, respectively. Sympathetic catecholamines are released very easily and quickly in response to stress (1-2s) and their half-life is very short (aprox. 70s) (McCarty, 1983). There are several ways of measuring the activity of the sympathetic nervous system, including changes in heart rate, pupillary diameter, skin resistance, and peripheral blood flow. Activation of the Sympathetic System stimulates cardiac rate and output in farm animals subject to painful procedures and these have been used in studies as a sign of stress (Lay *et al*, 1992b; Grondal-Nielsen *et al*, 1999), although bradycardia is seen in some situations (Broom and Johnson, 2000). Catecholamines have been used to assess pain after painful procedures in calves and lambs (Mellor *et al*, 2002). Recently Steward *et al* (2008) have looked at calves heart rate and eye temperature after disbudding as an indirect measure of Sympathetic activation.

Other substances whose concentrations may change in cattle after painful experiences are: ACTH (Graf and Senn, 1999), CRH as a stimulator of ACTH/β-endorphin secretion (Johnson *et al*, 1992), endogenous opioids, vasopressin (Graf and Senn, 1999); acute phase proteins (Fisher *et al*, 1996; Earley and Crowe, 2002; Ting *et al*, 2003a); substance P (Cotzee *et al*, 2008), oxytocin, prolactin (Parrot, 1990), glucose, LH, TSH, hepatic enzymes (Broom and Johnson, 2000) etc…

The rectal temperature may increase after stressful events such as transport (Trunkfield and Broom, 1990). For example, psychological stressors, such as placement of rats in open-field settings or conditioned aversion stress, was found to trigger cytokine release and its associated fever response before there was a rise in glucocorticoid concentration (Zhou *et al*, 1993). However, because automatic nervous system may cause peripheric vaso-constriction, this parameter has to be analysed with caution.

Changes in blood white cells (WBC) and especially the ratio between leukocytes (Stress Leukogram) have been used in cattle to measure pain related distress (Chase *et al*, 1995; Ting *et al*, 2003a; Ting *et al*, 2004; Doherty *et al*, 2007). Stress leukogram in cattle will show, in some stressful situations, an increase in neutrophil to lymphocyte ratio as was found by Doherty *et al* (2007) in disbudded calves.

Changes in antibody production during periods of stress have been studied (Zanella *et al*, 1991b) and, although it does not seem to be a very reliable measure, it has been established that animals do produce less antibodies when vaccinated under intense stress.

Some authors have been using more recent technology in assessing stress and pain in humans (Winterhalter *et al*, 2008). Some of these techniques are also being tested for animal pain assessment. For example, Anderson and Muir (2005) have been trying to quantify clinical pain in animals submitted to surgery by measuring skin impedance. Gibson *et al* (2007) used electroencephalogram (EEG) to assess noxious sensory inputs in scoop dehorned heifers.

Finally some words on the use of performance (daily weight gain, milk yield etc…) for assessing chronic pain. Although good performances by production animals do not necessarily correspond to good welfare, the contrary in frequently true. Low milk yield, loss of body condition, reduced daily weight gain, reduced fertility, etc. have all been shown to occur in animals in pain (Fisher *et al*, 1996; Dobson and Smith, 2000; Ting *et al*, 2003a; Bretschneider, 2005; Rust *et al*, 2007; Van Borell, 2007). The use of performance measures has to be done with care and using control groups because many other factors may confound the results. When analysing farm or herd performance data, extra care has to be taken because of the danger of missing individuals whose welfare is greatly threatened.

1.4. Controlling pain in cattle.

Feeling pain is considered essential for survival and has endured natural selection exactly because of that. With a properly working pain system animals are better able to cope with the environment because (adapted from Bateson, 1991):

1 – It allows them to distinguish, at peripheral level, potentially harmful stimuli from harmless sensations essential to daily activities.

2 – They learn to correlate certain circumstances to harm and, as a result, to act preventively. Pain stimulates "fight or flight" behaviour even if this means inhibiting competing activities.

3 – They avoid activities that might delay recovery, without inhibiting crucial behaviours, like eating, hiding or fighting.

However, although pain has some beneficial facets this does not mean it is unavoidable or even acceptable. For example, there is no real advantage for a cow to endure the pain of dehorning because: she will not be able to escape; most certainly she does not need to learn that it is a painful procedure in order to avoid it next time; and it has no advantage in precluding other activities. Similarly a calf with a broken limb will not walk around on that leg just because some pain-killer is given. If pain is so essential to safeguard an injured tissue or organ we should ask: why does analgesia facilitate recovery from surgery?

In contrast, the benefits, which ensue from the prevention of suffering in farm animals, supersede any advantage that might arise from them feeling pain. This is especially true for pathologic pain that leads, as we have seen, to anorexia, chronic stress, immunodepression, frustration and hopelessness.

There are a few problems that are specific to cattle pain management. Practitioners have identified some of them in surveys published (Watts and Clarke 2000; Whay and Huxley, 2005; Huxley and Whay, 2006; Hewson *et al*, 2007; Huxley *et al*, 2008). The flaws more commonly acknowledged are: reduced cost-effectiveness, low practicability (few long acting drugs available), long withdrawal periods and lack of legal license (e.g. opioids). These are the reasons pointed out by practitioners, who acknowledge severe pain in some pathologic conditions but admit seldom using analgesics to deal with it. For example, non-steroid-anti-inflammatory-drugs (NSAID) were given to only 50% of cows that underwent caesarean section, 55% of claw amputations, and 1% of cases of dehorning (Whay and Huxley, 2005) and only 68% of respondents in another survey gave postoperative analgesic drugs to cows that underwent caesarean section (Watts and Clarke, 2000). In another survey, of the 605 respondents 1.7% and 4.6% said they used NSAID after disbudding and castration respectively. Also significant was the number of practitioners that used xylazine (17%), lidocaine (74%) or no drug (25%) for these procedures (Huxley and Whay, 2006). A large US survey reported that dairy owners use anaesthetic and analgesia for dehorning in 12.4% and 1.8% respectively (Fulwider *et al*, 2008). Mish *et al* (2008) found that seventy-eight percent of dairy producers dehorn their own calves but only 22% use local anaesthetics and it was also shown that producers who used local anaesthetics were 6.5 times more likely to have veterinary involvement in their dehorning decisions. An appraisal in Ontario showed that, although most veterinarians found sole ulcers to be painful or very painful, very few used any analgesics in cows affected by this claw disorder (Anderson, 2005). The use of

analgesia after castration also differs with age and specie: 0.001% of piglets received analgesia, compared with 6.9% of beef calves and 18.7% of dairy calves under 6 months of age, 19.9% of beef calves and 33.2% of dairy calves over 6 months of age, and 95.8% of horses (Hewson *et al*, 2007).

We suggest that two of the most important reasons for not using adequate analgesia in cattle are: 1) humans are not able to identify pain or its signs are suppressed/hidden by cattle; 2) the economic consequences of not using analgesia are negligible.

Effective cattle pain management should combine the reduction of primary and acute pain with the prevention of secondary (central or peripheral) hypersensitivity (Nolan, 2000). Practitioners and farmers are usually more concerned with the first pain but will often neglect the control of pathologic or chronic pain. This happens because it is less obvious, its' control is more expensive and it does not pose safety problems for the operator.

Acute pain in cattle practice is usually addressed by local or regional anaesthesia, sometimes combined with a sedative (Edmondson, 2008). Sedatives, such as xylazine, are used more often in cattle for safety reasons rather than for their analgesic properties. The use of long acting analgesics is usually not used at all or limited to one injection after a particularly painful procedure.

Studies in humans and small animals have shown that a combination of drugs acting at different points in the nociceptive system provide a greater effect than individual drugs on their own (Woolf and Chong, 1993; Nolan, 2000). This means that the combination of a local anaesthetic, with an adrenergic agonist and NSAID is a sound approach. In humans the association with opioids is common but not as much in cattle due to legal restrains.

The idea of anticipatory control of pain was first introduced in experiments with animals under general anaesthesia by showing the benefits of central sensitization prevention by infiltrating an area with local anaesthetics (Coderre *et al*, 1990). These results led to the concept of 'preemptive analgesia' that can be described as: initiating an analgesic regimen before the onset of the noxious stimulus to prevent central sensitization and limit the subsequent pain experience (Woolf and Chong, 1993). Unfortunately the drugs most frequently recommended for pre-emptive are opioids, which are not licensed for cattle, and non-steroid-antiinflammatory drugs might have dangerous side effects if used in dehydrated or severely hypotensive patients.

To summarize, the drugs most often used in cattle pain management are, by order of importance, local anaesthetics, 2α adrenergic agonists (e.g. xylazine) and several NSAIDs. Opioids, ketamine and other general anaesthetics are very seldom used in field conditions.

1.4.1. Local and regional anaesthesia

Most of the surgical procedures can be performed safely and efficiently in dairy cattle using a combination of physical restraint, mild sedation and regional or local anaesthesia.

Local anaesthetics block the initiation and propagation of action potentials by preventing the voltage-dependent increase in Na+ conductance. Their main action is to block sodium channels by physically plugging the transmembrane pore (Rang *et al*, 2003b). Local anaesthetics are usually injected in an acid solution as the hydrochloride salt (pH 5). Following injection, the pH increases as a result of buffering in the tissues and a proportion of the drug dissociates to release free base. As it is lipid soluble the free base is able to pass through the cell membrane to the interior of the axon where reionization takes place. The reionized portium is used to plug the sodium channels (Wildsmith, 1996). In case of inflammation and C fibres hypersensitivity there is an accumulation of neurotransmitters and increased expression of the sodium channels that reduces significantly the efficacy of local anaesthetics. The efficacy of these drugs is also reduced in low pH environment and in the presence of pus, necrotic material and tissue debris.

Two percent lidocaine hydrochloride and 2% mepivacaine hydrochloride have become the most commonly used local anaesthetic agents in cattle because of low cost and limited toxicity (Edmonson, 2008). Lidocaine is three times more potent than procaine, diffuses more widely in the tissues and its effects last 90 to 180 minutes (Muir *et al*, 1995; Edwards, 2001; Smith *et al*, 2002; Anderson and Muir, 2005; Edmonson, 2008). Most of the studies on cattle castration and disbudding have been made with 2% lidocaine, with the exception McMeekan *et al* (1998) in which bupivocaine was used for scoop dehorning and Doherty *et al* (2007) in which 5% lidocaine was used for hot-iron disbudding.

Regional anaesthesia (RA) involves the injection of a local analgesic solution into connective tissue around a sensory nerve trunk, sometimes quite far from the origin

of the noxious stimulus. RA techniques are usually simple, not expensive and provide a reversible loss of sensation to a relatively well-defined area of the body. It is sometimes preferred to local infiltration because it is more efficient, requires less volume and does not cause distortion of tissue that can result in local pain. For example, Graf and Senn (1999) noted that the injection puncture and pressure of liquid at the site resulted in indications of transient acute pain in calves before dehorning. It does, however, require exact knowledge of the anatomy of the nerve(s) in question, including the structures they innervate, their location and their relationship to other structures (Edwards, 2001).

Three RA techniques will be presented in detail: cornual nerve blocking, caudal epidural anaesthesia and scrotum anaesthesia.

1) Cornual nerve anaesthesia

The horn and the skin around its base are innervated in cattle by the cornual branch of the lachrymal (zygomaticotemporal) nerve which is part of the ophthalmic division of the trigeminal nerve (Edmonson, 2008). The corneal nerve leaves the lachrymal nerve within the orbit and passes through the temporal fossa (easily palpated with the fingers). After emerging from the orbit it ascends just behind the lateral ridge of the frontal bone and terminates at the base of the horn where it divides into a number of branches. On the upper third of the lateral edge of the frontal bone it lies relatively superficially covered by skin and only a thin layer of temporalis muscle. A 2-5 cm, 19-20 gauge needle is inserted ventromedially close to the frontal bone approximately half way between the base of the horn and the eye lateral canthus. With small calves the needle only has to perforate the skin before the anaesthetic can be injected. If the needle goes too deep the injection will be made beneath the aponevrosis of the temporalis muscle. The cornual artery and vein lie in close proximity to the nerve and therefore aspiration ensures that the needle tip has not been placed intravascularly inadvertently. Five mL of 2% lignocaine on each side is usually enough to block the nerve in calves. Larger cattle with well-developed horns may require additional anaesthetic infiltration along the caudal aspect of the horn, in the form of a partial ring block, to desensitize subcutaneous branches of the second cervical nerve (Noordsy and Ames, 2006).

Cornual nerve block has shown beneficial effects with amputation and hot-iron disbudding (Petrie *et al*, 1996; Grøndahl-Nielsen *et al*, 1999; Graf and Senn, 1999; Sutherland *et al*, 2002a; Sylvester *et al*, 2004; Doherty *et al*, 2007). In one study, trials with caustic paste disbudding suggested that cornual nerve anaesthesia does not reduce

pain significantly (Vickers *et al*, 2005). This led to the suggestion that nerve block before caustic paste disbudding is unnecessary (Duffield, 2007).

2) Epidural anaesthesia

Caudal epidural anaesthesia is an easy and inexpensive method of analgesia that is commonly used in cattle. Caudal epidural block is performed by insertion of the needle between the last sacral (S5) and first coccygeal (Co1) vertebrae or between the first two coccygeal vertebraes. The volume injected varies with the weight and objective of the procedure (usually 0.5 mL per 45 kg of body weight) (Edmonson, 2008).

A high caudal epidural at the sacrococcygeal space (S5–Co1) desensitizes sacral nerves S2, S3, S4, and S5. The low caudal epidural at first coccygeal space (Co1–Co2) desensitizes sacral nerves S3, S4, and S5 (Noordsy and Ames, 2006; Edmonson, 2008). For castration the area desensitized by epidural injection may not be enough because the spermatic cord includes some nerves that leave the spinal cord at a higher level (S1). The onset of muscular paralysis of the tail occurs after 60 to 90 s after the injection. When 2% lidocaine is used analgesia attains its maximum extent in 5-10 min and persists for about one hour, after which there is progressive recovery (Hall *et al*, 2001).

3) Scrotum anaesthesia

Infiltration of the skin along the incision lines desensitizes the scrotum but it does not block the nerves running in the spermatic cord. These fibres can be blocked by direct injection into each cord at the neck of the scrotum or by injecting 5 to 10 mL of local anaesthetic into the parenchyma of each testicle (the drug is drained by lymph vessels and diffuses along the spermatic cord). Alternatively the skin at the neck of the scrotum may be ring infiltrated along with a deep injection into the spermatic cord (Hall *et al*, 2001).

1.4.2. Non-steroid-anti-inflammatory-drugs-

Non steroidal anti-inflammatory drugs (NSAID) include a variety of different agents of different chemical classes. Most of these drugs have three major types of effect (Rang *et al*, 2003a) of which the first two are essential for pain management:

1- anti-inflammatory effect – modification of the inflammatory reaction

2- analgesic effect – reduction of some types of pain.

3- antipyretic effect.

All of these effects are related to the primary action of these drugs: cyclo-oxygenase enzymes inhibition, reducing inflammation by decreasing the production of

prostaglandins, tromboxane A2 and other inflammation mediators (Lees *et al*, 2004; Nolan, 2000). The last decade has seen a tremendous increase in the study of the ciclo-oxygenase class of enzymes. Two of them (COX-1 and COX-2) are now well known. COX-1 is a constitutive enzyme expressed in most tissues and involved in tissue homeostasis and COX-2 is induced in inflammatory cells when they are activated, being responsible for inflammation activation and mediation (Vane and Botting, 2001). Conventionally it was assumed that almost all unwanted effects of NSAID were due to the COX-1 inhibition (Rang *et al*, 2003a). Recent data suggests than this may be an oversimplification and that COX-2 may also have some constitutive activity (Livingston, 2000).

Thus NSAID are mainly effective against pain associated with inflammation or tissue damage because they decrease production of mediators (PGE_2 and PGI_2) that sensitise peripheral nociceptors terminals producing localized pain and also hypersensitivity (Stock *et al*, 2001). They also reduce other components of the inflammatory and immune response that cause pain, namely vasodilatation and oedema.

However, some NSAID show analgesic effects other than those due to inflammation reduction (Rang *et al*, 2003a; Bunsberg, 2008). These drugs show activity at nervous central level (spinal nociception and central sensitization) by inhibiting COX-2 activated PGE_2 that lowers the threshold for neuronal depolarization and increases the number of action potentials and repetitive spiking (Lees *et al*, 2004). Some NSAID also may also reduce pain through centrally mediated mechanisms involving α2 and μ opioid receptors (George, 2003). Chambers *et al* (1995) showed that flunixin-meglumine does have an analgesic central effect (spinal cord), being blocked by adrenoreceptors antagonists. Efficacy of some NSAIDs is comparable to opioids in many cases of surgical, musculoskeletal and visceral pain (Nolan, 2000; Bunsberg, 2008).

The pre-emptive use of NSAID shows some drawbacks in severely ill, dehydrated or general anaesthetized animals because of inhibition of prostaglandins that are necessary for adequate renal function. However, the use of pre-emptive NSAID in healthy animals, such as those which are exposed to farm routine procedures, may be advantageous. Unfortunately this is rarely done in large animal practice probably because of lack of knowledge or habit. Only one study has looked at this effect on cattle (Zulauf *et al*, 2003) showing that if NSAIDs were administered before Burdizzo

castration (along with sedation and local anaesthesia), there would be lower serum cortisol, greater feed intake and less scrotal swelling during the first 72 hours.

Worldwide NSAIDs are used in farm animals mostly for their anti-inflammatory and anti-toxic activity (Lohuis *et al*, 1991; Stilwell, personal observations). Although NSAIDs have been shown to be effective in controlling pain in many clinical situations e.g. post-surgery, arthritis, colic, mastitis and traumatic lesions, practitioners usually agree that there are not enough cost-effective, long-acting analgesic drugs and very few use them following castration or disbudding (Stilwell, 2007a; Hewson *et al*, 2007).

In cattle, ketoprofen and carprofen have been studied following castration (Earley and Crowe, 2002; Mollony *et al*, 2002; Stafford *et al*, 2002; Ting *et al*, 2003a; Ting *et al*, 2003b; Pang *et al*, 2006). The effects of ketoprofen (McMeekan *et al*, 1998; Sutherland *et al*, 2002a; Stafford *et al*, 2002), phenylbutazone (Sutherland *et al*, 2002a), sodium salicylate (Coetzee, 2007) and meloxicam (Heinrich *et al*, 2008) have been studied in disbudded calves. The effect of diclofenac has been studied in lambs castrated by use of a castration clamp (Mollony *et al*, 1997).

Flunixin meglumine is a NSAID known to inhibit mainly Cyclo-oxygenase-1 and is considered to have excellent analgesic properties. Flunixin meglumine has a short half-life of 7 hours (Landoni *et al*, 1995) but its analgesic effect usually lasts longer as a result of accumulation and slow release from inflamed tissues (Nolan, 2000). Although i.v. injection is preferred, the s.c. route is the only practical solution in field conditions and is equally effective.

Carprofen is a NSAID with a mode of action that is not entirely understood and, although it is considered a relatively poor cyclo-oxygenase inhibitor, it is COX-1 sparing drug (Kay-Mugford *et al*, 2000). It has been shown to inhibit production of interleukin 6 (IL-6) (Armstrong and Lees, 2002) Several studies on cats, dogs and horses (Johnson *et al*, 1993; Lasceles *et al*, 1998; Al-Gizawiy and Rudé, 2004) reveal that it is an excellent post-surgical analgesic and also controls arthritic pain. The half-life of carprofen depends on the species, but has been established to be > 34 hours in 17-week-old calves and 44 to 64 hours in adult cows (Lohuis *et al*, 1991; Delatour *et al*, 1996; Lees *et al*, 1996). A long-lasting anti-inflammatory effect of carprofen has been found for cattle (Balmer *et al*, 1997). In horses it was compared to ketoprofen showing 16 to 20 time's higher plasma half-life (Armstrong *et al*, 1999). Carprofen administered prior to surgery versus after surgery in dogs shows better analgesic effect (Welsh *et al*,

1997; Lascelles *et al*, 1998). The duration of the analgesic effect of carprofen in calves has not been established.

1.4.3. 2α Adrenergic agonist drugs

An adrenergic alpha-agonist is a drug which selectively stimulates alpha adrenergic receptors. The alpha-adrenergic receptors are divided into two subclasses: α1 and α2. Xylazine is an analogue of clonidine and it is an agonist at the α_2 class of adrenergic receptor. This drug is the most used sedative in cattle practice because ruminants are the most sensitive of domestic animals to the action of xylazine (10 times more sensitive than horses) (Gross and Tranquilli, 2001; Törneke *et al*, 2003).

Xylazine is classified as an analgesic as well as a sedative and muscle relaxant. It acts upon the CNS (spinal cord and brain) by simulating the effect of noradrenaline released by inhibitory descending pathways (Sullivan *et al*, 1987; Pertovaara, 2006). This activation or stimulation of α2-adrenoreceptors decreases sympathetic discharges, reduces the release of norepinephrine and leads to a potent antinociceptive activity which is not blocked by the opioid antagonist, naloxone (Sullivan *et al,* 1987).

Xylazine exerts its sedative effects at alpha2 adrenergic postsynaptic receptors localized in the cell bodies of the locus coeruleus (Hsu, 1981). The muscle relaxant properties are related to inhibition of the interneural transmission of impulses in the central nervous system (Gross and Tranquilli, 2001).

Other described effects of xylazine in cattle are: reduction in heart rate, cardiac output and arterial blood pressure, probably caused by, among other factors, the depressant effect on cardiac contractility; slowing of the respiratory rate (Gross and Tranquilli, 2001); increase in urine volume or output for about 5 hours; transient hypoinsulinemia, due to its direct effect on alpha-2-adrenoceptors of pancreatic islet beta cells resulting in an inhibition of insulin release (Hsu and Hummel, 1981), hyperglycaemia and glicosuria, that is detected after 15 to 30 minutes and peaks at 2 hours (Eichner, 1979; Raptopoulos and Weaver, 1988; Scholtysik *et al*, 1998; Lima *et al*, 2001); reduction in plasma adrenaline (Scholtysik *et al*, 1998); increase in body temperature (+1.9 C) when the dose of 0.2mg/kg was used (Young, 1979) but a decrease when 0.4mg/kg was used (Gross and Tranquilli, 2001); reduction in reticular rumen activity that can lead to bloat (Ruckebusch and Toutain, 1984; Ruckebusch and Allal, 2008); transient reduction in haematocrit values and haemoglobin concentration

(Eichner, 1979); and increase uterine tone in late gestation (Abrahamsen, 2008) that can lead to abortion.

Several studies have looked at different species cortisol responses after being sedated with xylazine (Thompson *et al*, 1988; Brearley *et al*, 1990; Brearley *et al*, 1992; Frank *et al*, 1992; Sanhouri *et al*, 1992). Most studies on cattle (Brearley *et al*, 1990; Brearley *et al*, 1992) found a lower cortisol level in sedated animals exposed to stress (e.g. transport, general anaesthesia) suggesting that alpha 2-adrenergic receptors are involved in the response of plasma cortisol concentrations to stressors. In another study, rested and transported goats showed lower plasma cortisol concentrations when given xylazine (Sanhouri *et al*, 1992).

After i.v. injection cattle tend to lie down immediately (depending on the dose) but the effect is short. After i.m. injection the absorption and distribution is rapid (although incomplete) but the half-life is short (36 minutes in cattle). The i.m. injection of xylazine (0.2 mg/kg) in calves caused deep sedation, recumbency, useful analgesia that is evident at 5 minutes and maximum at 10 minutes. Analgesia usually last for 30-40 minutes (Jones, 1972; George, 2003).

It should be recalled that xylazine is not an anaesthetic drug, that its analgesic effect is dose dependant and that analgesia is not present except in deeply sedated animals (Nolan, 2000; Gross and Tranqulli, 2001). Also the sedation produced by α2-adrenergic agonists can be overridden by elevated sympathetic tone in anxious or unruly patients (Abrahamsen, 2008) and by other unknown factors. This means that it is difficult for the practitioner to predict the effect of a certain dose on an individual animal. Usually xylazine at the dose of 0.05 mg/kg IV to 0.1 mg/kg i.m. results in recumbency in 50% of tractable cattle and 0.2 mg/kg i.m. causes recumbency in most cattle (Abrahamsen, 2008).

In cattle practice xylazine is used on its own for restraining, physical examination of aggressive cattle, transport and minor surgeries (Stilwell, personal observations). It is also used associated with regional or local anaesthesia in major surgeries. Faulkner *et al* (1992) showed a beneficial effect on performance and health of castrated bulls when butorphanol and xylazine are administered. Sometimes it is the only drug used for castrations and this suggests that its use is more frequently related to safety reasons than to its analgesic effect. This is evident when looking at the answers of a survey in which practitioners admitted using more xylazine than lidocaine when castrating calves (Hewson *et al*, 2007). Xylazine is sometimes used for hot-iron disbudding (Vickers *et*

al, 2005; Faulkner and Weary, 2000; Mish *et al*, 2008; Stilwell, personal observations) because its sedative effect facilitates handling and reduces activity after the procedure, giving the idea that distress is low. By giving xylazine the hot-iron disbudding may be performed by just one person**.** Vickers *et al* (2005) suggest that it suffices when paste disbudding.

The use of caudal epidural xylazine has been studied in heifers and they show that there is less intra-operative distress during abdominal surgery (less reaction to lidocaine injection, more sedation and ataxia) compared with controls, although no differences were found in signs of pain after the surgery (Chevalier *et al*, 2004). However, the authors do not make clear if the decrease in reaction is due to the analgesic properties of xylaxine or its sedation and muscle relaxation effects. The thoracolumbar epidural injection of xylazine has been used as the sole drug (or in combination with lidocaine) in the anaesthesia of cattle for laparotomic surgeries (Lee *et al*, 2004).

1.4.4. Other artificial methods.

General anaesthesia may be thought of as being the ideal for pain-free surgery but is seldom used in field conditions in cattle. However, it is interesting to refer to a study comparing various methods of castration that found that general anaesthesia consistently caused the most severe rise in serum cortisol (Anderson and Muir, 2005). Bearley *et al* (1992) also considered that recovery from general anaesthesia was a very stressful period. Some authors have suggested that general anaesthesia may be intensely distressful to a patient despite the absence of pain stimulus from surgery (Anderson and Muir, 2005; Muir, 2008).

Opioids are exogenous molecules that bind to μ, κ or δ receptors located on the neural cell membranes (George, 2003) and are the drug-class of choice to treat severe acute or chronic ongoing pain. They are also a preferred choice for pre-emptive analgesia for surgery in humans, experimental animals and small animals (cats and dogs). Unfortunately they are rarely used in cattle practice because of several factors: lack of studies on efficacy and drug residues; cost; legislation restrictions; short acting; considerable number of side effects (respiratory depression, ruminal stasis etc…).

Butorphanol is a drug with mixed μ and κ affinities and the only opioid that is regularly used in bovine pain management, mainly in experimental conditions or when an excruciating pain is predicted. It is a potent analgesic but does not provide local or regional anaesthesia. Grøndahl-Nielsen *et al* (1999) showed a positive effect in

controlling pain after hot-iron disbudding. It has been used (0.05–0.1 mg/kg IV or IM in smaller ruminants, 0.02–0.05 mg/kg IV or IM in larger ruminants) associated with xylazine. Legal restrains will probably avert the routine use of this drug in most countries.

Morphine has poor analgesic properties in food animals. It is unclear if the low efficacy is due to a scarcity of μ receptors in the CNS or to poor drug disposition following parenteral injection. Because dorsal horn neurons express opioid receptors, morphine has been used as an epidural injection with fairly good analgesic effects after castration and other surgeries (George, 2003). Methadone has been used in humans for chronic pain arising from chemical burn injuries but the effect of this drug has never been tested in animals (Altier *et al*, 2001).

Ketamine is a dissociative anaesthetic commonly used in veterinary medicine. It is sometimes used as a general anaesthetic in young calves but because it is expensive it is only used for very painful surgeries in adults when recumbency and general anaesthesia are needed. Its short anaesthetic activity is also a drawback. However ketamine possesses potent analgesic effects when administered at sub-anaesthetic doses. Adding a small dose of ketamine to more traditional chemical restraint (i.e. xylazine) greatly enhances the level of sedation and analgesia.

Although corticosteroids are not considered drugs with analgesic properties they deserve a mention because they are the anti-inflammatory agents that provide a greater control of all elements of both acute and chronic inflammation than any other class of drugs. They reduce oedema, preserve cell integrity, reduce the metabolism of the arachidonic acid reducing the production of several inflammation mediators (are nociceptors activators). Unfortunately they also possess several side effects that are dangerous in many of the situations causing pain – they may have immunodepressing effects.

1.4.5. Self induced analgesia

We could not finish a chapter on pain control without addressing the various possibilities of endogenous analgesia. “Stress-induced hypoalgesia” is the term used for an increase in nociceptive thresholds after exposure to acute stressors (Amit and Galina, 1986). For example, Herskin *et al* (2004) showed that stressors like novel surroundings or head fixation led to hypoalgesia when dairy cows were exposed to laser stimulation.

This concept is important because it may influence the response of animals in studies on pain management, especially if stress like restraining is occurring.

Research on the modulation of pain transmission throughout the central nervous system has shown that a number of endogenous substances can produce analgesia when administered to mammals. Among neuropeptides, the endogenous opioids (enkephalins, dynorphins and β endorphins) are the most potent analgesics (Hughes *et al*, 1975). These molecules are synthesized in the spinal cord neurons and adrenal medulla in response to the nociceptive activation of N-methyl-D-aspartate (NMDA) receptors. However, the classical neurotransmitters norepinephrine, dopamine, acetylcholine and serotonin also exhibit analgesic action (Yaksh *et al*, 1985). These endogenous systems are activated by a broad range of environmental stimuli of varying complexity, such as fear, fighting, shock and restrain (Lewis *et al*, 1980; Rodgers and Hendrie, 1983; Lester and Fanselow, 1985; Porro and Carli, 1988).

It is well known that some situations in which the individual is involved in very vigorous activity of brain and body, frequently associated with emergency physiological responses, severe trauma or injury is not noticed. The traditional example is that of the horse that finishes a race with a broken limb. The immediate response to a painful condition may not occur when a stressful situation is going on because endogenous opioids which act as analgesics are released (Bodnar, 1984). Rushen and Ladewig (1991) detected an opioid-based stress induced analgesia in restrained pigs and found that naloxone (a generalized opioid antagonist with a strong affinity for μ-opioid receptors) increased HPA responses and vocalisation in response to restraint. Wylie and Gentle (1998) found that chickens deprived of food for some hours showed very few signs of lameness when given an intra-joint injection just before being fed and that this feature was reversed by naloxone injection. In a review, Porro and Carli (1988) suggest that immobilization leads to partial reduction of the behavioural and hormonal responses, with transient modifications of neurotransmitter systems in the brain.

However, this possibility needs to be studied further because there might be many other factors involved. For example, Melzack (1982) says that only around 40% of humans experience such a syndrome of stress induced analgesia in an emergency situation and Schwartzkopf-Genswein *et al* (1997, 1998) were not able to detect stress-induced analgesia in cattle being branded.

Amniotic fluids increased periparturient opioid-mediated analgesia, and cows ingesting amniotic fluid had higher thermal threshold 1 hour postpartum (Pinheiro Machado *et al*, 1997).

The release of endogenous opioids when young calves are disbudded or castrated is a possibility. This has not been studied but some changes in behaviours could be explained by this self induced mechanism as will be discussed further on.

1.5. Painful procedures on farms.

1.5.1. Castration

Thousands of cattle are castrated worldwide each month (Ewoldt, 2008). Because of the number of animals involved and the pain the procedure causes it should be seen as a very important welfare issue. Castration is usually done to reduce aggressiveness and sexual activity and to modify carcass characteristics by increasing marbling and reducing the incidence of dark-cutting meat (Field, 1971) Castration of cattle is not an usual procedure in Portugal but is done with two main purposes: to reduce sexual behaviour in fattening animals and to produce "cabrestos" used in bullfights to help herd the bull out of the arena, The Portuguese consumer does not appreciate the bullock's marbled meat so very few farmers castrate beef cattle. In the north of the country bloodless castration was performed to produce work-oxen (Stilwell, personal observations).

Castration should be carried out at a young age because cortisol levels, weight loss and pain increase dramatically when it is performed after puberty. A review by Bretschneider (2005) indicates that weight loss increases quadratically as the age of castration is increased and is independent of the method used. Castration methods described for cattle are those that involve surgical removal of the testicles, application of a constricting elastic band (rubber ring) at the base of the scrotum and bloodless castration by external clamping. Rubber ring castration of cattle is seldom done in Portugal (Stilwell, personal observations). All these methods are known to cause long periods of severe swelling, discomfort, pain-related behaviours, leukogram changes, increased levels of acute-phase proteins, reduced appetite and loss of body condition (Chase *et al*, 1995; Molony and Kent, 1997; Fisher *et al*, 1996; Murata, 1997; Earley

and Crowe, 2002; Stafford *et al*, 2002; Ting *et al*, 2003ab; Ting *et al*, 2004; Bretschneider, 2005; Stafford and Mellor, 2005a).

Surgical castration is the more reliable method but is associated with severe complications such as infections, tetanus and haemorrhages (Turner and McIlwraith, 1989; Ewoldt, 2008). Accordingly to European legislation it can only be performed under anaesthesia by a veterinary surgeon, but in the USA it is traditionally done by lay people in a chute under a restraining method called "tail-block" (Ewoldt, 2008; Stilwell, personal observations). There are several surgical techniques that vary:

a) in the number of incisions (one incision: longitudinal over the scrotum midline or two incisions: longitudinal over each testicle, either laterally or caudally),

b) the localization of the incision (longitudinal as explained or transversal, cutting the bottom one third to one half of the scrotum).

c) the haemostatic measures applied (pulling, torsion, emasculator, ligature etc…) (Stafford *et al*, 2002; Ewoldt, 2008). Transverse cutting and pulling the spermatic until it breaks is only carried out on small calves as it can lead to complications in older animals (Wolfe *et al*, 1987).

While the one incision technique offers the advantage of speediness and less tissue damage the two incision method facilitates the access to both testicles. According to some authors drainage is also improved with two large longitudinal incisions (St Jean, 1995; Ewoldt, 2008) but no comparative study on the inflammation, pain and distress resulting from the application of these different techniques has been done.

External clamping (also know as Burdizzo castration) is done by closing the clamp at both lateral edges of the scrotum crushing the internal structures of the testicles (ie, spermatic cord, blood vessels and cremaster muscle). Castration by use of an external clamping technique produced the least severe responses of the methods tested in a study by Stafford *et al* (2002). Some authors consider use of a castration clamp to cause only short-term pain in young calves (Robertson *et al*, 1994). Castration by use of an external clamping technique is sometimes preferred because it is quick and bloodless, with therefore less likelihood of an infection. Nevertheless it has some important disadvantages, which include signs of distress and pain, reduced appetite, loss of body condition, severe inflammation and leukogram changes (Fisher *et al*, 1996; Murata, 1997; Stafford and Mellor, 2005a; Bretschneider, 2005) and it is considered the method with the least certainty of success (Kent *et al*, 1996). Dangerous complications, which are possible when erroneous application of the castration clamp occurs (e.g. crushing

across the median raphe of the scrotum disrupting the scrotum blood supply), are necrosis and gangrene of the scrotum (Baird and Wolfe, 1999; Stafford and Mellor, 2005a; Ewoldt, 2008).

Application of elastic bands is done in young calves (almost since birth) and causes ischemic necrosis of the scrotum and testicles, eventually resulting in sloughing of the scrotum and contents. This method is much more popular for lambs and because it is easily performed in this specie it is preferred to the other methods. In this species there are several studies suggesting that it causes less pain and distress than other methods (Shutt *et al*, 1988; Barnett, 1988; Mellor and Murray, 1989; Kent and Molony, 1993; Kent *et al*, 1995)

It is to be expected that castrated calves will continue to experience pain or at least some "irritation", as described by Stafford (2007), for days or weeks, regardless of the method used. This is particularly true for calves that are kept in large paddocks interacting with other animals while still in a state of primary and secondary hyperalgesia. Recommendations by the European Commission (European Commission, 2001) are that protocols to control pain (local anaesthesia and analgesia) should be used when calves are castrated. However, Ewoldt (2008) states that in the USA "*…when castrating a large number of calves, the provision of analgesia is often inconvenient and expensive*". In a guest editorial paper Stafford (2007) recognizes that "*while we can gauge the relative severity of the pain caused by different methods of castration in the hours following castration and know how to lessen it, there remains a number of important questions relating to our understanding of the experience of the long lasting pain caused by castration and its alleviation*" and "*if it is shown that the pain is significant we will need to develop protocols to prevent this pain or to alleviate it…*" This suggests that for welfare and economical reasons, even where legislation is not specific, analgesia should be maintained for the period during which intense pain is probable.

Some anaesthesia and analgesia protocols have been studied for different castration methods (Robertson *et al*, 1994; Fisher *et al*, 1996; Earley and Crowe, 2002; Stafford *et al*, 2003; Ting *et al*, 2003a; Ting *et al*, 2003b; Stafford and Mellor, 2005b; Coetzee *et al*, 2007). It is well documented that castration causes behavioural (e.g. changes in gait, posture during standing and lying, foot stamping, restlessness, tail wagging, scrotum licking and feeding behaviour) and physiologic (e.g. increases in blood catecholamines, cortisol and acute-phase proteins concentrations and reduction in

immune function) pain-related changes for the first 8 hours and that these responses can be reduced by administering a local anaesthetic and an analgesic drug (e.g. ketoprofen) (Robertson *et al*, 1994; Fisher *et al*, 1996; Stafford *et al*, 2002; Ting *et al*, 2003a). Coetzee *et al* (2007) showed that i.v. sodium salicylate was effective in controlling pain in young calves but only for a few hours.

Very few studies on long-term pain (over 24 hours) after castration have been carried out. In one study (Ting *et al*, 2003b) the effects of repeated ketoprofen injection after surgical castration were evaluated. However, under field conditions it is unlikely that two injections of an analgesic drug would be given. Therefore, because inflammation and pain associated with the castration will probably last for more than 24 hours (Chase *et al*, 1995; Molony *et al*, 1995; Fisher *et al*, 2001; Ting *et al*, 2003a; Pang *et al*, 2006), it would be advantageous to study longer-lasting ways to reduce pain (Stafford and Mellor, 2005a; Stafford, 2007).

Lidocaine infiltration along the incision line together with lidocaine intra-testicular injection is not very efficient in controlling pain after surgical castration (Stafford *et al*, 2002) but Thüer *et al* (2007) showed that a local anaesthetic injected into the spermatic cord and s.c. at the neck of the scrotum reduced acute pain during and immediately after calves were castrated by use of a castration clamp. However, these injections are not practical or safe for the operator in the field especially when many calves have to be castrated.

Epidural injection between the last sacral and first coccygeal vertebra, is a simple and safe procedure in cattle. Lidocaine epidural injection has been used as regional anaesthesia for the first hour after castration (Ting *et al*, 2003a; McMeekan *et al*, 1998) and is more efficient in reducing pain-related behaviours than local ring-anaesthesia (Stafford *et al*, 2002). No study has been conducted to look at the efficacy of lidocaine epidural injection in reducing pain at the moment of external clamping of the scrotum/spermatic cord.

1.5.2. Disbudding

Disbudding refers to the process of destroying the tissue responsible for the generation of the horn with or without the removal of already existing horn bud. This is usually done in calves under the age of 4 to 5 months. The word dehorning is applied to total or partial removing the horns from adolescent or adult animals.

Disbudding of calves is approved in European legislation under certain conditions (Council Directive 91/629/EEC of 19 November 1991, amended by Council Directive 97/2/EC).

Although in some countries, like the USA, feed-lot cattle are disbudded/dehorned when they are weaned and separated from their dams, this is not usual in most of Europe. A few studies have shown that, when horns are left on feedlot cattle, the amount of bruised trim from the carcasses is twice the amount measured from equivalent hornless groups (Meischke *et al*, 1974; Grandin, 1980). A Canadian Beef Quality Audit (Van Donkersgoed *et al*, 2001) has estimated that bruising costs the industry $10 million a year. For this reason dehorning beef cattle may still increase in some countries. The disbudding of beef female calves is rare but is done in some Portuguese farms (Stilwell, personal observations).

In contrast, disbudding of dairy female calves is very common in Portugal and Europe. A survey carried out at the Portuguese Buiatric Association meeting in 2007 (Stilwell, 2007a, data not published), shows that all farms disbud at least part of their calves and 40% of farms disbud all of the female calves (Fig.1.2). Dehorning adult cows is still frequently done in Portugal (see Fig.1.2) and results mainly from two situations: 1) negligence in disbudding routine, leaving a few replacement animals to be dehorned later; and 2) farms changing from tie-stall system, in which dehorning was not necessary, to a free-stall system.

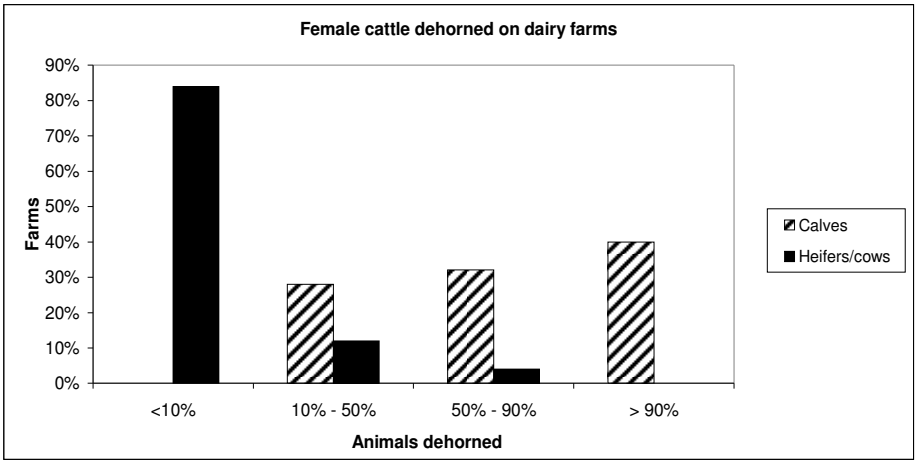

Figure 1.2. - Percentage of dairy farms that dehorn/disbud young or adult female animals (Stilwell, 2007, data not published)

The justification for this mutilation has been that it reduces the potential for traumas and lesions (especially serious in the udder because they can lead to mastitis)

caused by horned animals and so increases safety to humans and other animals in the herd. There are also reports of increased lameness and hoof pathologies when agonist behaviour is prevalent in a dairy herd. This is important in dairy herds kept in free-stalls or straw yards because animal density is usually high and confrontation is much more common than in pasture (Menke *et al*, 1999; Baars and Brand, 2000; Stilwell, personal observations). Maintaining some horned animals in these systems is an important welfare issue because horned animals tend to be more aggressive sometimes even preventing other cows from getting to cubicles, water trough or feed (Stilwell, personal observations).

The disbudding methods more commonly employed are: amputation (scoop, knife, guillotine, embryotomy wire and saw), chemical cauterization (caustic paste) and cauterization by heat. Figure 1.3. shows which disbudding methods are used in Portugal accordingly to a 2007 practitioners survey (Stilwell, 2007, data not published). A North American survey showed that 34.5% of calves were already disbudded at 8 week-old and 78.8% were disbudded at 12 weeks. In the USA the majority of calves were dehorned by hot iron (67.3%) and the remainder were dehorned by scoop (8.8%), paste (9.7%), saw (3.5%), or unknown by calf owner (10.6%) (Fulwider *et al*, 2008).

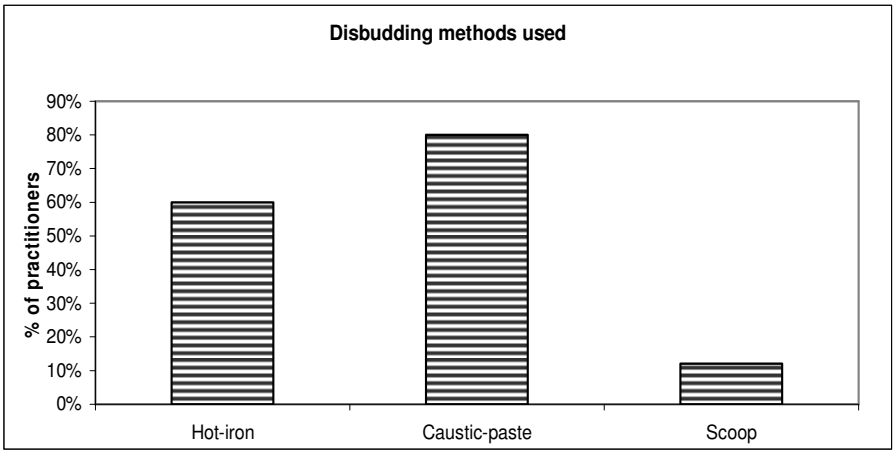

Figure 1.3. – Method of disbudding used in Portuguese dairy farms with which surveyed practitioners work (Stilwell, 2007, data not published).

Note: Forty percent of practitioners work with farms that use more than one method.

Amputation dehorning is not usual in dairy farms except when farmers postpone the procedure for too long. Compared with the other two methods presented here, amputation is the only one that is applicable when horns are more than a few

centimetres long. Because of this it is a preferred method for older calves (over 4 months) and regularly used when weaning calves from beef herds.

One of the amputation methods is called "scoop-dehorning" that causes a curved cut on the calves' head (Fig.1.4.). Because the device has to be vigorously pushed against the animals head and rapidly closed it is very difficult to predict the precise extension of tissue that is going to be cut specially with animals that are struggling. For this reason some skin and bone are frequently cut with the horn bud and horn-growing tissue (Fig. 1.4.). This should be avoided, although a study showed that pain was similar, regardless of the depth of the cut (McMeekan *et al*, 1997). Haemorrhage is frequent, especially in older animals, but usually haemostasis is spontaneous after a few minutes.

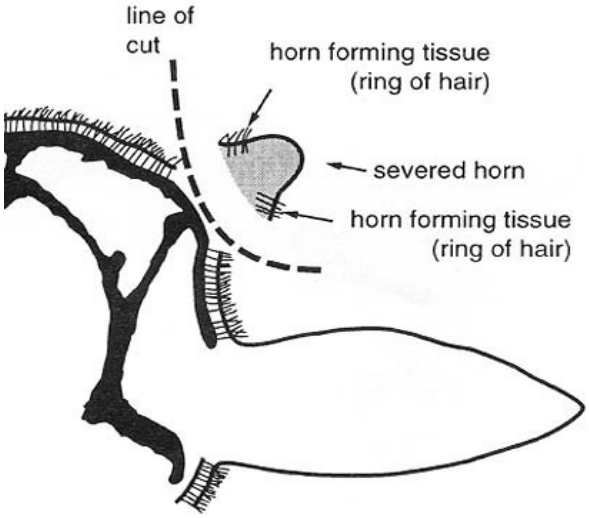

Figure 1.4. – drawing of scoop disbudding showing line of amputation.
Adapted from: http://www.agric.nsw.gov.au/reader/beefmanage/a024.htm

Scoop dehorning has been proven to cause intense and prolonged pain. Most of the studies with this method were done by measuring cortisol after disbudding with anaesthesia alone or with ketoprofen (Petrie *et al*, 1996; McMeekan *et al*, 1998; Sylvester *et al*, 1998a; Stafford *et al*, 2003; Sutherland *et al*, 2002ab; Sylvester *et al*, 2004). Only Sylvester *et al* (2004) looked at behaviour after scoop-dehorning (but not at cortisol). Two studies looked at the effect of heat cauterization of the scoop wound and found a lower cortisol concentration compared to non-cauterized dehorned animals (Sylvester *et al*, 1998b; Sutherland *et al*, 2002b).

Figure 1.5. – Deep chemical burn following caustic paste disbudding.

Caustic paste disbudding is caused by the chemical burn of underlying tissue. The active ingredient used for disbudding is usually sodium hydroxide alone or with calcium hydroxide. These strong and corrosive alkalis (pH 14) cause liquefactive necrosis, resulting in saponification of fats and denaturation of proteins, which allows deeper penetration of the chemical (Fig. 1.5). With caustic burns, tissue damage continues to increase as long as the active chemical is in contact with the tissue (Yano *et al*, 1993) and alkalis tend to penetrate deeper and cause worse burns than acids (Hettiaratchy and Dziewulski, 2004). Yano *et al* (1993) showed that after using sodium hydroxide to inflict alkaline injury on rats, the subcutaneous tissue pH reached its peak value at the 32nd minute and had not recovered to the pre-experimental level by the 90th minute. Histological findings after alkali burns in pigs revealed full-thickness epidermal necrosis and superficial dermal necrosis (Cowart *et al*, 2000). The effects of chemical tissue damage on nociceptors are not fully understood. The pain caused by an alkali is described by humans as “itching pain” or “marked pain” (Ma *et al*, 2007) or sometimes as a chronic and severe pain (Kumbhat *et al*, 2004). Malenfant *et al* (1996) found that 36% of chemical burn patients complain about pain whereas 71% of them experience paresthetic sensations. After a period of acute pain most humans affected by alkali burns develop what as been described as “neuropathic-like abnormal sensations” (Khedr *et al*, 1997). However, all these references deal with more extensive areas of injury than those caused by disbudding. Caustic paste disbudding is usually done in very young calves (2 to 4 weeks of age) as long as the horn-growing tissue is readily identified. Farmers sometimes favour this method because it is easily performed and may give the idea of

being less painful because there is little struggling during the procedure (Stilwell, personal communications), although care has to be taken to prevent paste running onto face and eyes. Neither of the two published studies on pain caused by caustic paste disbudding (Morisse *et al*, 1995; Vickers *et al*, 2005) have looked at the signs of pain in calves disbudded after the injection of local anaesthesia associated with analgesia (non-steroidal-anti-inflammatory drug) or the animals' responses within the first hour post-disbudding. Vickers *et al* (2005) did the study with xylazine sedated animals and suggest this treatment is enough to control pain.

Figure 1.6. – Hot-iron disbudding in which a heated device is pressed against the horn bud for 30 – 45 seconds.

Hot-iron disbudding is done by applying to the horn base an electric or butane-gas heated device (Fig. 1.6) usually attaining temperatures above 600º C, for approximately 30-45s. Thermal disbudding leads to the destruction of all the epidermal and dermal layers extending down to the subcutaneous tissue, but it may also cause tissue damage and oedema that extends beyond the burn site increasing the sensitized area (Junger *et al*, 2002; Doherty *et al*, 2007). Thermal burns induce pain at the site of injury and mechanical intradermal IL-6-induced hyperalgesia (Summer *et al*, 2007a). Hot-iron disbudding is best performed when horn-buds are evident by palpation corresponding to the age of 4 to 8 weeks. Although some stockpersons think that the removing of the bud is essential (Stilwell, personal observations) this is not the case providing the horn-growing tissue is completely destroyed. Pain after hot-iron

disbudding has been evaluated by measuring plasma cortisol and/or behaviour (Boandl *et al*, 1989; Morisse *et al*, 1995, Grøndahl-Nielsen *et al*, 1999, Graf and Senn, 1999, Milligan *et al*, 2004; Vickers *et al*, 2005; Doherty *et al*, 2007). These authors have studied the effect of regional anaesthesia and the association of a NSAID (ketoprofen) with local anaesthesia.

As already mentioned, paste-disbudding is the disbudding method most used in Portugal, followed closely by hot-iron disbudding. In the United Kingdom the use of chemicals for disbudding is only permitted for calves under the age of 7 days (Animal Welfare Act, 2006) although no proof exists than it is less painful at these ages. Figures 1.7 and 1.8 illustrate the attitudes of Portuguese farmers and practitioners towards disbudding and dehorning (Stilwell, 2007a, data not published). The graphics clearly demonstrate that pain management after disbudding is seldom done in young animals and, in the case of adult cows, only done in half of the farms. These results are very similar to the ones found in other surveys in Europe and USA (see above).

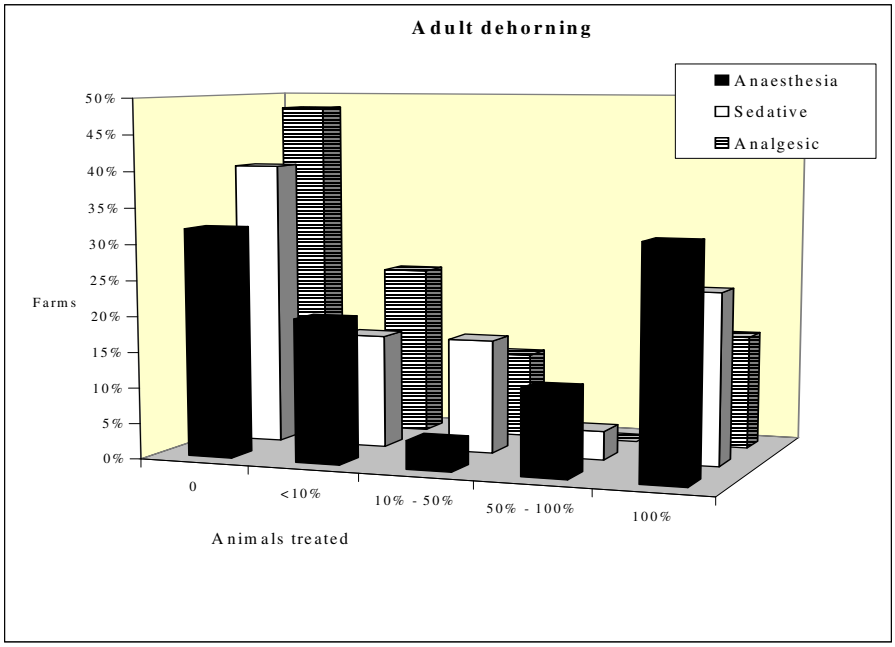

Figure 1.7. – Percentage of farms that use anaesthetic, sedative or analgesic when dehorning adult dairy cows (Stilwell, 2007, data not published).

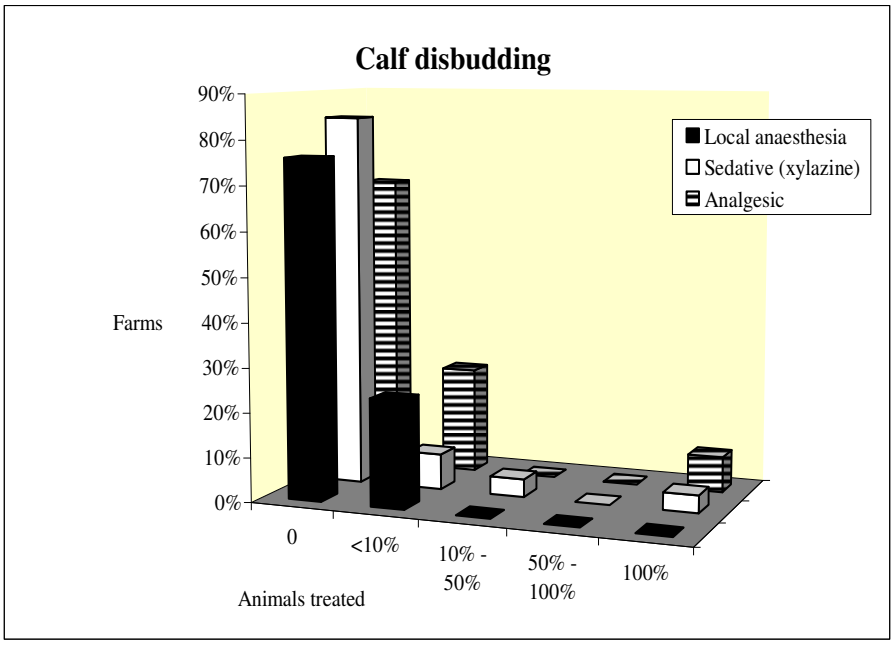

Figure 1.8. – Percentage of farms that use anaesthetic, sedative or analgesic when disbudding dairy calves (Stilwell, 2007, data not published).

1.5.3. Other painful interventions.

Although we did not study other painful conditions suffered by cattle in modern farms we would like to refer to some of them so that the discussion on pain assessment, pain control and the ethics behind pain management will be more comprehensive.

Lameness is probably the most important malaise in cattle in terms of pain. It is not only very prevalent in dairy farms but it causes severe acute and chronic pain leading to a state of primary and secondary hyperalgesia. Lameness is generally recognised to be the most severe welfare problem facing the dairy cow and the European dairy industry (EFSA report on Dairy Cow Welfare, 2008, not published). Because of this high prevalence and because lameness is a reason for the decline in production and fertility and a predisposing factor for many other diseases (ketosis, mastitis…), hoof trimming and hoof surgery is fairly common in dairy farms. These interventions can be very painful and analgesia is advisable, although not usual, through regional anaesthesia during the procedure and analgesics following the procedure. Providing pain relief to cows that are treated for hoof problems improves animal health, welfare and production (Stilwell, 2007a, data not published).

Obstetric interventions, like obstetrical manoeuvres to correct dystocia, episiotomy, pulling large calves etc, can also cause severe pain to the dam and the foetus. Competent management of calving, epidural anaesthesia during procedures and

NSAID administration following calving may reduce pain caused by calving and lead to better performance (Shubert H, 2007, data not published).

Although caesareans are less common than they used to be because of genetic evaluation of bulls for calving performance and because of improved calving conditions, other surgeries are getting much more frequent (e.g. abomasums displacement). Surgery results in acute pain which is experienced during the procedure and for some hours or possibly days afterwards and may lead to the development of hyperalgesia where the response to a painful stimulus is exaggerated or allodynia when stimuli that are normally not painful, become painful (Anderson and Muir, 2005; Stafford and Mellor, 2005a). Pre-emptive analgesia is seldom used in cattle practice but the association of a sedative (usually xylazine) with local or regional anaesthesia is common. Short acting NSAID are usually given for one day after the intervention but this may not be enough to control pain after major surgery.

Many of these procedures would profit greatly from a long acting analgesia protocol. Practitioners claim that the cost, lack of scientific data and some legal restrictions are the major obstacles for a suitable pain management in cattle after surgeries (Stilwell, 2007a, data not published). The need for more studies on the benefits of long acting analgesia after these interventions is, therefore, crucial.

Unfortunately we would also add carelessness as another reason for lack of pain control. This is particularly surprising in view of the already mentioned positive relationship between pain management and recovery, healing and other diseases (e.g. ketosis) reduction.

CHAPTER 2 – Objectives.

In conformity with the statement on the first pages of this thesis that was transcribed from J. Webster book "Animal Welfare: A Cool Eye Towards Eden", it is hoped that the work done might have an impact on cattle welfare.

A concerned farmer or practitioner is often confronted with a dilemma – faced with the need to perform certain procedures how can he reduce the resulting distress? Research may help us respond, in a more competent way, to this dilemma.

We have seen that we can block nociception, at least temporarily, we can fight inflammation and we can reduce pain with analgesics, but we know very little on how cattle show pain and on the efficacy and duration of the effect of these drugs after painful procedures are performed in the field. So, the objectives of our study were:

a) To determine which physiological or behavioural signs are useful to evaluate pain in cattle.

b) To determine the severity and duration of pain after some routine procedures performed in cattle farms.

c) To determine which analgesic protocols were more efficient and well as practical.

CHAPTER 3 – Essay: Why control pain in animals?

Concern for animal pain – the background.

Although Aristotles had already addressed the issue of animals' *telos* - the 'cowness' of the cow or the 'pigness' of the pig (Rollin, 2000) - in an attempt to demonstrate the moral intrinsic value of animals, Voltaire was perhaps the first eminent figure to address the issue of animal pain and human beings' duty towards animals. In his "Dictionnaire Philosophique" (1764), the French philosopher countered the beliefs, held by his contemporaries, that animals were no more than automatons, creatures without a soul, and therefore deprived of the capacity to suffer. Adapting the famous Descartes analogy, contemporaneous Cartesians suggested that animals 'did not think, therefore they did not exist'. This concept freed many scientist from any remorse associated with causing pain to animals and influenced all future human-animal relationship.

Addressing those followers of Descartes who stated that a dog's howls, when pinned to a board to be dissected, were the result of the release of mechanical strings, Voltaire asked: *"You find in them the same sense organs as in yourselves. Answer me mechanists, did nature set up all these sense strings so that the animal, at the end, did not feel a thing?"*

A few years later Immanuel Kant wrote in his "Duties to Animals and Spirits" (~1790): '*so far as animals are concerned we have no direct duties. Animals are not self-conscious and are merely means to an end. That end is man. Our duties to animals are merely indirect duties to mankind. If he is not to stifle his human feelings he must practice kindness towards animals for he who is cruel to animals becomes hard also in his dealings with men'*. Although expressed in a different way and not referring directly to pain, the disdain for animals' interests was still present – being cruel to animals was bad but only because it increased the likelihood of cruelty towards other humans.

Also at the end of the 18th century, Jeremy Bentham, gainsaying this widespread line of thinking, presented his famous reasoning: "*The question is not 'Can they reason?', nor 'Can they talk?', but 'Can they suffer?*" (Introduction to the principles of morals and legislation, 1780). This probably marks the beginning of society's concern for animal welfare.

It is only fair, however, to credit another author who a few years earlier, suggested that *"pain is pain, whether it is inflicted on man or beast; and the creature that suffers it whether man or beast, being sensible of it whilst it lasts, suffers evil"* (Humphrey Primatt, 1776). Previously, Hutcheson (1775) in "Systems of Moral Philosophy" had already condemned cruelty towards animals and presented the idea of animals' rights: "*brutes have a right that no useless pain or misery be inflicted on them*".

Charles Darwin (1838-1840) brought, for the first time, some scientific enlightenment to the, until then philosophic, discussion by suggesting that there were similarities in origin, evolution and functioning between animals and men. He advocated, in the "The expression of the emotions in man and animals" (1872), that resemblances were not limited to muscles, bones or internal organs but should include "superior mental competences" such as different degrees of memory, reasoning and emotions. Science was establishing a new era by stating that man was not alone in the capacity to suffer and that the idea of a living automaton no longer made any sense. Consequently the first welfare guidelines and legislation emerged at this time in Europe: "animals should be slaughtered as quickly and painlessly as possible"; "animals should not be overloaded with burden or work"; "food should be assured"; "shelter and adequate care should be provided" (examples taken from Mahele, 1994).

All through the twentieth century science continued to establish anatomical and physiologic similarities between humans and other mammals, including the functioning of the nervous system. Although pain management continued to be very limited, respect and care grew towards farm animals because of close dependency – this has been called the "fair contract between humans and animals", by which both parties are better off in virtue of the relationship (Rollin, 2000).

After World War II the extraordinary call for food in an economically devastated world and the escalating demand for research in new technologies, lead to profound changes in farming systems and an increased need for laboratory animals. Although management, nutrition and medicine improved some aspects of the farm animals' life – "*on balance ... the animal is better cared for; it is certainly much freer from disease and attack by its mates; it receives much better attention from the attendants, is sure of shelter and bedding and a reasonable amount of good food and water*" (Taylor, 1972) – genetic selection and the very intensive industrialized production brought about new welfare challenges. Farm animals were no longer seen as living partners but as pieces in an insensitive production machine. Pain and suffering, for example, were no longer a

result of excessive work or negligence but of deliberate mutilations (disbudding, castrations, beak trimming, tail docking, teeth clipping etc…) or the inability of an individual to adapt to the environment and production levels (e.g. lameness in dairy cows; mastitis, injury, liver lipidosis, dystocia, abomasum displacement, etc…). Similarly, an enormous increase in research and testing exposed animals to disease, injury and extreme pain without providing them with any benefits in compensation.

With time, consumers became more aware of some of the negative effects resulting from intensive farming and became more informed, judicious and sensitive. Three reasons are behind this increasing concern for animal welfare during the last 50 years (adapted from Fraser, 2003): 1) the change to a less humane way of confining animals, sometimes named "factory farms"; 2) a prevailing urban population that saw animals as sentient beings rather than as instruments and a media that constantly "humanized" animals and showed some terrible conditions in which animals were kept; 3) a reaction to all "non natural" conditions that could put food safety at risk. This was particularly evident after the BSE outbreak.

Rollin (2000) also suggests that "s*ociety was more ready to think ethically about animals*", because of what we would call an "ethic wave" that engulfed society during the second half of the twentieth century and included moral crusades such as those in favour of civil rights, equal treatment for women, the integration of coloured people, immigrants and the disabled, and even nature conservation. We would add another reason that may explain why some countries, namely northern Europe, headed this ethical wave for animals: prosperity and stability in the more developed countries allowed for financial and intellectual investment in improving animal welfare – "*…wealth (…) allowed us to behave towards (animals) with responsibility and altruism*" (Webster, 2005).

So, although some of the consumers' demands sprung from the idea that intensive farming produces less "natural" and safe food, it is also true that ethical concerns played a considerable part. Retailers (and legislators) quickly responded to this situation by demanding stringent welfare policies from animal producers.

In 1964 the British Farm Animal Welfare Council published the first guidelines destined to guarantee better living conditions for farm animals. These are widely known as the Five Freedoms that include, obviously, "Freedom from pain and discomfort". Since then welfare guidelines and legislation have been developed all around the world but mainly in Europe, (98/58/EC, 91/629/EEC, 64/432/EEC and 93/119/EC). In these

and other European recommendations concern for pain management is evident: "Floors (…) designed as not to cause injury or suffering to calves standing or lying on them." (Council Directive of 19 November 1991, 91/629/EEC, laying down minimum standards for the protection of calves); "Castration is painful, regardless of the surgical procedure" and "Analgesia should be used to prevent pain in piglets which are castrated" (EFSA, 2004); "When providing pain relief no distinction should be made on the basis of age as animals from as early as 4 hours after birth exhibit cortisol responses to mutilations" (European Commission, 2001).

Meanwhile animal rights movements became more popular and one of the most important reasons for this expansion was the idea that humans caused a great deal of pain and suffering to animals (especially in experimentation, intensive farming and at slaughter). Andrew Linzey, Peter Singer and Tom Regan are three of the most important authors behind these movements that consider that causing pain and suffering to animals is unacceptable because these have intrinsic moral value and interests. Not suffering pain is, of course, considered a superior interest. "*We are each of us the experiencing subject of a life, a conscious creature having an individual welfare that has importance to us whatever our usefulness to others*" (Regan, 1985). Singer in his book 'Animal Liberation - A New Ethics for our Treatment of Animals' (1975) introduces the concept of 'speciesism' that is the exploitation of other species for the sole benefit of our own.

Although public pressure and science have brought about many improvements in the living conditions of animals it has to be said that the Kantian philosophy is still profoundly embedded in our way of thinking. This is evident in the text of some welfare guidelines. For example the US Animal Welfare Act leaves out cold-blooded animals and warm-blooded animals not "used for research, teaching, testing, experimentation, exhibition purposes, or as a pet, [and] farm animals used for food, fibre or production purposes". Also many research codes of practice admit that some element of pain and suffering is acceptable if the nature of the research makes it impossible to control it. Like Webster (2005) says "*we make decisions as to the quality of an animal's life, indeed its very existence, almost entirely according to our view of its utility, its beauty, its entertainment value or its value as a friend*". So, apparently, in the eyes of the law and society, animals continue to have no intrinsic value and are important solely as instruments or resources for human benefit. For example, very rarely is someone prosecuted for cruelty except if this involves damage to the property of another human

being. This may be changing in some countries (New Zealand and Spain) but only for some species (great apes).

Pain management in animals – yes or no?

After this brief preamble on the history of human concern about animal suffering we shall address the reasons that may substantiate farm animal pain management.

We have already transcribed the statement by the Interagency Research Animal Committee (IRAC) recommending equal consideration for pain and distress in humans and animals. If we accept this rule, even with the exceptions that we have addressed, it is implicit that concern with pain prevention and its management should be the same for both. Although more than 20 years old, it continues to be a sound ethical code that should be followed by researchers, practitioners and farmers.

There is also a widely held belief that animal pain should not be a concern because animals are not able to appraise its meaning, they cannot perpetuate the emotions associated with physical pain and are unable to anticipate future suffering. As Rollin (2000) puts it "*those who would minimise our moral obligations (…) affirm that animals (…) live only in the now, in the moment*". This would be an argument in favour of, for example, dehorning animals without anaesthesia and analgesia – the acute pain caused by cutting is short lived and so would cause little distress.

However, even if we admit that animals do not possess the capability to understand or anticipate the future, we should be aware that some of these shortcomings may be the cause of greater distress and not of its exclusion. For example, animals, because they do not know what is happening, are much more susceptible to fear of the people that are trying to help them, will panic more easily when restrained in order to be treated and are easily frightened by smells. Not understanding why and for how long a pain will have to be endured may be more distressful than being aware of its consequences. As Rollin (2000) puts it: "*By living only the 'now' its entire universe is pain*".

If animals were just an organic machine capable of transmitting only electric signals and responding instinctively to them, we should only be concerned with pain in as much as it impacts on our safety or on the animals' performance. But because evidence shows that this is not the case it seems clear that before studying pain evaluation and alleviation the basic question should be answered: why is it correct to control pain in animals?

The reasons in favour of pain management in farm animals

The reasons why farm animals' pain should be properly managed can be grouped in the following three sets.

- Ethical reasons.
- Health reasons.
- Performance reasons.

All of these reasons are intertwined but for reasons of clarity in this presentation they will be addressed independently.

Modern society and especially the European public have demonstrated, without a shadow of a doubt, that animal welfare is one of their main concerns when discussing animal production (European Commission, 2005) and sentience is the fundamental, morally important basis, upon which worry for animal welfare rests (Kirkwood, 2006). As Duncan (1993) puts it"... *neither health nor lack of stress nor fitness is necessary and/or sufficient to conclude that an animal has good welfare. Welfare is dependent on what animals feel*".

Figure 2.1 shows how animal welfare is important for the consumers all over European Union. In fact, it was the public pressure to improve animal's quality of life associated to indisputable scientific evidence that animals do suffer, that prompted the revolutionary sentence in the EU Amsterdam Treaty: "*…animals should be treated as sentient beings*" and "*…European Institutions are now obliged to pay full regard to the welfare requirements of animals when formulating and implementing Community legislation*". In the same way, professional ethics now include incontestable reference to the veterinarians' (and other professions) duty towards animal welfare protection (Código Deontológico da Ordem dos Médicos Veterinários[b]; Anthony, 2003).

[b] "(…) impõem aos Médicos Veterinários, o dever de exercer a sua actividade com os adequados conhecimentos científicos e técnicos, o respeito pela vida e bem estar animal, a prossecução da sanidade animal, a conservação, o melhoramento, e a gestão do património animal, incluindo o da fauna selvagem, a salvaguarda da saúde pública e a protecção do meio ambiente." (Art. 2 Código Deontológico da Ordem dos Médicos Veterinários.

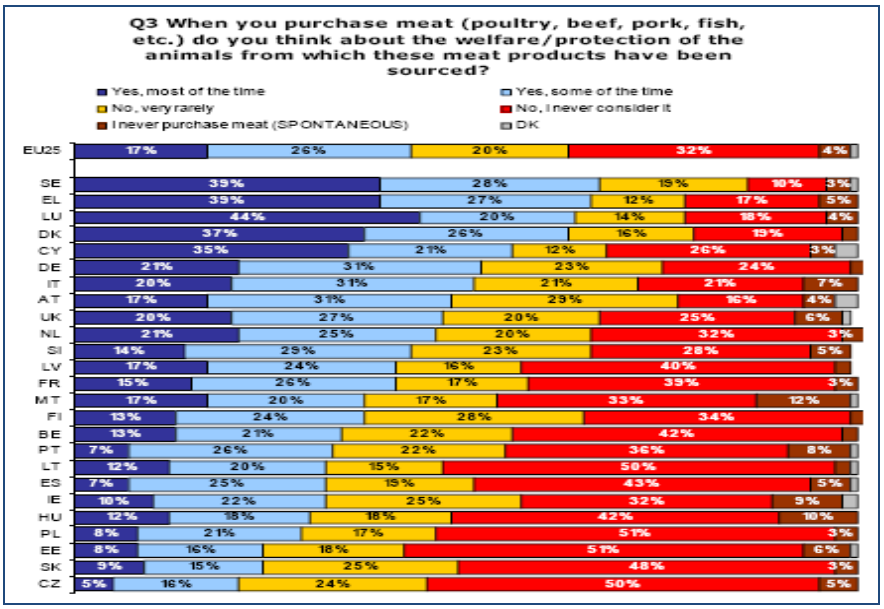

Figure 2.1. – European attitude towards farm animal welfare (European Commission, 2005).

At this moment it should be said that, although farm animal welfare studies should be based on sound science and the process of welfare should be independent from the ethical judgment it generates (Broom, 1991), we should not be reluctant to use ethics in the discussion and application of the results (Sandøe *et al*, 2003). We have seen that there are no real differences in pain mechanisms between humans and animals, and that pain causes not only physical suffering but also long lasting mental distress. If this is so why do we seek hidden differences? Although excessive anthropomorphic associations are to be avoided – '*we must avoid the anthropomorphic projection of our own conception of suffering onto other species*" (Webster, 2005) – empathy is sometimes an important factor when attributing pain to other animals (Bekoff, 2006). This is fabulously depicted in Miguel Torgas' short story about the feelings and emotions of a bull called Miura being fought in the arena (1940)[c]. Many people cannot resist placing themselves inside the head of an animal and good animal husbandry often depends on it as does good science. We also infer subjective experiences in other human beings from objective behavioural observations (Baars, 2001) such as, for example, the pain

[c] "Parou. Mas quando acabaria aquele martírio? Não haveria remédio para semelhante mortificação? Num último esforço, avançou quatro vezes. Nada. Apenas palmas ao actor.
Quando? Quando chegaria o fim de semelhante tormento?
Subitamente, o adversário estendeu-lhe diante dos olhos congestionados o brilho frio dum estoque.
Quê?! Pois poderia morrer ali, no próprio sítio da sua humilhação?! Os homens tinham dessas generosidades?!
Calada a lâmina oferecia-se inteira.
Calmamente, num domínio perfeito de si, Miura fitou-a bem. Depois, numa arremetida que parecia ainda de luta e era de submissão, entregou o pescoço vencido ao alívio daquele gume".

experienced by someone yelling after cutting a finger with a knife. We do not need to hear an explanation about the feelings associated with the injury to know that pain was felt. If we come across so many significant similarities between humans and non-human animals, validating our use of animals for medical experimentation, why cannot we use extrapolation to explain many other findings such as reaction to pain (Stilwell, 2005)?

In conclusion, although in most developed countries there is a strong feeling towards our moral obligation to control pain in animals, we are still far from implementing it due to cultural, practical and economic reasons. This will change and we have to use science to lead us in the more adequate path.

Ironically there are also more worldly reasons to justify timely and efficient pain management – prolonged pain may reduce profit and food safety and quality.

There is countless evidence showing that animals are less likely to maintain adequate performance under persistent stress and pain. For example, a high and prolonged cortisol level may lead to a decrease in milk production because of alteration in glucose metabolism, it may delay the healing process after surgery or it may predispose the animal to infectious disease because of immunodepression (Van Borell, 1995; Sapolsky *et al*, 2000). Pain may also prolong the time needed for recovery from an underlying condition (Muir and Woolf, 2001) because of reduced normal behaviour (e.g. grazing), gait and movement alteration, decreased appetite and probably because of alternative resource allocation. Additionally there is some evidence that acute stressors impair reproductive performance during critical periods of the reproductive cycle, early pregnancy and lactation (Dobson and Smith, 2000; van Borell *et al*, 2007). It is also well known that catecholamines and opioids reduce oxytocin production during lactation and may negatively influence milk ejection and yield (van Borell, 1995).

Several studies have shown decreased dry matter intake, reduced daily gain and loss of weight following castration and disbudding (Faulkner *et al*, 1992; Molony *et al*, 1995; Fisher *et al*, 1996; Fisher *et al*, 2001; Rust *et al*, 2007). See review by Bretschneider (2005). The effects of these procedures on the immunity system activity has also been shown (Chase *et al*, 1995; Murata, 1997; Ting *et al*, 2003a; Ting *et al*, 2003b; Ting *et al*, 2004; Aubry, 2005).

Pain and distress also have an impact on food quality and safety, for direct and indirect reasons. For example: by increasing susceptibility to infectious disease it will cause an increase in antimicrobials use and abuse; animals submitted to stressors are

known to shed more bacteria responsible for zoonosis (Barham *et al*, 2002); pain reduces movement and this may have an effect on muscle quality; hormones and other metabolites produced in an organism subject to pain may reduce meat characteristics and preservation properties.

Having proven that there are economical, health and food safety reasons, as well as ethical motives for pain control in cattle, we should determine why analgesia is still so seldom used. Two reasons may explain this: research has not provided clear and convincing enough figures about these losses, and so practitioners and farmers are not convinced; or costs with drugs, man-power and time are still superior to the losses. This last reason would justify why some authors assert that it is neither practicable nor economically possible to control long-lasting pain after some painful procedures (Stafford, 2007). Hopefully ethical concern and the consequent increased use of analgesics will bring about more research and new, more efficient, long-lasting and cheaper drugs.

CHAPTER 4 – Validating the use of plasma cortisol for the experiments.

STUDY 1

The effect of duration of manual restraint during blood sampling on plasma cortisol levels in calves.

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Abstract:

Many studies on stress and pain rely, solely or mainly, on plasma cortisol assessment.

Confounding factors, such as handling, may cause a release of cortisol making the interpretation of the results difficult. We looked at the influence of duration of restraint on the plasma cortisol levels of one-to-two month old calves. Forty-three calves were divided into four groups according to the time-space between restraint and blood sampling: i) Group 0, immediate blood-sampling; ii) Group 0.5M, half a minute restraint; iii) Group 1M, one minute restraint and iv) Group 2M, two minutes restraint. The only increase in plasma cortisol compared with all the other groups, was seen with blood sampling after two minutes of restraint. This study provides evidence to suggest that cortisol released as a result of handling stress is not evident if blood sampling is carried out within one minute of restraining calves.

Keywords: animal welfare, calves, cortisol, handling, stress, restrain

Introduction – edited and included in Chapter 1.

Objectives - This study was designed to measure the effect of time between first restraint and blood sampling, on plasma cortisol levels of young calves.

Materials and methods

The study site

This study was carried out at a large cattle rearing unit which receives between 100 and 200 young calves each month from dairy farms. The great majority are Holstein-Friesian, but some crossbreeds are seen (Holstein × Limousine and Holstein × Belgian Blue). Transport distances from farms of origin range from 2 to 200 km and on arrival all calves are put in individual boxes and receive an electrolyte solution. Animals are fed twice a day with a commercial milk-replacer in individual buckets. Water and concentrate are available all day. New straw is added every three-to-four days, but bed material is only completely removed when the calf is weaned and moved to group paddocks. Calves have close contact with herdspersons at feeding, adding of bedding and during twice daily individual visual monitoring. Weaning occurs at approximately two months of age.

Study animals

Forty-three male Holstein-Friesian calves were included in the study. They were housed in the same building along four rows and although age varied between 31 and 67 days (Table 4.1) all calves were milk fed.

The animals underwent systematic allocation to different groups. Starting at one end of the first row every four calves were distributed to the following four groups, according to the time between entering the individual pen and blood sampling: i) Group 0, immediate blood sampling; ii) Group 0.5M, 0.5 min restraint; iii) Group 1M, one minute restraint and iv) Group 2M, two minute restraint. Restraint was carried out by squeezing the calf gently against the pen wall with a knee, while holding the head with one hand. This was done by an experienced veterinary surgeon and no excessive force was needed with any of the animals. A second person, five metres from the pen, measured the time and advised when venipuncture and blood sampling should be done. One calf that should have been included in Group 0.5M was excluded because of signs of illness. The last three calves of the last row were included in Group 2M. There were no age differences between groups.

Blood samples (7 ml) were taken into a heparinised tube by left jugular venipuncture. Blood was immediately centrifuged and frozen (–20ºC). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using commercial kits (Coat-A-Count ®, Diagnostic Product Corporation,

Los Angeles, CA, USA). The inter-assay coefficient of variation for cortisol was 5.5% for the level of 1 μg dL–1 and 1.9% for the level of 5 μg dL–1.

Statistical analysis

The distributions and variance of the data were shown not to be normally distributed by Levene and Shapiro-Wilks tests. Significant differences between the four groups were then determined by the Mann Whitney *U* test following a Kruskal Wallis, one-way analysis of variance. Computer software 'SPSS 14.0 for Windows' was used for the analysis.

Results

The results (Table 4.1.) showed a significant difference between Group 2M and Group 0 (P = 0.002), Group 0.5M (P = 0.03) and Group 1M (P = 0.021). Individual variation in blood cortisol levels was very large within each group but especially in the 2M group. Within each group we also compared cortisol levels of animals that were younger than the mean age with those that were older than the mean age and found no differences (data not shown in table).

	Time to blood sampling			
Animals	0	0.5M	1M	2M
n	10	9	10	13
Age (mean ±SD)	47 ±11	46 ±13	54 ±10	49 ±11
Cortisol ±SD	11.71±7.97^{A}	18.39±13.85^{A}	16.34±14,33^{A}	40.12±31.10^{B}

Different superscripts indicate difference for which P < 0.05

Table 4.1 – Mean age (days) and blood cortisol levels (nmol L-1) of calves restrained for different lengths of time.

Discussion and conclusions

The question, 'how long after an animal has been stressed by handling and restraint, will the cortisol response be evident?', has not been answered for young calves used for studies on pain associated with disbudding, dehorning, tail docking and castration.

Hopster *et al* (1999) found that initial collection within one minute of restrain did not alter baseline cortisol in dairy cows, but repeated venipuncture at 15 min intervals

caused an increase in cortisol in primiparous cows less used to handling. Our study used young dairy calves that had been accustomed, since birth, to human proximity and contact. Although restraint was easy and the animals did not show any evidence of distress, we did show that handling alone does cause a significant cortisol response, even in very young calves that were used to human contact. However, we also showed that cortisol levels are not affected if blood sampling is done immediately after restraint (up to one minute of restraint, at least). This suggests that when studies on distress and pain in calves are carried out, non-treated control groups may give reliable information on baseline plasma cortisol levels, providing that blood sampling is carried out by an experienced operator and takes place within one minute of first handling and restraint.

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CHAPTER 5 – Castration.

5.1. Evaluating and controlling long lasting pain.

STUDY 2

Effects of nonsteroidal anti-inflammatory drugs on long-term pain in calves castrated by use of an external clamping technique following epidural anesthesia.

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Objective—To compare efficacy of flunixin meglumine versus carprofen in controlling pain under field conditions following castration by use of an external clamping technique in calves that received epidural anesthesia.

Animals—40 male 5- to 6-month-old calves.

Procedures—Calves were allocated to 4 groups: castrated only (control calves; n = 8); castrated 5 minutes after epidural injection of 2% lidocaine (epidural-alone treated calves; 8), castrated after epidural anesthesia and SC flunixin meglumine administration (epidural-flunixin treated calves; 12), and castrated after epidural anesthesia and SC carprofen administration (epidural-carprofen–treated calves; 11 [1 calf not included]). Plasma cortisol concentration was measured before and 6, 24, and 48 hours after castration. Time of arrival at the feed trough at 24 and 48 hours was observed. Calves were observed at 24 and 48 hours for 4 pain-related behaviors.

Results—At 6 hours, control calves had significantly higher plasma cortisol concentrations, compared with baseline values and those of epidural-flunixin– and epidural-carprofen–treated calves. At 24 hours, epidural-carprofen–treated calves had

significantly lower plasma cortisol concentrations, compared with control calves. At 48 hours, epidural-carprofen–treated calves had plasma cortisol concentrations that were similar to baseline values and significantly lower than epidural-flunixin– and epidural-alone–treated calves. At 24 and 48 hours, epidural-carprofen–treated calves were first to arrive at the feed trough and had fewer pain-related behaviors.

Conclusions and Clinical Relevance—SC carprofen administration in combination with epidural injection of lidocaine may improve the welfare of calves castrated by use of an external clamping technique for up to 48 hours.

Introduction – edited and included in Chapter 1.

Materials and Methods

Animal housing and management—The study was conducted in a Holstein-Friesian farm that is used for the fattening period. Calves are bought at dairy farms at 8 to 15 days of age, milk fed and weaned at 2 months of age at another farm, and then transported at 4 months of age to the fattening farm. Castration in this unit is performed on all calves between the ages of 5 and 6 months. Calves entering the farm are usually grouped by age and size in 2-hectare, outdoor, sandy-soiled paddocks.

Pine trees provide shade. Food is distributed once a day (10 am) and consists of a total mixed ration of wheat straw and concentrate (corn, soybean meal, and barley). As a rule, most calves are already near the feed trough when food is delivered.

Experimental procedures—Forty calves with a mean age of 173 ± 11 days and an estimated weight of 180 kg were taken 2 days before the study began from the large paddocks and placed in a smaller pen with an adjacent chute. The feed trough area was large enough for all calves to access. Water was permanently available. Weather conditions during the study were dry with mild temperatures (22° to 26°C). The day before castration, calves were moved once through the race and chute to reduce the effect of novelty on the study.

This study was approved by the Committee of the Interdisciplinary Centre of Research in Animal Health of the Lisbon Veterinary Faculty, which is responsible for approving studies that involve experiments with animals. The study was performed on a farm that does not use anesthesia or analgesia for castration by use of an external clamping technique in 5- to 6-month-old calves. Owner consent was obtained from the farmer before the start of the study. At the conclusion of the study, results obtained were useful

in convincing the farmer to use anesthesia and analgesia for calves undergoing castration.

On the day of the castration, calves were moved, 4 at a time, through the race to the chute where treatmentsand castrations were performed beginning at 9am. The order of entrance in the race depended only on the location of the calf in the pen, and calves were driven quietly by stockmen blinded to the treatments. Each of the 4 calves were allocated to 1 of the following 4 groups according to their order in the chute: control group, calves were castrated and treated SC with 5 mL of saline (0.9% NaCl) solution; epidural anesthesia (epidural-alone) group, calves were castrated 5 minutes after a caudal epidural administration of 2% lidocaine (4 mL) and SC administration of 5 mL of saline solution; epidural anesthesia plus flunixin meglumine (epidural-flunixin) group, calves were castrated 5 minutes after caudal epidural administration of 2% lidocaine (4 mL) and SC administration of 8 mL (approx 2.2 mg/kg) of flunixin meglumine; and epidural anesthesia plus carprofen (epidural-carprofen) group, calves were castrated 5 minutes after caudal epidural administration of 2% lidocaine (4 mL) and SC administration of 5 mL (approx 1.4 mg/kg) of carprofen[b]. The remaining 8 calves were allocated alternatively to the epidural-flunixin group or epidural-carprofen group. One calf that was originally assigned to the epidural-carprofen group was not included in the study because of severe lameness. Therefore, 8 calves were assigned to the control group, 8 to the epidural-alone group, 12 to the epidural-flunixin group, and 11 to the epidural-carprofen group. All SC injections were given on the neck region, and epidural injection was given between the last sacral and the first coccygeal vertebrae. It was assumed that the NSAIDs would not have taken effect until after the castration had been performed, but the epidural anesthesia was confirmed before castration by absence of resistance to tail lifting.

Castration was performed by closing a castration clamp[c] on each side (first left then right) after assuring that the spermatic cord was pushed to the edge of the scrotum. The procedure was done with the calves standing. The efficacy of castration was not possible to assess, but no complication (eg, necrosis) was detected in any of the calves in the following weeks. After leaving the chute, all calves were left free in a study pen with water and feed available.

Blood samples were collected immediately after the calves entered the chute into 7-mL heparinized tubes[d] by venipuncture of the coccygeal vein at the following times: 5 minutes before castration (baseline) and 6, 24, and 48 hours after castration. Plasma was

separated from blood samples by use of centrifugation and subsequently stored at −20°C until assayed.

Plasma cortisol concentration—Cortisol was assayed in duplicate and measured with a validated solid radioimmunoassay without extraction[e]. The intra-assay coefficient of variation for all samples was 6.98%, and the interassay coefficients of variation were 11.4% for 1 µg/dL and 4.4% for 5 µg/dL.

Behavioral assessment—Behavioral assessment was all done by 1 experienced observer, who was blinded to the study. Identification of the calf was made by ear-ring number with the help, when needed, of binoculars[f]. Behavior was recorded at the time of castration and at 24 and 48 hours after castration at the time of feeding and also at the time of blood sample collection. At the time of castration, behaviors were recorded during and just after external clamping of the spermatic cord. The 3 behaviors recorded were vocalisation, kicking with hind limbs, and lifting forelimbs off the ground. At feeding time at 24 and 48 hours after castration, the time of arrival of calves at the feed trough was recorded during the 15 minutes following feed distribution at 10 am. A score was given to each calf according to the following specifications: a score of 1 was assigned to calves with immediate arrival at the feed trough (already at or near the trough when feed was distributed), a score of 2 was assigned to calves with an early arrival to the feed trough (walked to feed trough within 5 minutes of starting food distribution), a score of 3 was assigned to calves with a late arrival to the feed trough (walked to feed trough after 5 minutes from the start of feed distribution), and a score of 4 was assigned to calves that did not approach the feed trough during the 15-minute observation period. At 24 and 48 hours after castration, the behaviour of calves was assessed after going through the race and chute for blood sample collection. Calves were observed for a 15-minute period for the following behaviors: abnormal walking with the hind limbs abducted, an arched back, raising the hind limbs, and looking at or licking the scrotum area.

Statistical analysis—Shapiro-Wilk and Levene tests were used to study the presuppositions of normal distribution and variance homogeneity of plasma cortisol concentration data. Because plasma cortisol concentration data fulfilled these presuppositions, a 1-way ANOVA model was used for each period and t tests for paired samples were used to compare periods within each group. When the ANOVA F test was significant, the Tukey test was used to compare plasma cortisol concentrations among groups and the Dunnett test was used to compare plasma cortisol concentrations of

groups with those of the control group. Distributions of these variables of gait behavior and arrival time at the feed trough were determined on the basis of results of the Shapiro-Wilk and Levene tests to be non-normal, so nonparametric analyses were used. Significant differences between the 4 calf groups at each time were determined by use of the Mann-Whitney U test followed by a Kruskal-Wallis 1-way ANOVA. Values of $P < 0.05$ were considered significant.

A correspondence analysis was also performed to find correlations between the treatment groups and different behaviors. This method aims at reducing multivariate data into a manageable number of variables to obtain a global view of the data that is useful for interpretation. A cluster analysis, considering Euclidian distance and the Ward method, was performed; graphics are not shown.

Results

Plasma cortisol concentrations—Baseline plasma cortisol concentrations were similar among all groups (P = 0.164; Table 5.1). Compared with control calves, both epidural-carprofen– (P = 0.009) and epidural-flunixin– (P = 0.025) treated calves had significantly lower plasma cortisol concentrations at 6 hours after castrations. Calves in all groups had a significant increase in plasma cortisol concentration at 24 hours after castration, compared with baseline values for each group; however, at 24 hours after castration, only epidural- carprofen–treated calves had a significantly (P = 0.016) lower plasma cortisol concentration, compared with control calves. At 48 hours after castration, epidural- carprofen–treated calves had a significantly lower plasma cortisol concentration, compared with epiduralflunixin– (P = 0.002) and epidural-alone– (P = 0.026) treated calves, but not compared with control calves (P = 0.129).

		Time after castration		
Treatment group	**5 min before castration**	**6 h**	**24 h**	**48 h**
Control (n = 8)	15.45±3.20^{aA}	36.78±5.24^{aBC}	46.99±7.15^{aC}	24.89±4.97^{abAB}
Epidural (n = 8)	16.22±3.45^{aA}	21.56±5.90^{abAB}	36.46±7.15^{aB}	36.28±4.07^{aB}
Epidural-flunixin (n = 12)	19.48±2.62^{aA}	17.69±4.28^{bA}	32.57±5.82^{abB}	32.45±4.06^{aB}
Epidural-carprofen (n = 11)	10.61±2.73^{aA}	15.12±4.47^{bAB}	24.66±6,07^{bB}	15.81±4.25^{bAB}

^{a,b} Means within each column that do not have a common lower-case letter superscript differ significantly ($P < 0.05$).
^{A,B,C} Means within each row that do not have a common upper-case letter superscript differ significantly ($P < 0.05$).

Table 5.1. - The effect of no treatment and treatment with epidural anesthesia alone, epidural anesthesia plus flunixin-meglumine, and epidural anesthesia plus carprofen on mean ± SD plasma cortisol concentrations of calves following castration by use of an external clamping technique.

Behavioral findings—Although 6 of 8 control calves had a detectable reaction of kicking (n = 3) or lifting forelimbs (3) to external clamping of the spermatic cord, no significant differences were found among groups because some calves that received epidural anesthesia also had behaviors indicative of pain. These reactions were observed immediately and only after external clamping of the first spermatic cord. In addition to observations in the control calves, 1 epidural-alone–treated calf, 2 epidural-flunixin–treated calves, and 2 epidural-carprofen–treated calves kicked when the clamp was applied. Vocalisation was rare; only 1 control calf (that also kicked) and 2 epidural-alone–treated calves vocalized. At 24 hours after castration, epidural-carprofen–treated calves arrived significantly sooner at the feed trough than control calves (P = 0.033) and epidural-alone–treated calves (P = 0.033; Table 5.2.).

		Treatment group			
		Control	Epidural-alone	Epidural-flunixin	Epidural-carprofen
Behavior	**Time**	**n = 8**	**n = 8**	**n = 12**	**n = 11**
Arrival at feed trough*					
	24 h	2.88 ±0.64^{A}	2.88 ±0.64^{A}	2.42 ±0.79^{AB}	2.00 ±0.77^{B}
	48 h	2.50 ±0.53^{AB}	2.63 ±0.74^{A}	2.33 ±0.65^{AB}	1.64 ±0.79^{B}
Gait alterations					
Abnormal walking (Nº)†	24 h	4	7	6	4
	48 h	1	1	5	1
Arched back (Nº.)†	24 h	4	3	2	1
	48 h	4	2	1	1
Lifting hind leg (Nº.)†	24 h	2	0	1	1
	48 h	0	2	0	0
Scrotum licking (Nº.)†	24 h	2	1	3	2
	48 h	2	1	2	1
Total gait alterations (mean ± SD)‡	24 h	1.50 ±0.53^{A}	1.38 ±0.98^{AB}	1.00 ±0.74^{AB}	0.73 ±0.65^{B}
	48 h	0.88 ±0.35^{A}	0.75 ±0.46^{A}	0.67 ±0.65^{AB}	0.18 ±0,40^{B}

*Mean ± SD score in which a score of 1 = immediate arrival at the feed trough, 2 = an early arrival to the feed trough,
3 = a late arrival to the feed trough, and 4 = no approach to the feed trough during a 15-minute observation period.
†Number of times each behavior was observed. ‡Number of times behaviors were observed divided by the number of calves in each group.
^{A,B}Different upper-case letters in the same row indicates significant ($P < 0.05$) differences.

Table 5.2. - The effect of no treatment and treatment with epidural anesthesia alone, epidural anesthesia plus flunixin-meglumine, and epidural anesthesia plus carprofen on behaviors indicative of pain in calves following castration by use of an external clamping technique.

At 48 hours after castration, epidural-carprofen–treated calves arrived significantly (P = 0.041) sooner at the feed trough than epidural-alone–treated calves. At either time after castration, no significant difference was found in the time to arrival at the feed trough between epiduralflunixin–treated calves and epidural-carprofen–treated calves. At 24 hours after castration, epidural-carprofen–treated calves had significantly (P = 0.033) less gait and posture abnormalities than control calves. At 48 hours after castration, epidural-carprofen–treated calves had significantly less gait and posture abnormalities than control calves (P = 0.009) and epidural-alone–treated calves (P = 0.041). Results of correspondence analysis revealed a close correlation between different behaviors and the treatment groups at 24 hours after castration. Arched back and non-arrival at the feed trough (score 4) were closely correlated with control calves. Late arrival at the feed trough (score 3) and abnormal walking were correlated with epidural-alone treated calves. Immediate arrival (score 1) at feed trough was not related to any of the groups at 24 hours.

At 48 hours after castration, results of correspondence analysis revealed correlations between different behaviors and treatment groups. Nonarrival at the feed trough (score 4) and raising hind limbs were closely related with epidural-alone–treated calves. Delay of arrival at the feed trough (scores 2 and 3), arched back, and licking the scrotum were closely related to control calves. Epidural-flunixin–treated calves had more instances of abnormal walking than other group calves. Immediate arrival at the feed trough (score 1) was closely related to epidural-carprofen–treated calves. No pain-related behavior had a close relationship to epidural-carprofen–treated calves at 48 hours.

Discussion

Acute pain is a known activator of the hypothalamic-pituitary-adrenal axis; therefore, measuring plasma cortisol concentrations has been extensively used to help evaluate the presence and severity of painful conditions (Stott, 1981; Moberg, 2005). Because other factors, such as fear and stress, may cause a similar increase (Mellor *et al*, 2005) it is important to reduce the effect of handling. For calves in our study, no significant differences were found in baseline plasma cortisol concentrations among groups and our measurements were similar to those of calves of the same age group in other studies (Chase *et al*, 1995; Fisher *et al*, 1996; Ting *et al*, 2003). This indicates that

initial handling had a negligible effect on plasma cortisol concentrations when calves of our study went into the race. Calves of our study were accustomed to humans and handling. Also, care was taken to obtain blood samples immediately after the calves had entered the chute because results of another study (see Study 1) indicate that the effect of handling on plasma cortisol concentrations is reduced if blood sample collection is performed within 2 minutes after a stressful event. In our study, control calves had an increase in plasma cortisol concentrations at 6 and 24 hours after castration, findings that are similar to those of Ting *et al* (2004) and Pang *et al* (2006). In other studies, it has been reported that plasma cortisol concentrations of calves castrated at 1 week (Robertson *et al*, 1994; Mollony *et al*, 1995), 1 month (Thüer *et al*, 2007), 2 to 4 months (Stafford *et al*, 2002), or 5.5 months of age (Fisher *et al*, 1996) return to baseline concentrations after just 2 to 3 hours. It is important to consider that these calves were either young or were kept in individual pens with little movement permitted and a reduced possibility of additional trauma. In contrast, Ting *et al* (2003a) and Pang *et al* (2006) found an increased plasma cortisol concentration at 24 and 72 hours after castration, respectively, compared with noncastrated calves. Results of other studies in calves indicate that surgical castration or castration by use of latex rings results in increased plasma cortisol concentrations at 2 days (Chase *et al*, 1995) or at 7 and 14 days (Fisher *et al*, 2001) after the procedures.

In our study, epidural-alone–treated calves did not have significantly different plasma cortisol concentrations at 6 or 24 hours after castration, compared with control calves. This finding suggested that epidural block with lidocaine, if effective, did not control nociception for more than a few hours or that it did not control pain originating from deep structures. This was to be expected because lidocaine nerve blocks do not last > 90 to 120 minutes (Stafford and Mellor, 2005a). In our study, epidural-alone– treated calves continued to have high plasma cortisol concentrations at 48 hours after castration, suggesting that calves castrated by use of a castration clamp can cause prolonged hyperalgesia and pain.

Low plasma cortisol concentrations of epidural-flunixin– and epidural-carprofen–treated calves at 6 hours after castration in our study suggest that these drugs are equally effective in controlling pain for the first 6 hours, as was found for ketoprofen in other studies (Stafford *et al*, 2002; Ting *et al*, 2003a). In contrast, Pang *et al* (2006) failed to find similar effects at 6 and 12 hours after castration in comparison to calves castrated by use of a castration clamp that did and did not receive carprofen IV. In our

study, the increase in plasma cortisol concentration from baseline at 24 hours after castration in calves of all groups indicates that the treatments studied here are not capable of totally controlling inflammation and pain. This was also suggested by Pang *et al* (2006) for calves and by Price and Nolan (2001) for lambs. Nevertheless, epidural-carprofen–treated calves in our study, but not the epidural-flunixin treated calves, did have significantly lower plasma cortisol concentrations at 24 hours after castration, compared with control calves and epidural-alone–treated calves, suggesting some analgesic effect of carprofen. This could be the result of the longer half-life of carprofen, compared with flunixin, or the accumulation of carprofen in inflamed tissues. At 48 hours after castration, the plasma cortisol concentrations in epidural-carprofen–treated calves was similar to baseline values and significantly lower than those of epidural-alone– and epidural-flunixin– treated calves, indicating that carprofen treatment reduced overall cortisol response for the duration of our study. These results are similar to those of Pang *et al* (2006). Although Pang *et al* (2006) did not find an effect of carprofen on plasma cortisol concentrations in calves from 0 to 6 hours after castration, they found that plasma cortisol and acute-phase protein concentrations remained high in castrated calves for 3 and 14 days, respectively, and that carprofen reduced overall cortisol response and inflammation.

In contrast, epidural-flunixin–treated calves of our study had high plasma cortisol concentrations at 48 hours after castration, indicating that the analgesic effect of flunixin does not last for 48 hours. The low plasma cortisol concentration of control calves at 48 hours after castration may be the result of reduced inflammation because of the powerful anti-inflammatory effect of high concentrations of glucocorticoids (Guyton, 1981) produced by these calves during the previous 24 or more hours.

Pain-related behaviors that indicated that all groups of calves in our study were affected were observed at 24 and 48 hours after castration. No calf group had a close correlation with immediate arrival at the trough (score 1) at 24 hours, suggesting that reluctance to move was increased in all calves. However, control calves and epidural-alone–treated calves had higher numbers of gait alterations and a significant delay (scores 3 and 4) in getting to the trough, compared with other calf groups. Epidural-carprofen–treated calves were the first to arrive at the feed trough and had fewer pain-related behaviors, compared with control calves, at 48 hours after castration. This finding supports the proposal, following the data on plasma cortisol concentrations, that calves that are reluctant to move are those that have more pain when forced to do it. We

suggest that the reduced appetite of castrated cattle, as described in a review by Bretschneider (2005) could be the consequence, among other factors, of this reluctance to move. No differences were found in the time of arrival at the feed trough and in the mean number of gait alterations between epidural-flunixin– and epidural-carprofen–treated calves. Although not complete, it appeared that some analgesia was provided by both these drugs.

Thüer *et al* (2007) observed definitive signs of pain in nonanesthetized 1-month-old calves during castration by use of an external clamping technique. In another study (Fisher *et al*, 1996) in which locally injected lidocaine was evaluated in the castration of older cattle, some differences were found in plasma cortisol concentrations for the first 2 hours after castration between lidocaine-treated and nontreated cattle, but no evaluation of behaviour was attempted. We could not find a significant effect of epidural injection on the behavior of calves at the time of castration. Two factors could explain this: control calves could not express more signs of pain because of the limiting effect of having 4 calves in the chute at the same time, or lidocaine epidural anesthesia is not efficient in blocking deeper pain caused by the clamping of inner structures. The fact that only the first application of the castration clamp on the left side elicited pain related behaviors could be explained by the fact that intense pain probably inhibits further reactions. It might also be that the first application of the castration clamp causes some kind of endogenous opioid–mediated analgesia, and as a result, the second application of the castration clamp on the right side elicits less pain.

External clamping of the spermatic cord by use of a castration clamp causes extensive tissue damage and severe inflammation (Ting *et al*, 2003ab; Pang *et al*, 2006) Although age of cattle and the method of castration are important issues (King *et al*, 1991; Fisher *et al*, 1996; Bretschneider, 2005; Stafford and Mellor, 2005a;) results of several studies (Chase *et al*, 1995; Mollony *et al*, 1995; Fisher *et al*, 1996; Ting *et al*, 2003a; Pang *et al*, 2006; Thüer *et al*, 2007) indicate that chronic pain occurs for several days following the use of a castration clamp on the basis of increases in acute-phase protein concentrations and pain-related behaviors.

It is well recognized that tissue and nerve damage, in association with the exposure of the nociceptors to an inflammatory environment, result in an increased sensitivity of the high-threshold nociceptors so that they will respond to low-intensity stimuli (Handwerker and Reeh, 1991; Tracey and Walker, 1995; Lascelles *et al*, 1997; Hellebrekers, 2000; Anderson and Muir, 2005). This pathologic pain process, possibly

causing allodynia, may also follow castration. The consequence of this is that walking and other daily activities of calves in the paddock could be responsible for regular activation of sensitized nociceptors and the release of cortisol in response to pain (Breazile, 1988). Molony *et al* (1995) suggests that it may be that only during the most intense peaks of the experience of chronic pain does behavior change enough to permit its recognition. Accordingly, we suggest that routine movement and interaction between hyperalgesic calves at the feedlot could be responsible for perpetuating the signs of pain and the high plasma cortisol concentrations that we found in calves of our study.

In conclusion, calves castrated by use of a castration clamp under field conditions in a feedlot have increases in plasma cortisol concentrations and painrelated behaviors at 6, 24, and 48 hours after the procedure. We suggest that external clamping of the spermatic cord causes prolonged inflammation and a state of hyperalgesia that is responsible for acute pain and, consequently, for an increase in plasma cortisol concentrations when calves have to move. In our study, SC carprofen administration in combination with epidural injection of lidocaine at 5 minutes before castration was efficient in improving the welfare of 5-month-old calves for at least 48 hours by reducing signs of pain. Further studies are needed to determine whether carprofen treatment alone is as efficacious as SC carprofen administration in combination with epidural injection of lidocaine in reducing signs of pain following castration in calves.

5.2. Evaluating pain after different surgical castration.

STUDY 3

Effects of surgical castration with one or two incisions on cortisol, rectal temperature, scrotum swelling and behaviour of calves.

To be submitted.

Abstract

There are several surgical castration techniques described but few studies have tried to compare their effect on welfare. We compared, at 1, 2, 3 and 6 hours, the effect of two different castration methods on cortisol, reaction to palpation, rectal temperature and scrotum thickness of 5-6 month calves given local anaesthesia and a sedative. Eighteen calves were randomnly allocated to two groups: castrated through one central scrotum incision (Cast1, n=9) or two longitudinal incisions over each testicle (Cast2, n=9). Of these eighteen animals, eleven were previously submitted to a sham-procedure and acted as a control group (Cast0, n=11). At 2h cortisol was higher in the Cast2 group but there was no difference between Cast0 and Cast1. At 1, 3 and 6 hours both castrated group showed higher cortisol than controls and at 6h calves in the Cast1 group had higher cortisol than Cast2. Scrotum was thicker in both castrated groups compared with control, at all times but at 6 hours Cast1 showed a thicker scrotum than Cast2. Rectal temperature was higher in both castrated groups than in Cast0 group at 2, 3 and 6 hours and at 2 and 6h Cast2 showed a higher value than Cast1. Both castrated groups reacted more to palpation than Cast0, and Cast1 reacted more at 6 hours than Cast2. We conclude that, although one incision causes less initial pain, the inflammation, probably due to reduced drainage, leads to more intense and prolonged pain than when performing two incisions.

Objectives – The objective of this study was to compare the effects of two surgical castration techniques (one or two incisions) on cortisol, rectal temperature, scrotum swelling and behaviour of 5-6 month calves.

Material and Methods –

Farm and Animals

Twenty nine 5-6 months Holstein-Friesian calves were used in this study. Animals were not weighed but live weight was calculated to be approximately 160 kg considering the calves' age and body condition. The animals belonged to a state owned experimental farm located in the center of Portugal (Estação Zootecnica Nacional), were housed during the study in a small pen (15x20 meters) with an adjacent chute, were fed grass hay and concentrate and were vaccinated against clostridium (8 strains), IBR, BVD, BRSV and PI_3 viruses. Weather conditions were similar on all study days – cloudy sky and low temperature (12 – 15 ºC)

Animals in Group Cast0 were handled one week before the beginning of the castration experiments. The eleven calves were sedated with xylazine, local anaesthetic was injected into the spermatic cord and the testicles were handled for one minute. The same animals that were used as a control group were later allocated to the castration groups. During the following weeks, eighteen calves (including the eleven that were previously used as controls) were then randomly allocated to the two castration groups: nine were surgical castrated through one incision (Cast1) and nine were surgical castrated through two incisions (Cast2). All calves were given 2% xylazine (4 ml i.m.; Rompun ®, Bayer) and when they went down local anaesthetic was injected into each spermatic cord at the scrotum neck and along the incision lines (centrally along the raffe in case of Cast1 or longitudinal over each testicle in case of Cast2). After five minutes one incision along the central raffe or two incisions over each testicle (each ~ 7 cm) were made and each testicle exposed at a time trough the incision(s). The adherent adipose tissue and connective tissue was stripped proximally to allow for good exposure of the cord and the tunica vaginalis was open and the retractor muscle and the vascular cord were separated. Then an emasculator was placed around the cord halfway between the testicle and the inguinal opening. Above the emasculator a ligature with Supramid nº3 was applied tightly around the vascular cord. The emasculator was kept for approximately 30 to 45 seconds before the same procedure was done on the other testicle.

At 1, 2, 3 and 6 hours after the procedure, including the control group, animals were quietly driven to a chute and blood (7 ml) was collected from the coccygeal vein into an heparinised tube and rectal temperature, reaction to scrotum palpation and scrotum thickness were assessed (always in this same order).

Cortisol

Blood was kept in ice then centrifuged and the plasma frozen (-20C). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). The lowest detectable concentration of cortisol was 1.0 nmol/l. The between-day differences for plasma cortisol concentrations within groups were not significant so data were pooled.

Rectal temperature

After blood sampling a thermometer was inserted and pressed against the rectum wall for 5 to 10 seconds (it was removed and the temperature recorded after the thermometer emited a "bip").

Reaction to palpation

With the calves inside the chute each testicle was palpated gently. Reaction was classified by an experimental observer blind to the surgical method of each calf as 0 = no reaction to 5 = extreme reaction. The behaviours observed were: vocalisation, lifting one or both hind legs, kicking, lifting front legs and lunging forward against the chutes' gate.

Scrotum thickness

Scrotum diameter was measured by using a cutimeter at the scrotum thickest portion (approximately midway between the apex and the base). The cutimeters' jaws were closed gently until touching the scrotum skin cranially and caudally. Each side was measured individually and the results are presented as the mean.

For the control group (Cast0) blood sampling and rectal temperature appraisal were done at all times but the reaction to palpation and scrotum thickness were evaluated only at 1 hour because no variation was to be expected.

Statistical Analysis

Distributions of these variables were shown by Levene and Shapiro–Wilks tests to be non-normal, so non-parametric analyses were used. Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between the groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance. *P*-values less than 0.05 were considered significant.

Results:

There were no differences in age between the groups.

The cortisol results (Table 5.3. and Figure 5.1.) show differences between both castration groups and control at all times except Cast1 at 2h. At 2h Cast2 has a significantly higher cortisol than Cast1 but at 6h it is Cast1 that shows higher cortisol compared with the other castrated group. Comparing cortisol levels along time shows that Cast1 group does not show difference at the first three moments but increases significantly at 6 h. In contrast, Cast2 shows an increase at 2 and 3h compared with 1 h but not at 6h. The control group shows a higher cortisol at 2h compared with 6h.

CORTISOL

Group	+ 1 h	+ 2 h	+ 3 h	+ 6 h
Cast1 (n=9)	40.74 ±15,40^{aA}	43.16 ±24.53^{aA}	60.36 ±19.20^{aAB}	98.74 ±54.58^{aB}
Cast2 (n=9)	50.68 ±36.52^{aA}	88.85 ±58.64^{bB}	79.67 ±57.53^{aBC}	46.16 ±20.77^{bAC}
Cast0 (n=11)	24.71 ±13.77^{bAB}	34.55 ±26.89^{aA}	20.04 ±13.34^{bAB}	21.04 ±12.15^{cB}

^{a,b,c} Means within each column that do not have a common lower-case letter superscript differ significantly ($P < 0.05$).
^{A,B,C} Means within each row that do not have a common upper-case letter superscript differ significantly ($P < 0.05$).

Table 5.3 – Mean ±SD plasma cortisol (nmol/ml) of calves castrated by different surgical techniques.

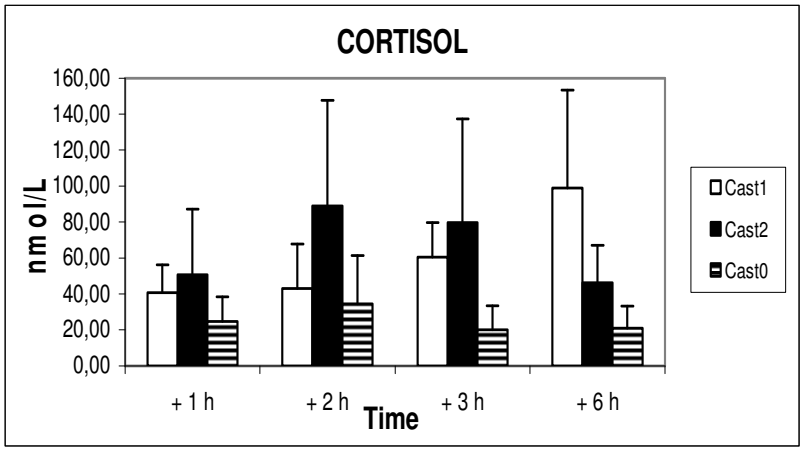

Figure 5.1. - Mean ±SD plasma cortisol (nmol/ml) of calves castrated by different surgical techniques.

Scrotum thickness results (Table 5.4. and Figure 5.2.) show that there were differences between both castrated groups and the control groups at all times (control group scrotal thickness was measured only at 1h). Comparing the two castrated groups it was shown that Cast1 had a significantly thicker scrotum at 6h. Along time Cast2 showed an increase of thickness at 2 and 3h but not at 6h compared with the first measure at 1h. Cast1 group showed a significantly increase between 1 and 2h and then between 2h and both 3 and 6h.

SCROTUM THICKNESS				
Group	+ 1 h	+ 2 h	+ 3 h	+ 6 h
One incision (n=9)	49.7 ±6.1^{aA}	60.7 ±8.4^{aB}	69.7 ±5.4^{aC}	70.4 ±7.7^{aC}
Two incisions (n=9)	51.4 ±6.3^{aA}	64.0 ±9.8^{aB}	68.3 ±8.7^{aB}	57.9 ±7.9^{bA}
Control (n=11)	29.7 ±4.1^{bA}	29.7 ±4.1^{bA}	29.7 ±4.1^{bA}	29.7 ±4.1^{cA}

^{a,b,c} Means within each column that do not have a common lower-case letter superscript differ significantly ($P < 0.05$).
^{A,B,C} Means within each row that do not have a common upper-case letter superscript differ significantly ($P < 0.05$).

Table 5.4. – Mean ±SD thickness of scrotum (mm) of calves castrated by two different surgical techniques.

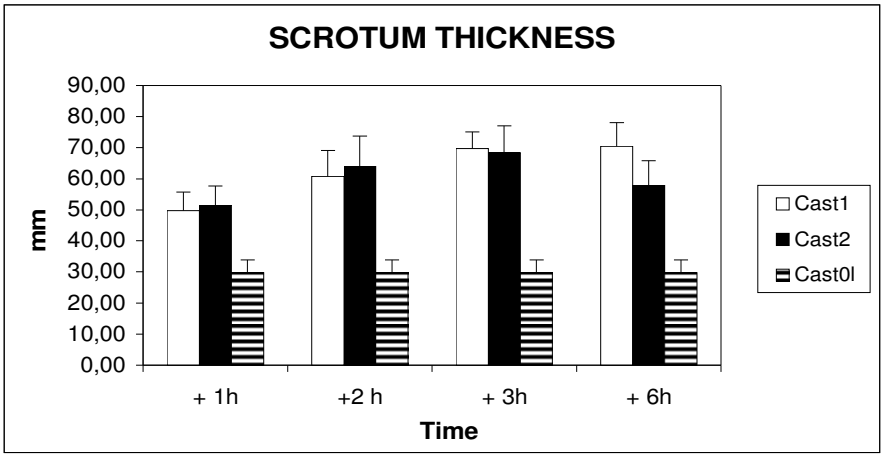

Figure 5.2. - Mean ±SD thickness of scrotum (mm) of calves castrated by two different surgical techniques.

Rectal temperature after castration results are shown in Table 5.5. and Figure 5.3.. Rectal temperature was higher in the two castrated groups except at 1h. At 2 and 3 h the Cast2 showed higher temperature than Cast1. Both castrated groups showed higher temperature at all times compared with 1h. There was an increase in temperature in the control group when comparing the values at 1 and 6h.

	RECTAL TEMPERATURE (C)			
Group	+ 1 h	+ 2 h	+ 3 h	+ 6 h
One incision (n=9)	38.7 ±0.1^{aA}	39.0 ±0.3^{aB}	39.5 ±0.2^{aC}	39.7 ±0.5^{aC}
Two incisions (n=9)	38.6 ±0.1^{aA}	39.7 ±0.5^{bBC}	40.0 ±0.4^{bB}	39.5 ±0.4^{aC}
Control (n=11)	38.7 ±0.2^{aA}	38.8 ±0.2^{cAB}	38.8 ±0.2^{cAB}	38.9 ±0.1^{bB}

^{a,b,c} Means within each column that do not have a common lower-case letter superscript differ significantly ($P < 0.05$).
^{A,B,C} Means within each row that do not have a common upper-case letter superscript differ significantly ($P < 0.05$).

Table 5.5. – Mean ±SD rectal temperature (C) of calves castrated by two different surgical techniques.

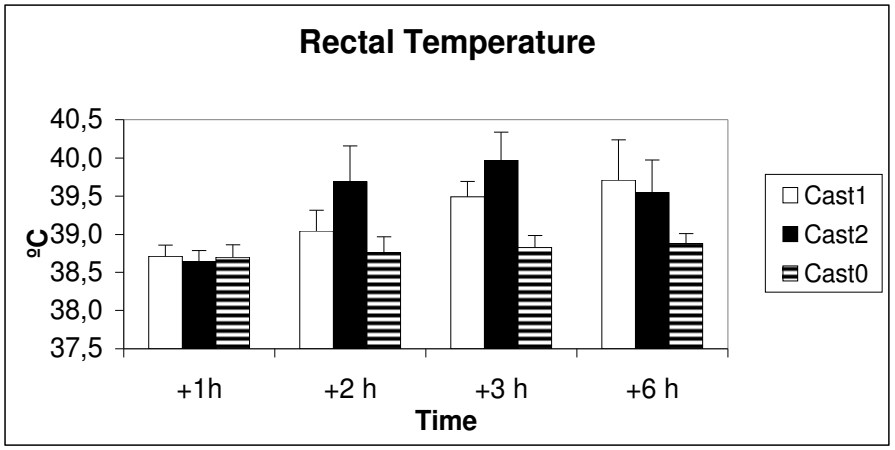

Figure 5.3. – Mean ±SD rectal temperature (C) of calves castrated by two different surgical techniques.

The degree of reaction to scrotum palpation is shown in Table 5.6. and Figure 5.4.. Compared to the control group the reaction to palpation was higher in the Cast2 at 2h and in the Cast1 at 3 and 6h. The castrated groups differed in reaction at 3 and 6h with the Cast1 showing more reactions.

	REACTION			
Group	+ 1 h	+ 2 h	+ 3 h	+ 6 h
One incision (n=9)	3.00 ±0.71^{aA}	3.67 ±0.71^{abA}	3.78 ±0.44^{aAB}	4.56 ±0.73^{aB}
Two incisions (n=9)	3.22 ±0.67^{aA}	4.33 ±0.71^{aB}	3.78 ±0.67^{b*AB}	2.78 ±1.09^{bA}
Control (n=11)	2.91 ±0.94^{a}	2.91 ±0.94^{b}	2.91 ±0.94^{b*}	2.91 ±0.94^{b}

^{a,b} Means within each column that do not have a common lower-case letter superscript differ significantly ($P < 0.05$). * trend p =0,052

^{A,B,C} Means within each row that do not have a common upper-case letter superscript differ significantly ($P < 0.05$).

Table 5.6.– Mean ±SD reaction to scrotum palpation of calves castrated by two different surgical techniques.

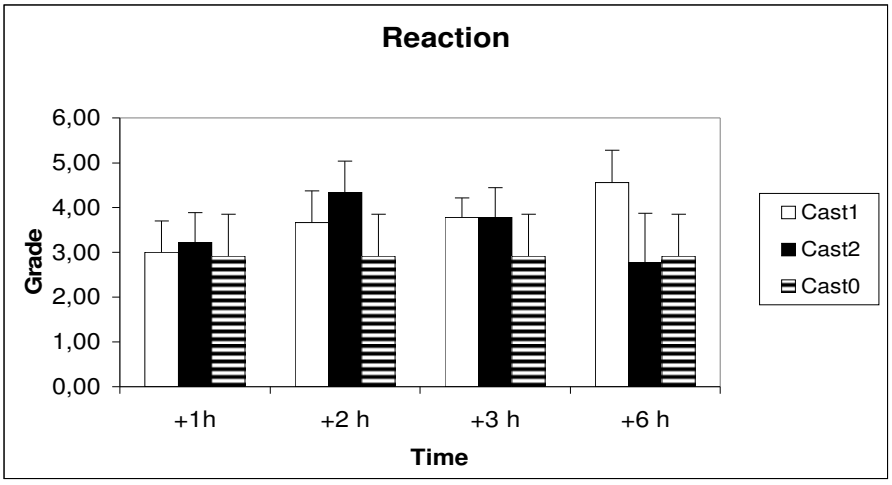

Figure 5.4. – Mean ±SD reactions to scrotum palpation of calves castrated by two different surgical techniques.

Discussion

It is well established that cattle learn to associate handling procedures and facilities with aversive events that have occurred previously so the fact that our calves were not castrated in the chute probably reduced the cortisol response to restrain and handling. This was patent in the control group that presented similar levels compared with other studies and did not show any difference in cortisol concentrations at any time.

Physiological and behaviour assessment was only done after 1 hour because it was expected that lidocaine would control pain response for at least 60 minutes (Muir *et al*,

1995; Edwards, 2001; Smith *et al*, 2002; Anderson and Muir, 2005). In addition, the fact that calves were given xylazine reduced their activity and the ability to be driven to the chute (although all were up 30 to 40 minute after being given the sedative). In our study, although both groups were given regional anaesthetics, the high cortisol and behaviour response to palpation shows that pain was already present at 60 min after the procedure. This result is similar to those of other studies (Fisher *et al*, 1996; Earley and Crowe, 2002) in which cortisol was already high at 60 to 75 min in castrated animals even when given local anaesthesia.

All the parameters measured were higher in the castrations groups compared with the control group showing that pain is felt at least from 1 to 6 hours. The duration of plasma cortisol increase after castration is consistent with the results of some other researches. Cohen *et al* (1990) recorded increased plasma cortisol at 3 and 6 h post-treatment in surgically castrated Holstein calves, Fisher *et al* (1996) showed a cortisol peak between 0.5 and 1.5 h after two-incision surgical castration, that was still significantly high at 8 hours and Ting (2003b) found a higher cortisol in castrated animals until 12 hours compared with controls. In contrast, Coetzee *et al* (2008) showed a very high baseline levels and consequently a very short significant increase in surgical castrated calves (less than 2 hours). The authors suggest that this was due to the fact that calves were moved to the research facilities less than 24 hours before the study started. Other authors have found cortisol to be still high at 48 (Chase *et al*, 1995) or 72 hours after castration (Faulkner *et al*, 1992). Fell *et al* (1986) showed higher cortisol levels at 6 days in surgical castrated calves when compared with female controls. In contrast, Fisher *et al* (1996) found a very small difference in plasma cortisol concentrations between castrates and controls probably because these calves were housed and blood-sampled in tie-stalls meaning that painful movements and interactions with other animals were less frequent.

Behavioural response to palpation has not been looked at in other studies with cattle. Behavioural assessment after surgical castration has been limited to gait, posture or lying behaviour changes (Robertson *et al*, 1994; Mollony *et al*, 1995; Ting, 2003b; Rust *et al*, 2007) or vocalisation during the procedure (Rust *et al*, 2007). Measuring mechanical nociceptive threshold responses has been tested in sheep showing a lower threshold in castrated lambs (Thornton and Waterman-Pearson, 1999). In our study castrated calves reacted slightly more at some moments when compared with controls, but not always. We suggest that this apparent similarity is not because there is little pain

and hyperalgesia in the swelled scrotum but because even untouched calves will try to escape to testicle palpation.

Other studies have looked at scrotum circumference after burdizzo castration (Fisher *et al*, 1996) and found significant differences for 35 days. We did not look at the circumference but at the diameter which was very much increased from 1 to 6 hours compared with the animals in the control group. This increase in diameter is due to inflammation, fluid extravasation and some haemorrhage and may be more important in causing pain than the extension of the skin cut as is evident by the differences found between surgical techniques.

Increase in body temperature results from endogenous pyrogens (IL-1 and prostaglandins) production and circulation such as follows non-infectious inflammatory conditions and extensive tissue damage (White, 1996). The low temperature (lower than reference rectal temperature for 6 month old calves) found in all groups at 1 hour after the procedure, was probably a xylazine side effect (Gross and Tranquilli, 2001). When xylazine had been cleared and inflammation mediators and metabolites got into the bloodstream, body temperature increased and was still higher than controls when the study ended.

One study looked at the effect of surgical castration with the spermatic cords broken by traction or cut by an emasculator on plasma cortisol (Stafford *et al*, 2005a), but no published study has compared the effect of the number of incisions on inflammation and pain. One-incision castration has the advantage of causing less tissue damage, less local anaesthetic use and reduced procedure time. On the other hand it may hold back drainage that is crucial when haemorrhage and oedema is considerable.

In our study we showed that cutting less tissue had no beneficial effect, compared with two incisions, except for a lower cortisol and rectal temperature two hours after surgery. In contrast, at six hours, plasma scrotum swelling was significantly bigger in the one-incision castration group and, consequently, so was pain, as was demonstrated by the higher cortisol and reaction to palpation found at 6h. Compared with the two-incision technique, in which the 6h values go back to 1h level after a temporary increase, all parameters increase continuously in the one-incision group. The two-incision castration overall cortisol profile is very similar to that of other studies – a quick but short cortisol increase (Cohen *et al*, 1990; Fisher *et al*, 1996; Stafford *et al*, 2005a), but although our study ended at 6 hours after surgery, we suggest that cortisol and pain would continue to increase for longer in the one-incision group. The substantial

and increasing swelling and hyperalgesia suggests that scrotum oedema was more severe in the one-incision group confirming that drainage was insufficient.

Conclusions

Our results show that surgical castration of 5-6 month old calves causes pain from 1 to at least 6 hours independently from the method used and even when sedation and local anaesthesia with lidocaine is used. Pain is demonstrated by behaviour, physical and physiologic changes. We also showed that cortisol, temperature, scrotum thickness and reaction to palpation differ in time and intensity between the two surgical methods – the one-incision causes a more intense inflammation and pain but later in time when compared to the two-incision technique. In view of these results we suggest that two-incision surgical castration is preferable in welfare terms to the one-incision method.

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Chapter 6 – Disbudding

6.1. Evaluating and controlling pain after scoop disbudding.

STUDY 4

O efeito de anestesia regional associada ou não a um anti-inflamatório não esteróide sobre o cortisol e comportamento de vitelas descornadas por amputação com alicate. (Effect of regional analgesia with or without a non-steroidal-anti-inflammatory analgesic on cortisol and behaviour of calves dehorned by amputation).

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(This paper was published in Portuguese and edited and translated by the author for this thesis)

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Abstract

Dehorning by amputation is still used in some dairy and beef farms because it allows for the dehorning of older animals. It is established that it causes severe and prolonged pain, so we wanted to study the effect of regional anaesthesia only or in association with a non-steroid-anti-inflammatory drug in controlling pain.

Fifteen female calves (mean age, 120 ±30 days) were randomly allocated to three different treatment groups: five dehorned by amputation with no treatment (C); five dehorned after regional anaesthesia (A); five dehorned after regional anaesthesia and i.v. flunixin-meglumine (AA). Plasma cortisol was measured before and 1, 3, 6 and 24 hours after dehorning. Five pain related behaviours were registered at 15 minutes, 1, 3, 6 and 24 hours after dehorning.

Compared with base-line levels, Group C showed increase cortisol at 1, 3 and 6 hours and Group A at 1 and 3 hours. Only Group AA showed no significant difference during the study. Group C showed higher cortisol levels than group AA at all moments post-dehorning except at 24 hours. At 3 hours group A had higher cortisol than both other groups.

Group C showed more pain-related behaviours than AA at 15minutes, 1 hour and 6 hours. At 6 hours Group A showed more pain-related behaviours than Group AA. At

24 hours all groups showed some behaviours of pain but there were no significant differences.

This study shows that regional anaesthesia is not sufficient to prevent cortisol rise and pain behaviours in calves dehorned by amputation. Only the association of local anaesthesia and a NSAID prevents signs of pain after scoop dehorning.

Introduction – edited and included in the Chapter 1.

Objectives:

The objectives of this study were to evaluate the intensity and duration of pain after amputation-disbudding (scoop) and to assess the effect of regional anaesthesia with or without an analgesic (flunixin-meglumine) in controlling pain-related distress.

Material and Methods:

Fifteen female Holstein calves aged 120 ±30 days, from a 300 dairy cow farm were used in this study. The calves were kept in a concrete-floored rectangular stable with sand bedded cubicles. One long side was occupied by a feed trough, with wheat straw and concentrate ad libitum, the other by a high wall and the short sides were closed with rails. Water was permanently available at two water troughs.

The calves were randomly allocated to the following treatment groups: C – scoop disbudded with no treatment (n=5); A – scoop disbudded after lidocaine (n=5); AA – scoop disbudded after lidocaine and i.v. flunixin-meglumine.

Five minutes before disbudding the animals were restrained, blood-sampled and treated. Restrain was done with care to reduce stress and blood-collection was done immediately after being caught by an experienced veterinarian. When applicable, lidocaine 2% (5 mL; Anestesin, Laboratório Farmacológico, Portugal) was given sub-cutaneously midway between horn-base and eye lateral canthus and flunixin-meglumine (6 mL, aprox. 2mg/kg; Finadyne, Shering-Plough) was given intravenously. The control calves were given 5 mL of saline instead of lidocaine. Animals were then spray marked with a number to be more easily identified when behaviour was recorded.

Scoop disbudding was done by applying the device on the horn bud and opening the handles so as to cut the horn-bud and all the horn growing tissue. Because of the need to close very rapidly the device it was not possible to guarantee that additional skin and subcutaneous tissue was not cut.

Blood sampling was subsequently done at 1, 3, 6 and 24 hours after disbudding, always in the same order. Blood was collected on-to a heparinised tubes, kept in ice,

then centrifuged and plasma frozen at -20ºC. Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA) at the Faculdade de Medicina Veterinaria by technicians blind to treatments. The lowest detectable concentration of cortisol was 1.0 nmol/L.

Four pain-related behaviours (Table 6.1.) were recorded for periods of 15 minutes at 15 minutes, 1, 3, 6 and 24 hours after disbudding, by an experienced veterinarian. Calves adopting an "inert lying" behaviour for more than 30 seconds during each observational period were also recorded.

Statistical Analysis

Distributions of the variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used (SPSS 15 for Windows ®). Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance.

Behaviour	**Description**
EAR flicking	Number of times the animal flicks its ears with no apparent reason (e.g. presence of flies around wound).
HEAD shaking	Shaking the head with evident rotation of the neck.
Head RUBBING	Using the hind limb to touch or scratch any part of the head or rubbing the head against objects.
TRANSITIONS	Lying and getting up immediately with no apparent purpose of resting.
INERT LYING	Sternal lying with head facing back resting on flank. No response to stimuli from the surroundings (e.g. other animals).

Table 6.1. – Description of the pain-related behaviours observed at 15 minutes, 1, 3, 6 and 24 after scoop-disbudding.

Results:

Plasma cortisol

Cortisol concentrations and differences after scoop disbudding are presented in Table 6.2. and Figure 6.1.

	TIME FROM DISBUDDING				
GROUP	-5 minutes	+ 1 h	+ 3 h	+ 6 h	+ 24 h
A (n=5)	21.74 ±7.75^{aA}	54.24±21.74^{a*bB}	103.23 ±30.68^{aC}	54.19±37.37^{aAB}	41.75±30.80^{aAB}
AA (n=5)	20.02 ±16.43^{aA}	34.84±33.66^{bA}	32.98±17.41^{bA}	14.33±5.96^{bA}	40.07±20.43^{aA}
C (n=5)	14.44 ±6.76^{aA}	90.13±19.71^{a*B}	66.62±10.54^{cBC}	47.26±16.20^{aCD}	30.42±17.23^{aAD}

Different lower case superscript letters indicate difference between groups for which $P < 0.05$.
Different upper case superscript letters indicate difference across time for which $P < 0.05$
Table 6.2. – Mean ±SD plasma cortisol concentration (nmol/L) of calves scoop-disbudded with no treatment (C), scoop-disbudded with regional anaesthesia (A) or scoop-disbudded with anaesthesia and analgesia (AA).

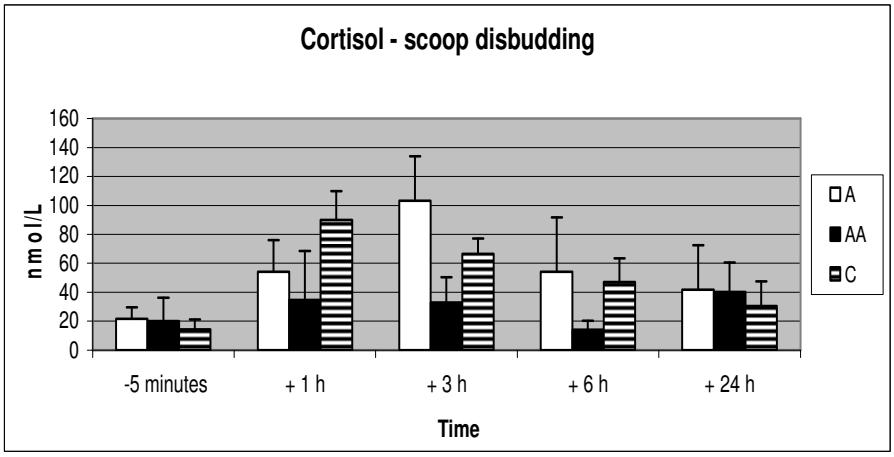

Figure 6.1. – Mean ±SD plasma cortisol concentration (nmol/L) of calves scoop-disbudded with no treatment (C), scoop-disbudded with regional anaesthesia (A) or scoop-disbudded with anaesthesia and analgesia (AA).

Comparing the baseline cortisol (- 5 minutes) to each group's values across time, we found the following differences:

- group A showed an increase at 1 and 3 hours after disbudding.
- group AA did not show any increase.
- group C (control) showed an increase at 1, 3 and 6 hours after disbudding.
- at 24 hours, although still numerical higher in all groups, cortisol concentrations were not statistically different from baseline.

Comparing the plasma cortisol between groups at each time we found that:

- there were no differences in baseline values.

- at 1 hour C group showed higher values compared to AA but not to A.

- at 3 hours post disbudding group A showed higher cortisol values compared to the other two groups and group C had higher cortisol than AA.

- there were no differences between groups at 24 hours.

Behaviour incidence

The incidence of the pain-related behaviours is presented in Table 6.3.and Figure 6.2.

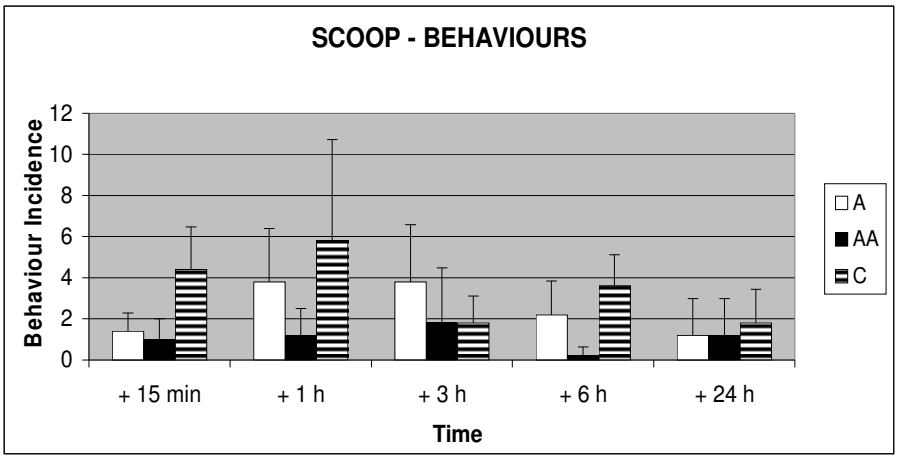

Figure 6.2. – Mean ±SD incidence of pain-related behaviours shown by calves after scoop-disbudding with no treatment (C), scoop-disbudded with regional anaesthesia (A) or scoop-disbudded with anaesthesia and analgesia (AA).

Signs of pain were evident immediately after disbudding (+ 15 m). The incidence at 15 minutes was higher in the calves not given regional anaesthesia (Group C) compared with the other groups. At 1 and 6 hours after disbudding we found a higher incidence of behaviours in group C compared to group AA but not to group A. At 3 hours Group A showed more behaviours than C and AA. At 24 hours there were no statistically differences between groups.

GROUP	BEHAVIOUR	TIME FROM DISBUDDING					
		+ 15 min	+ 1 h	+ 3 h	+ 6 h	+ 24 h	Total
A (n=5)	HEAD	2	6	12	1	1	22
	EARS	4	7	2	6	4	23
	RUBBING	1	6	4	2	1	14
	TRANSITIONS	0	0	1	1	0	2
	INERT LYING	0	0	0	1	0	1
	TOTAL	7	19	19	11	6	62
	INCIDENCE	1.4^{a}	3.8^{ab}	3.8^{a}	2.2^{a}	1.2^{a}	12.4
AA (n=5)	HEAD	2	4	1	0	4	11
	EARS	0	1	1	1	2	5
	RUBBING	2	1	5	0	0	8
	TRANSITIONS	1	0	2	0	0	3
	INERT LYING	0	0	0	0	0	0
	TOTAL	5	6	9	1	6	27
	INCIDENCE	1^{b}	1.2^{a}	1.8^{b}	0.2^{b}	1.2^{a}	5.4
C (n=5)	HEAD	10	16	5	5	4	40
	EARS	4	3	1	7	3	18
	RUBBING	7	9	3	6	2	27
	TRANSITIONS	1	0	0	0	0	1
	INERT LYING	0	0	0	0	0	0
	TOTAL	22	28	9	18	9	86
	INCIDENCE	4.4^{c}	5.6^{b}	1.8^{b}	3.6^{a}	1.8^{a}	17.2

Table 6.3. – Number of each behaviour and total incidence of pain-related behaviours at different times during the first 24 hours after scoop disbudding. Groups: A – disbudded after regional anaesthesia; AA – disbudded after regional anaesthesia and analgesia; C – disbudded with no treatment. Different lower case superscript letters indicate difference between groups for which $P < 0.05$.

Inert lying was only seen in one animal belonging to the non-treated group and only at 6 hours after disbudding.

Discussion

The fact that there were no differences in cortisol baseline values between groups and that they are similar to other studies, shows that restrain had no effect on this physiological measure. This may have been the consequence of gentle handling and quick blood sampling, as was demonstrated in our other study on the effect of handling (see Study 1).

The high cortisol and pain-related behaviour incidence showed by the control group soon after the procedure (15 min) demonstrates that this method causes very severe pain. Our results also show that this pain continues for, at least, 6 hours. The increase level and duration are very similar to those found in other studies until 3 hours

(Sylvester *et al*, 1998a) or 7 hours (Petrie *et al*, 1996; McMeekan *et al*, 1997; McMeekan *et al*, 1998). Our study shows a reduction in the incidence of pain-related behaviours in the control group at 3 hours after the procedure (no difference to the animals given analgesia) followed by a significant increase at 6 hours. This did not happen with the plasma cortisol levels. These changes can have several explanations: it may be an indication that the animals had to rest after a period of severe pain and high incidence of very active behaviours; or the effect of endogenous opioids released after an intense period of pain; or the result of a depressive state caused by the frustration of not being able to cope with intense pain (Sutherland *et al*, 2002a; Tse, 2004; Sumida *et al*, 2004); or the coincidence of a distractive episode at the time of behaviour recording that was not evident to the observer.

The lower incidence of behaviours immediately after disbudding in the anaesthesia group shows that the nerve block did reduce pain caused by the procedure but the increase in cortisol compared to baseline and similar incidence of behaviours compared to control at 1 hour, shows that the effect does not protect calves for long. The return of intense pain in group A is evident at 3 hours, at which time cortisol is higher than in the non-treated control group. This result had been previously shown in other studies in which only cortisol was assessed (McMeekan *et al*, 1998; Stafford *et al*, 2003). Our study shows that pain-related behaviour also increase in the A group at 3 hours although, because of high variation between individuals, we could only show a trend towards difference ($P = 0.054$) when compared with the other two groups. The reason for such a difference in cortisol of animals treated only with anaesthesia and the control group may be explained by the preparative and anti-inflammatory effect of cortisol released very early by the animals not treated (Sapolsky *et al*, 2000; Mostl and Palme, 2002). We suggest that, because anaesthesia treated animals did not release cortisol soon after the mutilation, a more severe inflammatory reaction occurred, followed by a state of hyperalgesia. The fact that the AA group did not show the same response at any time reinforces this idea, because flunixin-meglumine would have reduced the production of prostaglandins and other inflammation mediators.

No differences were found in the cortisol and behaviour-incidence at 24 hours, however the results seem to indicate that pain was still being felt by some individuals. The lack of statistical differences may be due to several factors: small number of animals in each group; high individual variation in mutilation severity; comparing groups that were all in the same condition (anaesthesia and analgesia effect had already

subsided). The inclusion of a sham-disbudded group could also have helped by showing that all disbudded groups had a higher incidence of pain-related behaviours (a total of 9 pain-related behaviours were recorded in the control group at 24 hours). No other study has looked at behaviour at 24 hours after scoop-disbudding.

In conclusion, this study shows that scoop-disbudding 4 to 5 month old calves causes immediate and intense pain. We also showed that pain is long-lasting (at least 6 hours) and may still affect the welfare of calves at 24 hours. Regional anaesthesia does not control pain for more than 1 hour and leads to more intense pain from 1 to 6 hours after the procedure. The association of lidocaine regional anaesthesia and analgesia by an anti-inflammatory drug (flunixin-meglumine) does control pain for the first 6 hours after scoop-disbudding.

We suggest that scoop-disbudding should not be used in small calves or, if absolutely necessary, only done under anaesthesia and analgesia.

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6.2. Evaluating and controlling pain after paste disbudding.

STUDY 5

Effect of caustic paste disbudding, using local anaesthesia with and without analgesia, on behaviour and cortisol of calves.

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Abstract

We looked at the effects of local anaesthesia with or without a non-steroidal-anti-inflammatory analgesic drug (flunixin-meglumine) on behaviour and plasma cortisol after caustic paste disbudding of 1-month-old calves: at 15 min, 1, 3, 6 and 24 h (Experiment 1; n = 32); at 10, 30 and 50 min (Experiment 2; n = 35); and after local anaesthesia effect subsided (90–180 min) (Experiment 3; n = 16).

In Experiment 1, cortisol was higher at 1 h in paste-disbudded calves than in all other groups. Paste-disbudded and paste-disbudded plus local anaesthesia calves showed higher cortisol at 1 h compared with baseline values. At 15 min paste-disbudded calves showed a higher incidence of pain-related behaviours than all other groups and those with anaesthetic or anaesthetic plus analgesia showed more pain-related behaviours than controls. At 1 and 3 h both paste-disbudded and paste-disbudded plus anaesthetic calves showed more pain behaviours than controls and those with analgesic.

In Experiment 2, paste-disbudded calves showed higher cortisol than all other groups at 30 and 50 min. No difference in cortisol was found between anaesthetic, anaesthetic plus analgesia and sham-disbudded calves. The incidence of pain-related behaviours was greater in paste-disbudded calves than in all other groups at all times. Calves

disbudded with anaesthetic or with anaesthetic and analgesic showed more pain-related behaviours than sham-disbudded animals during the first 10 min post-procedure.

In Experiment 3, paste-disbudded only calves had higher cortisol at 90 min and the anaesthetic-only group had higher cortisol at 180 min, when compared with control animals. Paste-disbudded calves showed more pain-related behaviours at 90, 120 and 150 min, and the anaesthetic-only disbudded calves at 180 min, when compared with sham-disbudded calves. In Experiment 1 and Experiment 3, several disbudded animals showed an "inert-lying" posture and this state may have reduced the display of the other more active behaviours.

The evidence indicates that caustic paste disbudding causes distress for at least 3 h and that local anaesthesia is efficient in controlling pain for the first hour but discomfort returns after the nerve blocking subsides. Overall, only local anaesthesia + NSAID provided effective reduction in pain as assessed by this method. Inert lying is a sign of distress in young calves after caustic paste disbudding.

Keywords: Calves; Caustic paste disbudding; Pain; Welfare; Pain-related behaviour; Cortisol; Analgesia

Introduction – edited and included in Chapter 1.

Objectives

Although pain and distress caused by disbudding is probably temporary, and the long run benefits for welfare are, as said, manifest, there are ethical reasons for studying more humane approaches to caustic paste disbudding. In order to assess the level of pain experienced after caustic paste disbudding and to understand which anaesthesia/analgesia protocols are better for calves' welfare, three experiments were designed Experiment 1 evaluated pain from 1 to 24 h in calves disbudded with: both local anaesthesia and a non-steroidal-anti-inflammatory analgesic, anaesthesia only, or neither; Experiment 2 compared pain during the first hour post-disbudding in calves in the same three conditions as in Experiment 1; Experiment 3 to assess the duration of action of effective local anaesthesia with lidocaine 2%.

2. Material and methods

2.1. Farm and animals

All experiments were carried out in the same 500 milking-cow dairy farm. All female calves were kept in a group pen (≃200 m^2) which consisted of a straw-bedded lying area and a solid floor feeding area. Animals were fed whole milk and concentrate

from two computer-controlled feeding stations. Calves were accustomed to human proximity due to routine care.

2.2. Common procedures

Different numbers of calves were included in each disbudding session, depending on the availability of calves of similar size and with a small horn bud. At each disbudding session calves were randomly allocated to the different groups.

The caustic paste used for disbudding (sodium hydroxide, SD-plus®) was applied with a spatula after clipping the hair around the base of the horn. Sham-disbudded animals were handled and hair-clipped in the same way, but an obstetric gel (VetopGel®) was applied on the horn instead of the paste. All calves were coloured-marked on both flanks with a randomly chosen number for easier identification when behaviour was assessed.

Cornual nerve anaesthesia needed for disbudding, was achieved by the injection of 5 mL of 2% lidocaine, without adrenaline, just ventral to the lateral edge of the frontal bone, midway from the base of the horn to the lateral canthus of the eye (Noordsy, 1994). Nerve blocking was confirmed by needle pricking, 5 min after injection when the hair was clipped. When applicable, 5 mL of saline was injected s/c by the same technique.

Blood sampling (7 mL) into a heparinised tube was by left jugular venipuncture. Blood was kept in ice then centrifuged and plasma frozen (−20 °C). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). The lowest detectable concentration of cortisol was 1.0 nmol/L. The intra-assay coefficients of variation was 9.2% for 1 ng/mL and 3.3% for 5 ng/mL and the inter-assay coefficients of variation was 3.4%, 1.4% and 1.2%, for Experiments 1, 2 and 3, respectively (Rodbard, 1974). The between-day differences for plasma cortisol concentrations within groups were not significant so data were pooled.

The frequency of four behaviours was recorded, namely: head-shaking, ear-flicking, head-rubbing (with hind feet or against objects) and transitions (quick transition from standing to lying and back to standing). Additionally, the number of animals adopting the “inert-lying” postures was registered if the calf showed the already described behaviour for more than 30 s in each observational period.

All disbudding procedures were done at the same time of day, in similar weather conditions and by the same operator. Behaviour recording and blood sampling (jugular venipucture) were by an experienced veterinarian, blind to treatments.

2.3. Experiment 1

Thirty-two female Holstein calves (mean age 27 ± 8 days; estimated weight of 60–70 kg), were randomly allocated to four groups: PD_1—caustic paste (SH-Plus®) disbudded 5 min after saline injection ($n = 8$); PDA_1—paste-disbudded 5 min after 2% lidocaine injection ($n = 9$); $PDAF_1$—paste-disbudded 5 min after i.v. injection of 3 mL flunixin-meglumine (approximately 2.2 mg/kg) and 2% lidocaine ($n = 7$); SD_1—sham-disbudded 5 min after saline injection ($n = 8$). Of the original nine calves belonging to SD_1 group, one was removed from the study because of respiratory disease signs.

Blood was collected at 5 min before (baseline values) and 1, 3, 6 and 24 h after disbudding. The incidence of the five pain-related behaviours was recorded throughout 15 min periods just after disbudding and then before each blood sampling.

2.4. Experiment 2

Thirty-five female Holstein calves (mean age 22 ± 4 days) were randomly allocated to four groups: PD_2—caustic paste (SH-Plus®) disbudded 5 min after saline injection ($n = 7$); PDA_2—paste-disbudded 5 min after 2% lidocaine injection ($n = 10$); $PDAF_2$—paste-disbudded 5 min after i.v. injection of 3 mL of flunixin-meglumine (approximately 2.2 mg/kg) and 2% lidocaine ($n = 10$); SD_2—sham-disbudded 5 min after saline injection ($n = 8$). Blood was collected at 5 min before (baseline values) and 10, 30 and 50 min after disbudding. Behaviour was recorded for 10 min periods, just before each blood sampling.

2.5. Experiment 3

Sixteen female Holstein calves (mean age 28 ± 6 days) were randomly allocated to three groups: PD_3—caustic paste (SH-Plus®) disbudded 5 min after saline injection ($n = 6$); PDA_2—paste-disbudded 5 min after 2% lidocaine injection ($n = 6$); SD_2—sham-disbudded 5 min after saline injection ($n = 4$).

Blood was collected at 5 min before (baseline values) and 90, 120, 150 and 180 min after disbudding. Behaviour was recorded throughout 15 min periods just after disbudding and then before each blood sampling.

2.6. Statistical analysis

Distributions of the variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used. Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance.

3. Results

There was no difference in mean age between groups in any of the experiments. Sham-disbudded calves did not show any changes in plasma cortisol levels at any time during the three experiments.

3.1. Experiment 1

Cortisol results from Experiment 1 are shown in Table 6.4.. There were no differences between groups in baseline values (−5 min) or at 6 h and 24 h after disbudding. At 1 h after the procedure, calves disbudded with no treatment (PD_1) showed higher cortisol compared with PDA_1 ($P = 0.006$) and with the other two groups ($P < 0.001$). At 1 h after disbudding PDA_1 calves showed numerically higher cortisol than $PDAF_1$, but this differences was not significant ($P = 0.055$). Only PD_1 ($P < 0.001$) and PDA_1 ($P = 0.015$) showed an increase at 1 h in relation to baseline. At 3 h $PDAF_1$ showed a lower cortisol level compared with PDA_1 and PD_1 (both $P = 0.005$) but equal to sham-disbudded animals.

The incidence of all pain-related behaviours (mean ± S.D.) recorded during Experiment 1, is presented in Table 6.5. Compared with sham-disbudded animals: PD1 showed more behaviours at 15 m and 1 h (both $P > 0.001$); PDA1 showed a higher incidence of pain-related behaviours at 15 min ($P = 0.008$), 1 h ($P = 0.008$), 3 h ($P = 0.006$) and 6 h ($P = 0.015$); PDAF1 only showed more pain-related behaviours at 15 min ($P < 0.001$). The non-treated disbudded calves showed more behaviours than PDA1 ($P = 0.001$) and PDAF1 ($P = 0.004$) at 15 min, but at 1 h only differed from PDAF1 ($P = 0.009$). The behaviours more commonly recorded after paste disbudding were head-shaking and head-rubbing at 15 min and 1 h (data not shown). At 3 h the “inert-lying” behaviour was recorded in three animals from the PDA1 and four animals from the PD1 group (Fig. 6.3.).

Group		Time from disbudding				
		- 5min	+ 1 hour	+ 3 hours	+ 6 hours	+ 24 hours
PDA_1	n=9	12.07 ±6.85^{aA}	32.88 ±26.59^{aB}	18.37 ±8.07^{aAB}	17.91 ±12.61^{aAB}	16.62 ±13.88^{aAB}
$PDAF_1$	n=7	12.94 ±10.27^{aA}	13.98 ±11.49^{aA}	6.25 ±5.74^{bA}	12.51 ±9.63^{aA}	9.18 ±8.56^{aA}
PD_1	n=8	16.86 ±11.15^{aA}	62.64 ±10.32^{bB}	19.44 ±14.14^{aA}	16.60 ±18.41^{aA}	12.34 ±12.05^{aA}
SD_1	n=8	13.78 ±9.81^{aA}	14.54 ±9.25^{aA}	12.32 ±12.32^{abA}	20.15 ±13.88^{aA}	13.26 ±14.09^{aA}

Different lower case letters indicate difference between groups for which $P < 0.05$.
Different uppercase letters indicate difference across time for which $P < 0.05$

Table 6.4. – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with caustic paste in Experiment 1. PDA_1: calves disbudded after treatment with lidocaine; $PDAF_1$: calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_1: calves disbudded without treatment; SD_1: calves sham-disbudded.

Group		Time from disbudding				
		+ 15 min	+1H	+ 3H	+ 6H	+ 24 H
PDA_1	n =9	2.67 ±1.66^{a}	2.11 ±1.54^{ab}	2.67 ±2.00^{a}	3.78 ±6.69^{a}	0.11 ±0.03^{a}
$PDAF_1$	n =7	3.57 ±1.27^{a}	0.57 ±0.79^{bc}	3.14 ±3.53^{ab}	1.57 ±2.94^{ab}	0.57 ±0.53^{a}
PD_1	n = 8	6.38 ±1.77^{b}	2.75 ±1.83^{a}	1.88 ±1.89^{ab}	0.50 ±1.07^{ab}	0.13 ±0.35^{a}
SD_1	n =8	0.63 ±0.74^{c}	0.13 ±0.35^{c}	0.38 ±0.52^{b}	0.13 ±0.35^{b}	0.38 ±0.52^{a}

Different lower case letters indicate difference between groups for which $P < 0.05$.

Table 6.5. – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with caustic paste in Experiment 1. Observational period: 15 min. Treatment groups: PDA_1 – calves disbudded after treatment with lidocaine; $PDAF_1$ – calves disbudded after treatment with lidocaine and analgesia (flunixin-meglumine); PD_1 – calves disbudded without treatment; SD_1 – calves sham-disbudded.

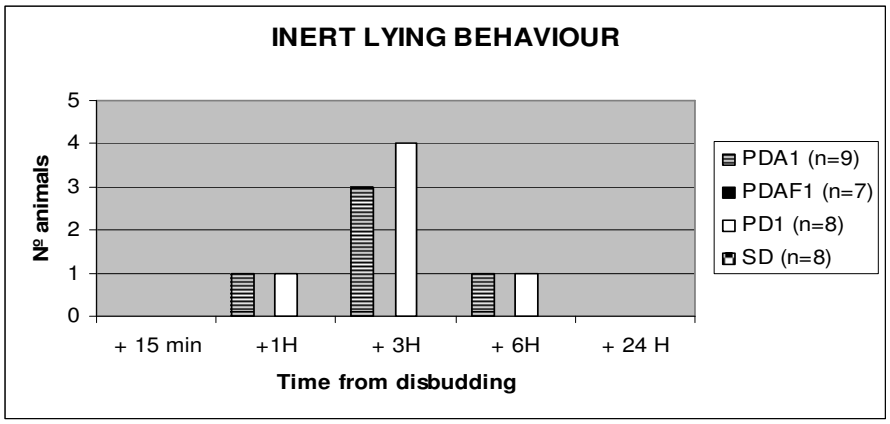

Fig 6.3. – Number of animals showing "inert lying" behaviour after caustic paste disbudding in Experiment 1. PDA_1: calves disbudded after treatment with lidocaine; $PDAF_1$: calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_1: calves disbudded without treatment; SD_1: calves sham-disbudded.

3.2. Experiment 2

No differences were found between groups in cortisol baseline values or at 10 min after disbudding (Table 6.6.). At 30 and 50 min after disbudding the PD_2 group showed a higher cortisol level compared with baseline ($P = 0.028$) and to all other groups (all $P < 0.05$).

Group		Time from disbudding			
		- 5 min	+ 10 min	+ 30 min	+ 50 min
PDA_2	n =10	11.16 ±7.9^{aA}	19.11 ±11.40^{aA}	16.71 ±10.69^{aA}	14.73 ±8.80^{aA}
$PDAF_2$	n =10	18.92 ±13.71^{aA}	23.14 ±16.67^{aA}	20.67 ±12.98^{aA}	19.80 ±9.67^{aA}
PD_2	n = 7	17.49 ±12.92^{aA}	25.54 ±15.15^{aAB}	41.39 ±14.85^{bBC}	42.32 ±14.47^{bC}
SD_2	n = 8	15.26 ±6.13^{aA}	16.84 ±7.06^{aA}	20.20 ±11.19^{aA}	14.34 ±8.57^{aA}

Different lower case letters indicate difference between groups for which P < 0.05. Different uppercase letters indicate difference across time for which P < 0.05

Table 6.6. – Plasma cortisol (mean ±SD) of calves disbudded with caustic paste in Experiment 2. PDA_2: calves disbudded after treatment with lidocaine; $PDAF_2$: calves disbudded after treatment with lidocaine and flunixin-meglumine; PD_2: calves disbudded without treatment; SD_2: calves sham-disbudded.

The incidence of pain-related behaviours (mean ± S.D.) observed during Experiment 2, is presented in Table 6.7. PD_2 showed more pain signs ($P < 0.001$, except PDA_2 $P = 0.005$) at all observation times compared with the other three groups. The PDA_2 showed more behaviours than sham-disbudded animals during the first ($P = 0.034$), second ($P = 0.034$) and third ($P = 0.016$) period of observation and more than $PDAF_2$ ($P = 0.035$) at 50 min. $PDAF_2$ showed the same behaviours as sham-disbudded animals at all times. The behaviours seen in disbudded animals were mainly head-shaking and head-rubbing. Sham-disbudded calves only showed ear-flicking. No inert lying was recorded during this experiment.

Group		Time from disbudding		
		0-10 min	20-30 min	40-50 min
PDA_2	n =10	1.60 ±0.97^{a}	0.60 ±0.52^{a}	1.60 ±1.26^{a}
$PDAF_2$	n =10	1.30 ±0.82^{ac}	0.50 ±0.53^{ac}	0.50 ±0.53^{b}
PD_2	n = 7	4.14 ±0.69^{b}	3.43 ±1.62^{b}	3.86 ±1.46^{c}
SD_2	n =8	0.63 ±0.52^{c}	0.00 ±0.00^{c}	0.25 ±0.46^{b}

Different lower case letters indicate difference between groups for which P < 0.05.

Table 6.7. – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with caustic paste in Experiment 2. Observational periods: 10 minutes. Treatment groups: PDA2 – calves disbudded after treatment with lidocaine; PDAF2 – calves disbudded after treatment with lidocaine and flunixin-meglumine; PD2 – calves disbudded without treatment; SD2 – calves sham-disbudded.

3.3. Experiment 3

Plasma cortisol variations from Experiment 3 are presented in Table 6.8.. Baseline cortisol levels were equal between groups. Calves disbudded with no treatment showed higher than baseline levels at 90 min ($P = 0.046$) and 180 min ($P = 0.028$). The PDA_3 showed an increase in cortisol at 180 min ($P = 0.028$) compared with baseline. At 90 min PD_3 showed higher levels than SD_3 ($P = 0.038$) but at 180 min it was the PDA_3 group that had higher cortisol levels than PD_3 ($P = 0.004$) and SD_3 ($P = 0.038$). At 120 and 150 min there were no cortisol differences between groups.

Group		Time from disbudding				
		-5 min	+ 90 m	+ 120 m	+ 150 m	+ 180 m
PDA_3	(n=6)	13.7 ±12.4^{aAB}	23.3 ±18.6^{aABC}	5.8 ±7.6^{aA}	28.1 ±15.7^{aBC}	43.3 ±9.8^{aC}
PD_3	(n=6)	14.9 ±8.4^{aA}	40.5 ±17.7^{bB}	11.9 ±16.4^{aAB}	20.1 ±8.5^{aAB}	27.2 ±5.3^{bB}
SD_3	(n=4)	12.7 ±12.3^{aA}	15.7 ±9.9^{aA}	12.8 ±12.9^{aA}	22.4 ±6.2^{aA}	16.5 ±14.3^{bA}

Different lower case letters indicate difference between groups for which $P < 0.05$. Different uppercase letters

Table 6.8. – Plasma cortisol (mean ±SD) of calves disbudded with caustic paste in Experiment 3. PDA_3: calves disbudded after treatment with lidocaine; PD_3: calves disbudded without treatment; SD_3: calves sham-disbudded.

Group		Time from disbudding			
		90 m	120 m	150 m	180 m
PDA_3	n =6	1.17 ±0.98^{a}	2.17 ±1.47^{ab}	2.17 ±1.94^{ab}	3.83 ±2.86^{a}
PD_3	n =6	4.33 ±2.58^{b}	2.33 ±1.37^{a}	2.83 ±1.05^{a}	0.83 ±0.75^{ab}
SD_3	n = 4	1.00 ±0.82^{a}	0.25 ±0.50^{b}	0.75 ±0.96^{b}	0^{b}

Different lower case letters indicate difference between groups for which $P < 0.05$.

Table 6.9. – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head rub and transitions from standing to lying) for calves disbudded with caustic paste in Experiment 3. Observational periods: 15 minutes. Treatment groups: PDA_3 – calves disbudded after treatment with lidocaine; PD_3 – calves disbudded without treatment; SD_3 – calves sham-disbudded.

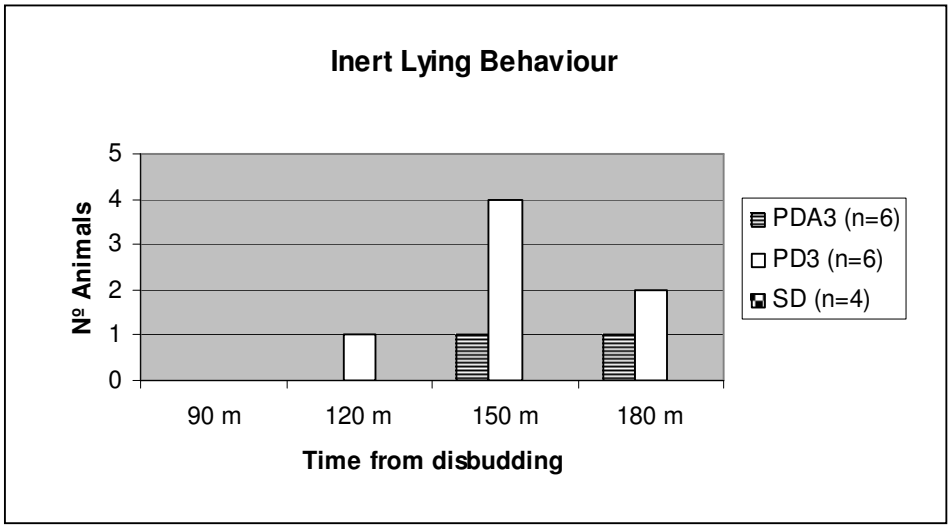

Fig. 6.4. -. Number of animals showing "inert lying" behaviour after caustic paste disbudding in Experiment 3. PDA_3: calves disbudded after treatment with lidocaine; PD_3: calves disbudded without treatment; SD_3: calves sham-disbudded.

The incidence of pain-related behaviours observed during Experiment 3, are presented in Table 6.9. Nontreated disbudded calves showed more behaviours than sham-disbudded at 90 (P = 0.019), 120, 150 (both P = 0.038) but not at 180 min (P = 0.114). Animals treated with local anaesthesia (PDA3) showed less behaviours at 90 min (P = 0.015) when compared with group PD3 and more at 180 min (P = 0.041) when compared with shamdisbudded calves (P = 0.038). At 180 min after disbudding PDA3 calves showed numerically more behaviours than PD3, but this difference was not significant (P = 0.065). The number of animals in each group showing ''inert lying'' is presented in Fig. 6.4..

4. Discussion

The division of the study into three separate experiments allowed the need for the second and third studies to be determined by analysing the results of the previous experiment. Also calf stress, due to frequent handling and blood sampling, was reduced by using three sets of animals.

No statistically significant changes were found in plasma cortisol or in behaviour where calves were sham-disbudded. This suggests that handling did not affect these measures.

Cortisol levels in Experiment 1 suggest that paste disbudding with no treatment only causes pain at 1 h. However, the incidence of pain-related behaviours at 3 h, in animals disbudded without analgesia, shows that some discomfort is felt until a later

period. Experiment 3 also shows a high incidence of pain behaviours until 3 h and a very high level of cortisol at 90 min, compared with baseline and sham-disbudded calves. Morisse *et al* (1995) and Vickers *et al* (2005) also showed a high number of pain-related behaviours up to 4 h, although Vickers' study was done with animals sedated with xylazine. All these results suggest that caustic paste disbudding causes distress in young calves for, at least, the first 3 h.

Graf and Senn (1999) showed a marked and immediate rise in plasma cortisol that peaked (80 nmol/L) 20 min after hot-iron disbudding. Likewise, Stafford *et al* (2003) found that cortisol increased rapidly and peaked at 30 min (90 nmol/L) after scoop-dehorning. Petrie *et al* (1996) and Grøndahl-Nielsen *et al* (1999) also showed plasma cortisol rises immediately after the procedures with hot-iron, suggesting intense and immediate pain. This is to be expected due to the strong restraint needed and the extensive and sudden damage of the tissues. The time from the application of caustic paste to the onset of measurable pain was only assessed in one study (Vickers *et al*, 2005) but was done in xylazine-sedated animals. In Experiment 1 we show that the highest incidence of pain-behaviours is seen almost immediately (0–15 min) after the disbudding suggesting that pain is felt very soon after the procedure. The results from Experiment 2 are in agreement with this suggestion because it shows behaviour modification soon after the procedure and a cortisol rise at 10 min, although it only becomes significant, compared with baseline and sham-disbudded animals, at 30 and 50 min. This suggests that behaviour analysis is a better indicator of very recent pain-induced distress possibly because the cortisol response is delayed.

The first two experiments show that plasma cortisol rises continuously reaching its highest level at 60 min after dehorning, in contrast to animals disbudded with hot-iron that show the highest cortisol level at 30 min (Doherty *et al*, 2007). This suggests that paste disbudding causes pain very soon after being in contact with the tissues, but is slower in triggering full nociceptor activity, probably due to the fact that caustic burns with strong bases, in contrast with the temporary activity of thermal burns, continues to cause damage as long as the active chemical is in contact with the tissue (Yano *et al*, 1993).

In contrast to other studies (Vickers *et al*, 2005), the present study shows that 5 mL of 2% lidocaine injected on the cornual nerve is efficient in reducing, but not preventing, the cortisol rise and pain-related behaviours that are seen in non-treated animals, for 1 h. However, the anaesthesia used in Vickers' study (1.5 mL to block the

cornual nerve and 3 mL s/c at the base of the horn) may not have been the more adequate, as was admitted by the authors. The control of pain in our study was incomplete during the first hour, for there were more behaviours than sham-disbudded and higher cortisol than baseline at 1 h, and was temporary since there was higher cortisol at 180 min and higher incidence of pain-related behaviours from 1 to 6 h as compared with sham-disbudded animals. This suggests that nerve-blocking does offer some protection to the calf disbudded with caustic paste but is not totally efficient and when it subsides additional discomfort is felt. Similar results have been shown after hot-iron and scoop-disbudding (Petrie *et al*, 1996; McMeekan *et al*, 1998; Sutherland *et al*, 2002ab; Stafford *et al*, 2003; Stilwell and Lima, 2004a), with cortisol and pain-related behaviours rising 1 or 2 h after the use of lidocaine regional anaesthesia.

The first two experiments of the present study show that flunixin-meglumine associated with the lidocaine block prevents the cortisol rise and pain-related behaviours. This suggests that the association of the two drugs is efficient in blocking early and intense stimuli arising from the chemically burned area. However, more behaviours related to pain (head shaking and head rubbing), are seen in the group disbudded after treatment with flunixin-meglumine (Fig. 6.5. and Fig. 6.7.) compared with sham-disbudded animals that just show ear-flicking (Fig. 6.6.) probably because of handling, hair-clipping or the contact of the gel with the skin. This difference indicates that some noxious sensations arise in disbudded and analgesic-treated animals during the first minutes of paste activity.

The three experiments show that the incidence of pain-related behaviours (Fig. 6.5. to Fig. 6.8.) is a useful indicator of pain in calves disbudded with caustic paste and should be used together with cortisol assessment. Our results show that head-shakes and head-rubs (Fig. 6.5. and Fig. 6.7.), especially rubbing the head with the hind feet, are the most common behaviours in calves disbudded with caustic paste and no pain-relief treatment. Ear flicks (Fig. 6.6.) were not easy to detect in those animals that were constantly shaking or rubbing their heads and this may explain the relatively low incidence of a very easy to perform behaviour. In contrast, this is the only behaviour that sham-disbudded animals show just after the procedure. Grøndahl-Nielsen *et al* (1999) found more ear-flicks in hot-iron disbudded calves compared with sham-disbudded but this could have been a result of a different type of tissue damage. In the present study, had this behaviour (ear-flicks) not been included, differences between disbudded and not-disbudded groups would have been greater. Mellor *et al* (2005)

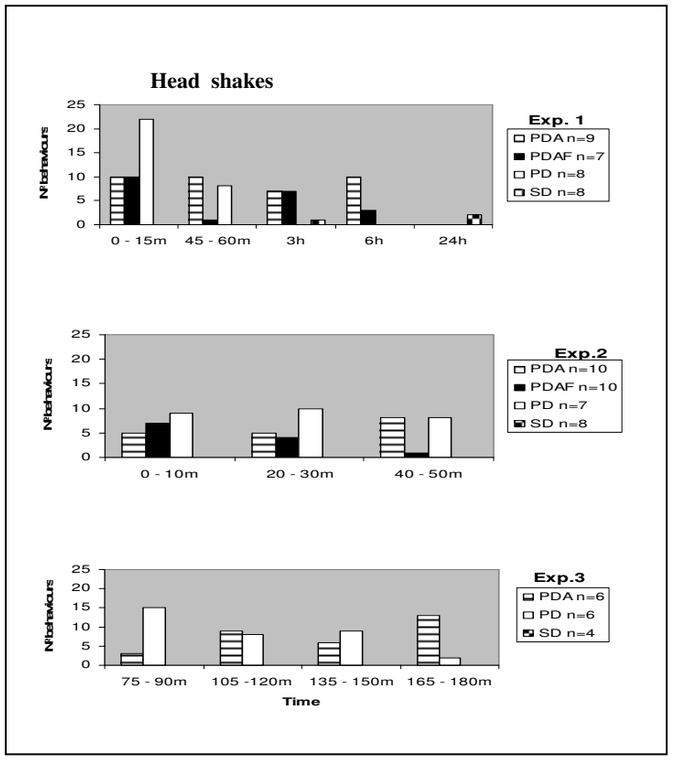

Fig. 6.5.. Incidence of "head shakes" for 1-month-old calves disbudded with caustic paste in three different experiments. PD: paste-disbudded; PDA: paste-disbudded with local anaesthesia; PDAF: paste-disbudded with local anaesthesia and analgesia (flunixin-meglumine); SD: sham-disbudded.

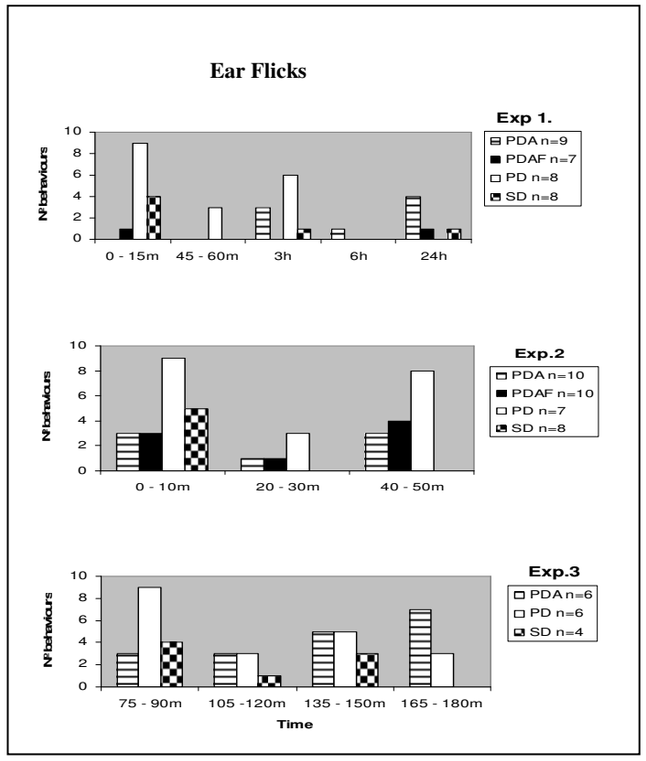

Fig 6.6. – Incidence of "ear flicks" for 1 month old calves disbudded with caustic paste in three different experiments. PDA: paste-disbudded with local anaesthesia; PDAF: paste disbudded with local anaesthesia and analgesia (flunixin-meglumine); PD: paste disbudded; SD: sham-disbudded. In Exp.1 recording at 3, 6 and 24 hours were equally done for a period of 15 min.

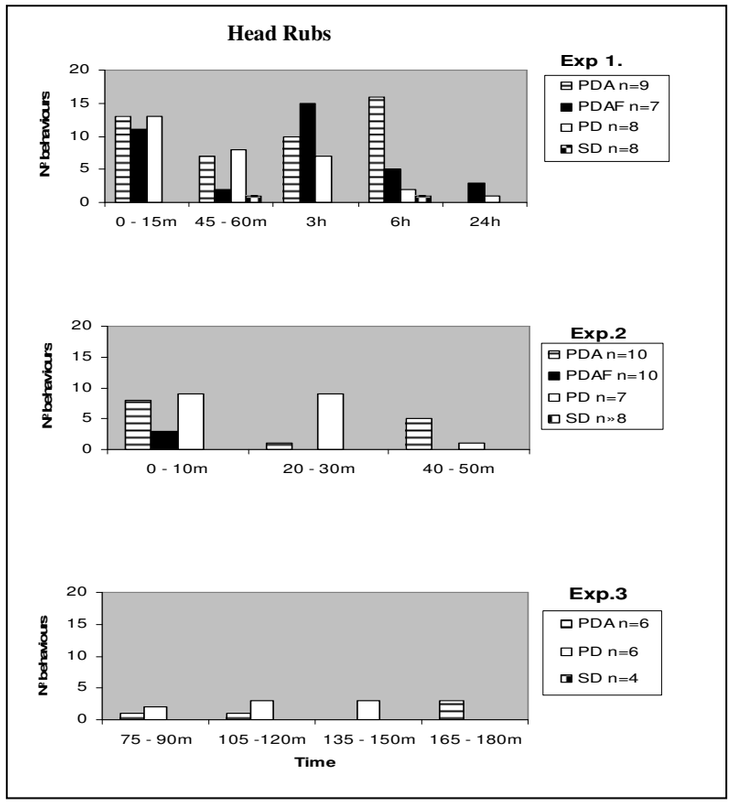

Fig 6.7. – Incidence of "head rubs" for 1 month old calves disbudded with caustic paste in three different experiments. PDA: paste-disbudded with local anaesthesia; PDAF: paste disbudded with local anaesthesia and analgesia (flunixin-meglumine); PD: paste disbudded; SD: sham-disbudded.

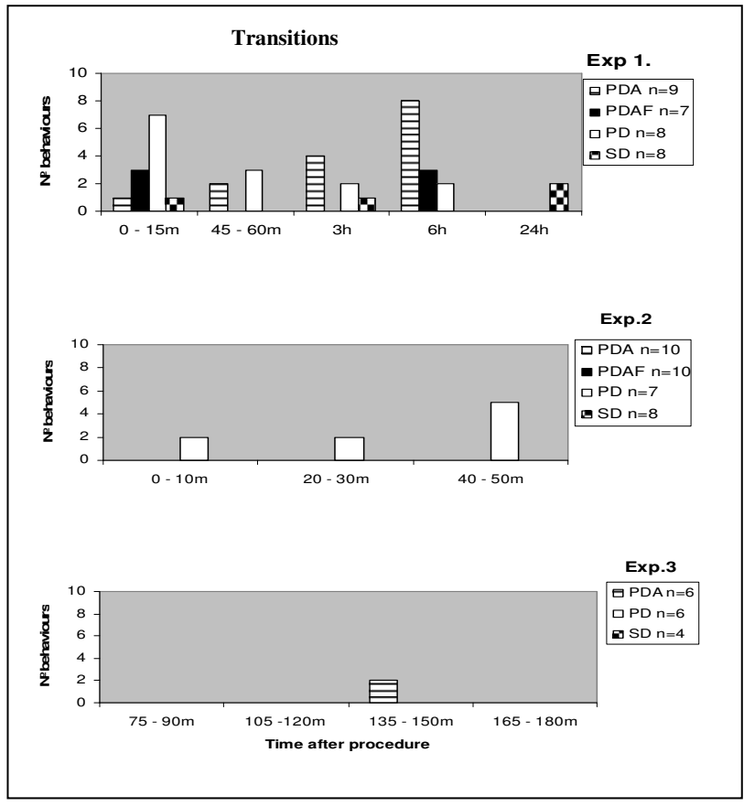

Fig 6.8. – Incidence of "transitions between lying and standing" behaviour for 1 month old calves disbudded with caustic paste in three different experiments. PDA: paste-disbudded with local anaesthesia; PDAF: paste disbudded with local anaesthesia and analgesia (flunixin-meglumine); PD: paste disbudded; SD: sham-disbudded.

suggest that behaviours that are shown by treated animals but not observed in controls or in animals subjected to some form of analgesia, is likely to be a useful index of noxious sensory input leading to pain and distress. Accordingly we suggest that ear-flick should not be used in studies on pain-induced distress after paste disbudding because of the risk of hiding important differences.

The results of the present study show that "inert lying" is an important behaviour that should be used to assess pain in young calves disbudded with caustic paste. "Inert lying" was never recorded during Experiment 2 but seen in several animals from Experiment 1 and 3 (Fig. 6.3. and Fig. 6.4., respectively). These results indicate that this behaviour relates to the intense distress felt during the first few hours after the caustic burn. However, this behaviour was not shown by animals in studies on scoop (see Study 4) and hot-iron dehorning (Stilwell *et al*, 2004b) even in animals showing very high cortisol values. The difference in age (younger in paste-disbudded animals) or the type of tissue damage, as suggested by Mellor *et al* (2005), may be responsible for the absence of this motionless state of calves. While assuming the inert-lying behaviour, the calves did not show any of the other monitored behaviours. By keeping almost immobile for the entire observational periods, four animals in the PD_1 group caused a reduction in the other recorded behaviours at 3 h, so that no difference was found when compared with sham-disbudded calves (Table 6.5.). A similar effect occurred for the PDA_3 and PD_3 at 150 min (Table 6.9.). Neglecting the significance of inert lying may lead to erroneous conclusions when evaluating the intensity and duration of distress in young calves.

5. Conclusions

Caustic paste disbudding causes intense pain from the first minutes after paste application and some behavioural signs of distress still remain at 3 h after the procedure. Behavioural analyses indicated that pain and distress are felt in calves treated with local anaesthesia and flunixin-meglumine during the first minutes after paste disbudding, although no significant increase in cortisol was shown. Treatment with local anaesthesia alone does control pain for the first hour but not from then to 6 h post-disbudding. We also suggest that inert-lying behaviour is a useful indicator in studies of pain in young calves although the further studies are needed to assess the relation between this behaviour and the intensity of pain.

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STUDY 6

Comparing plasma cortisol and behaviour of calves dehorned with caustic paste after non-steroidal-anti-inflammatory analgesia.

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Abstract

Caustic paste is frequently used for disbudding young female dairy calves. Nerve blocking may not be completely effective after such chemical tissue damage. Regional anaesthesia, together with a non-steroidal-anti-inflammatory drug (NSAID), was shown to reduce plasma cortisol in calves disbudded using caustic paste. To find out whether pre-emptive NSAID alone could control pain or whether NSAID reduces cortisol response by a mechanism other than by pain control, we compared cortisol levels and behaviour of 10 chemically disbudded calves treated with IV flunixin-meglumine, five of which were injected at 5 min (F0) and five injected at 60 min before dehorning (F1), with 5 sham-dehorned (ND) and 5 non-treated chemically disbudded animals (CD). There was a higher ($P < 0.001$) cortisol level in both NSAID-treated groups compared with ND at 1 h after disbudding, but no differences from control animals (CD). Non-treated disbudded animals showed higher cortisol at + 3 h compared with ND. A higher incidence of pain-related behaviours was shown in disbudded animals up to 3 h post-disbudding.

We concluded that pre-emptive analgesia treatment by itself is not effective in controlling pain and does not prevent blood cortisol increase after disbudding of calves with caustic paste.

Keywords: Dehorning; Calves; Analgesia cortisol; Behaviour

Introduction – edited and included in Chapter 1.

1. Objectives: This study was designed to answer two questions. Firstly, if local anaesthesia is not very efficient in controlling pain after chemical disbudding but local anaesthesia associated with NSAID is, can pain-induced distress be prevented by the pre-emptive use of flunixin-meglumine? Secondly, does NSAID reduce cortisol levels, after paste disbudding, by means of mechanisms other than its analgesic effect? We studied this by comparing the effect of treatment on blood cortisol and pain-related behaviours of young dairy calves.

2. Material and methods

2.1. Experimental procedures

The study was carried out on a 700 adult cow dairy farm, 50 km north of Lisbon, Portugal.

Twenty, 10 to 40 days of age (no difference in age between groups), female Holstein–Friesian calves were included in this study. The calves were kept in a group pen which consisted of a straw-bedded lying area and a solid-floor feeding area. An outside exercise area was usually available but was closed for the duration of the study. Animals were fed whole milk and concentrate from two computer-controlled feeding stations.

The calves were allocated randomly (5 numbers taken from a bag) to each treatment group. Treated calves were given 4 mL flunixin-meglumine (Finadyne, Schering-Plough ®, dose ± 2.2 mg/kg) intravenously, 1 h before disbudding (Group F1; mean age 27 ± 12 days) or 5 min before disbudding (Group F0; mean age 25 ± 12 days). Non-dehorned (Group ND; 30 ± 6 days) animals were injected IV with 4 mL of saline solution after first blood sampling (5 min before disbudding). The control group calves (Group CD; mean age 24 ± 10) were chemically disbudded with no treatment. The study was carried out in two different days but the pen, time of day, weather and stockman performing the disbudding, were exactly the same for all calves.

Five minutes after first blood collection, calves were forced to lie down, hair was clipped around horn buds and the caustic paste (SH-Plus® — Sodium Hydroxide) was applied with a spatula (following the normal procedure at this farm). ND animals were handled in the same way (including hair clipping) but instead of paste the horn buds were rubbed with an obstetric gel (VetTop Gel ®) for the equivalent time. Animals were coloured-marked on both sides with a randomly chosen number for easier identification

when behaviour was assessed. The observer was an experienced veterinary surgeon blind to the treatments.

Blood (7 mL) was collected into a heparinised tube by left jugular venipucture at 5 min before disbudding and at 1, 3, 6 and 24 h after disbudding. Blood was kept in ice then centrifuged and plasma was frozen (– 20 °C). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using commercial kits (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA). The inter-assay coefficients of variation for cortisol were 5.5% for the level of 1 μg/dL and 1.9% for the level of 5 μg/dL.

Five distress-reactions were registered while the calf was lying and the disbudding procedure was carried out: trying to stand on front legs (Stand), extending hind legs (Extend limbs), head shaking (Head shake), open mouth with no sound (Open Mouth) and vocalisation (Vocal).

Behaviour observations after disbudding were made for periods of 15 min, at 15 min, 1, 3, 6 and 24 h. We recorded the incidence of the following behaviours that have been previously used to evaluate pain after disbudding (Vickers *et al*, 2005, Grøndahl-Nielsen *et al*, 1999 and Morisse *et al*, 1995): a) head shake, b) ear flick, c) hind-limb scratching head or head rubbing against objects, c) quick transition from standing to lying and back to standing. During each observational period we also registered the occurrence of "inert lying", that we describe as calves lying with muzzle on flank, eyes closed and showing no reaction to surroundings.

2.2. Statistical analysis

Distributions of these variables were shown by Levene and Shapiro–Wilks tests to be non-normal, so non-parametric analyses were used. Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between the four groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance. *P*-values less than 0.05 were considered significant.

3. Results

Plasma cortisol changes over time for the four groups of calves are shown in Table 6.10.and Fig. 6.9. There were no differences ($P = 0.55$) in cortisol base-line values between groups. At 1 h after disbudding the two NSAID-treated groups showed an increase in cortisol compared with the base-line values ($P < 0.05$) and compared with the ND group ($P < 0.001$) but did not differ from control animals (CD). At + 3 h the CD

group cortisol level was higher than the ND but similar to both NSAID-treated groups. There were no cortisol differences between groups at six or 24 h. The sham-dehorned calves showed a lower cortisol level at + 24 h compared with + 6 h.

	TIME FROM DISBUDDING				
Group (n)	-5 minutes	+ 1 h	+ 3 h	+ 6 h	+ 24 h
ND (5)	10.54 ± 7.16 ^{aAB}	10.18 ± 4.14 ^{aAB}	6.50 ± 7.55 ^{aAB}	15.68 ± 13.06 ^{aA}	4.38 ± 2.98 ^{aB}
F0 (5)	11.63 ± 9.14 ^{aA}	67.07 ± 29.27 ^{bB}	10.27 ± 7.67 ^{abA}	10.58 ± 12.08 ^{aA}	14.10 ± 7.74 ^{aA}
F1 (5)	9.19 ± 10.06 ^{aA}	61.42 ± 25.40 ^{bB}	24.19 ± 39.01 ^{abA}	26.90 ± 31.21 ^{aA}	20.12 ± 28.35 ^{aA}
CD (5)	14.81 ± 7.53 ^{aA}	66.82 ± 11.26 ^{bB}	24.73 ± 14.18 ^{bA}	22.08 ± 22.18 ^{aA}	10.24 ± 12.28 ^{aA}

Column **+1h**: Different lower case letters indicate difference between groups for which $P < 0.01$.
Column **+3h**: Different lower case letters indicate difference between groups for which $P < 0.05$.
Different uppercase letters indicate difference ($P < 0.05$) across time for each group.

Table 6.10 - Mean ± SD plasma cortisol concentrations (nmol/L) for calves disbudded with caustic paste. ND: calves sham-disbudded; F0: calves disbudded + NSAID at -5 min; F1: calves disbudded + NSAID at -60 min; DC: calves disbudded without treatment

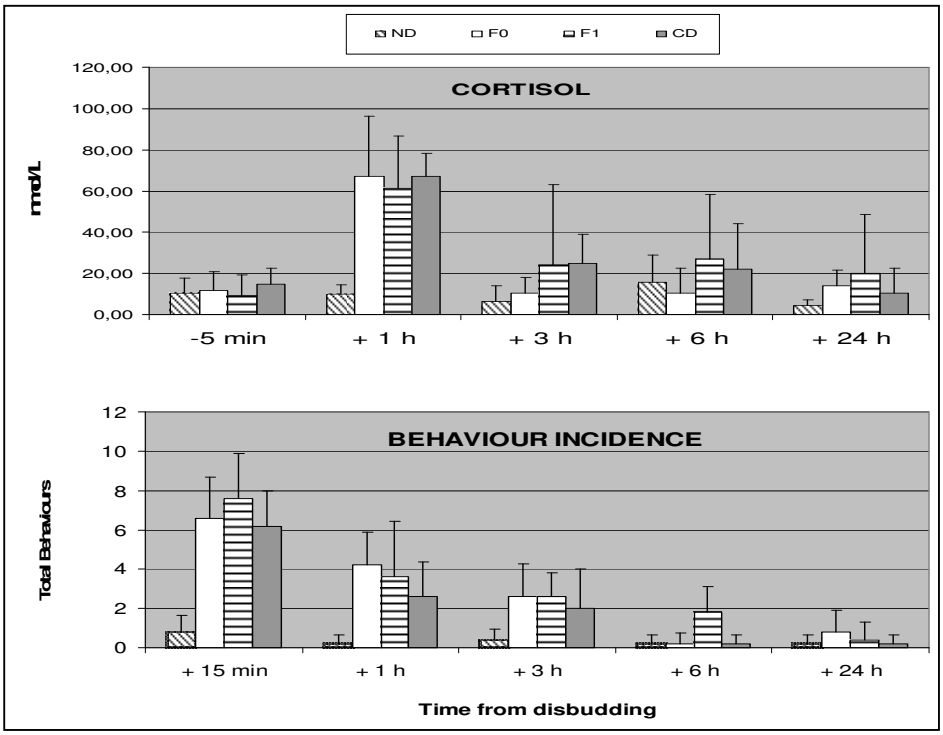

Fig. 6.9. Mean ± SD plasma cortisol concentrations (nmol/L) and mean incidence ± SD of pain-related behaviours for calves disbudded with caustic paste. ND: calves sham-disbudded; F0: calves disbudded + NSAID at -5 min; F1: calves disbudded + NSAID at -60 min; CD: calves disbudded without treatment

The analysis of the behaviour during the disbudding procedure (Fig. 6.10.) showed very few reactions (trying to stand, head shaking and hind-limb extension) to the restraining and paste/gel application in any of the groups. Two of the behaviours (open mouth and vocalisation) were not performed by any of calves.

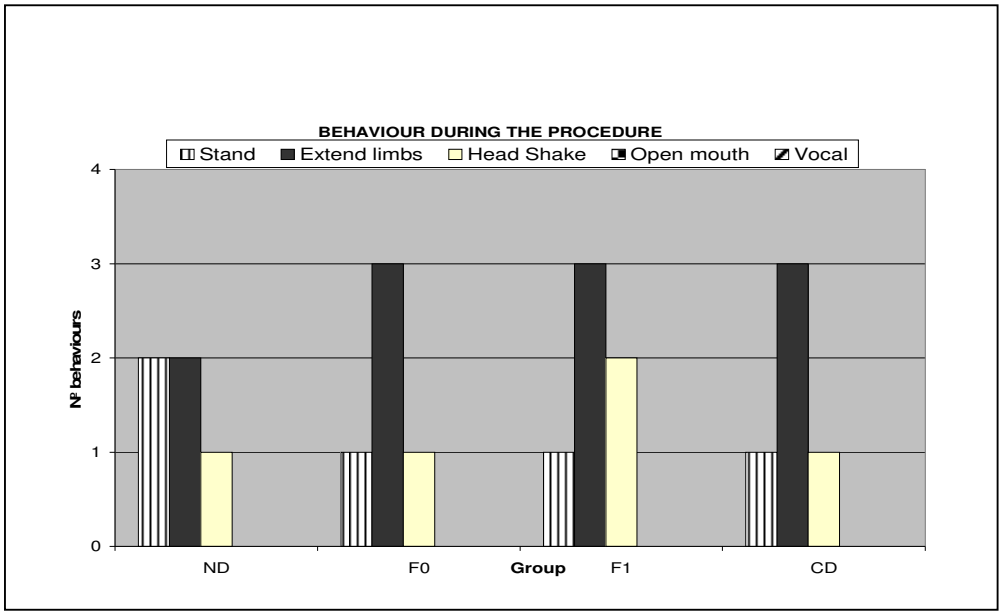

Fig.6.10. Number of behaviours shown by calves during the caustic paste disbudding procedure. ND: calves sham-disbudded (n=5); F0: calves disbudded + NSAID at -5 min (n=5) F1: calves disbudded + NSAID at -60 min (n=5); CD: calves disbudded without treatment (n=5).

The changes in incidence of the four pain-related behaviours recorded after disbudding are shown in Table 6.11 and Fig. 6.9.. There was a significant difference in total pain-behaviour incidence between each of the disbudded groups and the ND group at 15 min. The incidence of "head shaking" and "hind-limb scratching" in all disbudded groups showed a very significant difference compared with ND ($P = 0.008$). At 1 h CD and F0 group showed more behaviours than ND animals but the F1 group did not show any difference in behaviour incidence compared with ND animals ($P = 0.056$). However, there was a difference ($P = 0.032$) in the incidence of "head shaking" between ND and F1 animals. At 3 h only F0 showed a higher incidence of total behaviours when compared with ND, but F1 showed more head shaking ($P = 0.032$).

There was no difference in behaviour frequency at any time, between any of the disbudded groups. All disbudded groups showed a significant increase in disturbed behaviours at 15 min, 1 h and 3 h compared with behaviours shown at 6 or 24 h. Group F1 was the only group that showed higher frequency of disturbed behaviours at 15 min compared with all other periods of observation.

		TIME FROM DISBUDDING				
Group	Behaviour	+ 15 min	+ 1 h	+ 3 h	+ 6 h	+ 24 h
ND (n=5)	Head	0	0	0	0	1
	Hears	3	0	2	0	0
	Hind limb	0	1	0	1	0
	Transition	1	0	0	0	0
	TOTAL	4	1	2	1	1
	Mean ±SD	0.8 ±0.84 [a]	0.2 ±0.45 [a]	0.4 ±0.55 [a]	0.2 ±0.45 [a]	0.2 ±0.45 [a]
F0 (n= 5)	Head	17	10	6	1	0
	Hears	7	6	5	0	3
	Hind limb	7	5	2	0	2
	Transition	2	0	0	0	0
	TOTAL	33	21	13	1	5
	Mean ±SD	6.6 ±2.07 [bA]	4.2 ±1.79 [bAB]	2.6 ±1.82 [bB]	0.20 ±0.45 [aC]	0.8 ±1.10 [aC]
F1 (n = 5)	Head	19	14	6	2	1
	Hears	12	0	4	3	1
	Hind limb	7	4	3	4	0
	Transition	0	0	0	0	0
	TOTAL	38	18	13	9	2
	Mean ±SD	7.6 ±2.30 [bA]	3.6 ±3.21 [abB]	2.6 ±1.67 [abB]	1.8 ±1.30 [aBC]	0.4 ±0.89 [aC]
C (n = 5)	Head	15	5	0	0	0
	Hears	3	3	3	0	0
	Hind limb	9	3	4	1	1
	Transition	4	2	2	0	0
	TOTAL	31	13	9	1	1
	Mean ±SD	6.2 ±1.79 [bA]	2.6 ±1.52 [bAB]	2.0 ±2.0 [abB(	0.2 ±0.45 [a(	0.2 ±0.45 [aC]

Different lower case letters indicate difference between groups for which $P < 0.05$
Different uppercase letters indicate difference ($P < 0.05$) across time for each group.

Table 6.11. - Pain-related behaviours incidence rate for calves disbudded with caustic paste. ND: calves sham-disbudded; F0: calves disbudded + NSAID at -5 min; F1: calves disbudded + NSAID at -60 min; CD: calves disbudded without treatment.

Inert lying was observed in a few animals from the disbudded groups at 1, 3 and 6 h post-disbudding (Fig. 6.11.). Although no animal of the ND group was seen showing this posture, no statistical difference was found between the groups.

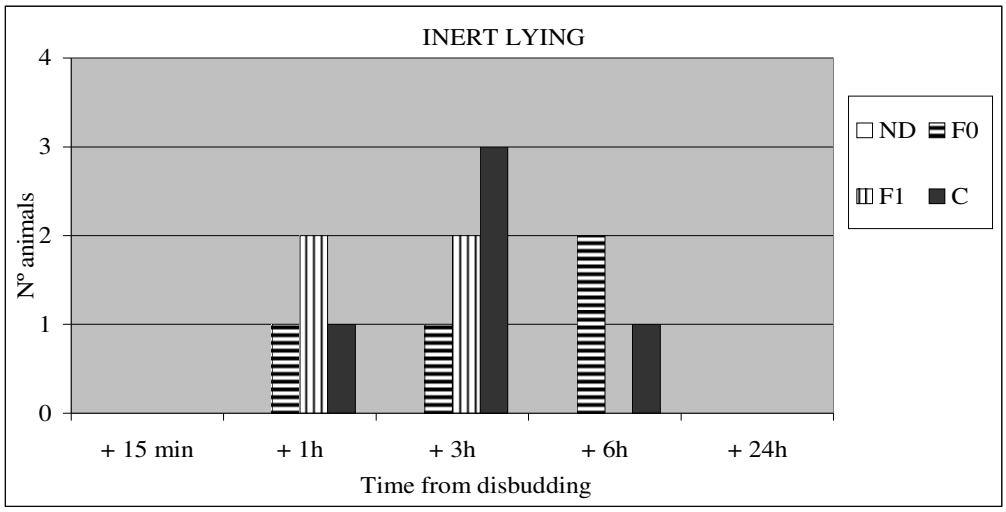

Fig.6.11. Number of calves showing inert lying behaviour after caustic paste disbudding. ND: calves sham-disbudded; F0: calves disbudded + NSAID at -5 min; F1: calves disbudded + NSAID at -60 min; CD: calves disbudded without treatment

4. Discussion and conclusions

The animals included in the study were of similar ages, had a common background and were handled so as not to cause them distress. Consequently all four groups involved in this study showed similar cortisol base-line levels.

Plasma cortisol concentrations peaked at 1 h in all disbudded animals, including those treated with NSAID, but did not change in non-disbudded animals, showing that the procedure does cause distress. Other studies found a similar increase in chemically disbudded non-treated animals (Stilwell *et al*, 2004b; and Morisse *et al*, 1995). The fact that cortisol levels in the control non-treated group were still higher than sham-disbudded at + 3 h shows that pain is present to a later time than was admitted by other studies (Morisse *et al*, 1995) and that the analgesia may have had some effect in alleviating the distress at this time. The cortisol results of our study, when we take account of the context, as proposed by Broom and Johnson (2000), show that caustic paste disbudding causes poor welfare in calves and that analgesic treatment, even if given in a pre-emptive way, is not sufficient to prevent the cortisol rise seen in disbudded but non-treated animals.

The high cortisol values at + 6 h (time of day: 16.00 h) compared with + 24 h (time of day: 10.00 h) in the ND group may be due to circadian variation or some husbandry factor not identified.

The small level of reactions shown by all the calves to the handling and actual disbudding can be explained as follows: these animals are used to the presence and even

contact with herdspersons; their size and strength are still easily subdued by an experienced operator; pain after tissue damage by chemicals only starts a few minutes after application, as explained by Choinière *et al* (1989). The fact that no calf vocalised during the procedure is also a sign of reduced distress because young animals usually vocalise when severe fear or pain is elicited (Watts and Stookey, 2000).

The post-disbudding behaviour observations support the idea of distress caused by caustic paste. At 15 min, all disbudded calves showed a very significant incidence of pro-active pain-related behaviours — especially head shaking. These may be intended to relieve the "itching pain" sensation that has been described in humans (Ma *et al*, 2007). Other behaviours also observed, but not recorded because of rarity, included backing and even falling after shaking the head vigorously. The higher incidence of pain-related behaviours at + 1 h and + 3 h (group F0), compared with sham-disbudded animals, shows that pain-induced distress is more prolonged than cortisol analysis suggested. This is in agreement with the study by Vickers *et al* (2005) that showed a higher level of three behaviours (head rub, head shake and transition) in paste-disbudded animals (sedated with xylazine) compared with sham-disbudded ones during the first 4 h.

Between one and 6 h after the procedure, some disbudded animals reduced their pro-active behaviour and assumed a passive one (inert lying). This behaviour, recorded in other studies with lambs after castration and described as the time during which it was difficult to elicit any evidence of conscious awareness (Molony *et al*, 1993), might be stress-induced and so an important indicator of an aversive experience (Gregory, 2004). Two of the calves that showed this behaviour at 3 h also had the highest cortisol level (110.26 and 111.05 nmol/L). Lane (2006) suggests that helplessness in animals is perhaps the closest correlate to a depressive state and very high levels of glucocorticoids have been found in animals suffering from this condition (Gregory, 2004; Sumida *et al*, 2004). This behaviour was not observed in our previous studies with animals dehorned by scoop or hot-iron (Stilwell and Lima, 2004a; Stilwell *et al*, 2004b). We suggest that this may be due to the different way each method damages the same tissue (Mellor *et al*, 2005) or the age of the calves (younger in the present study). The "inert lying" also had a collateral effect: the inactive animal does not perform as many behaviours and so reduces the group total count for that time period. The consequence of this is evident if we compare behaviour incidence between ND *vs* F1 at 1 h post-disbudding and ND *vs* CD at 3 h (Table 6.11.). The lack of statistical difference was due to the fact that two animals in the F1 group and three in the CD group adopted the "inert lying" posture

(Fig. 6.11.) and so reduced the frequency of behaviours. Overlooking some of these animals, because they do not show active behaviours, may lead to wrong conclusions regarding distress caused by some procedures.

Previous studies (Stilwell and Lima, 2004a) have shown that flunixin-meglumine in combination with lidocaine does reduce cortisol response to chemical disbudding, but this could be a direct effect of the NSAID on cortisol production and not necessarily through its analgesic effect. Only one study (McMeekan *et al*, 1998) has looked at the effect of a NSAID on its own (ketoprofen injected 20 min before amputation dehorning). These authors showed that during the first 1.30 h after dehorning the mean cortisol concentrations did not differ significantly from non-treated scoop-dehorned calves. Likewise, the present study shows that flunixin-meglumine, as a sole treatment, does not prevent cortisol rise after caustic paste disbudding, suggesting that it is likely that activation of damaged nerves in the chemically burned area is the major cause of distress and not an inflammation-related pain.

We concluded that analgesia with flunixin-meglumine alone, even if administered in a pre-emptive way, is not efficient in controlling pain-induced distress resulting from tissue damage in chemically disbudded young calves. Also, the study shows that pain evaluation should include cortisol and behaviour data so as to avoid overlooking animals in pain.

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6.3. Evaluating and controlling pain after hot-iron disbudding.

STUDY 7

Effect of hot-iron disbudding, using regional anaesthesia with and without analgesia, on cortisol and behaviour of calves.

Submitted for publication.

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Abstract:

The objective of the experiments included in this study was to assess plasma cortisol concentration and behaviour changes in calves hot-iron disbudded after different analgesic protocols. Experiment 1 assessed the response at 1, 3, 6 and 24 hours after disbudding with regional anaesthesia, with or without carprofen analgesia. At 1h after disbudding, cortisol and pain-behaviour incidence was higher in disbudded only than in sham-disbudded or carprofen-treated animals. After 1 hour, disbudded plus anaesthesia calves had higher cortisol than sham-disbudded. Immediately after the procedure pain-related behaviours were more frequent in the disbudded only group than in any other group. At 1h, disbudded only and disbudded plus anaesthesia calves showed more pain behaviours than the other groups. At 3h disbudded plus anaesthesia calves showed more pain behaviours than all other animals. In Experiment 2 the response at 10, 30 and 50 minutes of calves disbudded after anaesthesia only, anaesthesia plus carprofen, carprofen only and no-treatment, was studied. All disbudded calves showed higher cortisol at 10 min compared with base-line and sham-disbudded animals, but disbudded plus anaesthesia calves had lower concentrations than disbudded only and carprofen-only calves. At 30 and 50 min disbudded only and disbudded plus carprofen-only animals had higher cortisol than sham-disbudded and disbudded plus anaesthesia (with and without carprofen) calves. At 30 min, cortisol peaked in disbudded only calves and

was higher than that of the disbudded plus carprofen-only calves. Disbudded animals that were not given anaesthesia struggled more during the procedure than sham-disbudded calves and those that were given a nerve block. At 10 min disbudded only animals and disbudded plus carprofen-only calves showed more pain-related behaviours than all other groups. Disbudded plus anaesthesia (with or without carprofen) showed more pain behaviours than sham-disbudded calves. At 30 and 50 minutes disbudded only calves showed more pain behaviours than all other groups and the pain-related behaviours were more in disbudded plus carprofen-only compared with sham-disbudded animals. In Experiment 3, pain in calves treated with regional anaesthesia at 90, 120 and 150 min was assessed. The overall plasma cortisol concentrations were higher in disbudded calves than in sham-disbudded calves. The incidence of altered behaviours was higher in disbudded calves at 90 and 120 min. There are clear indicators of pain in calves disbudded with a hot-iron after the effect of regional anaesthesia has subsided unless an analgesic is given. Carprofen without anaesthesia does not reduce cortisol or prevent pain-related behaviours even if given in a pre-emptive way.

Key words: disbudding, pain, cortisol, behaviour, welfare

Introduction – edited and included in Chapter 1 – General Introduction.

Objectives

The objectives of the three experiments included in this study were to assess pain-related distress in calves after hot-iron disbudding by measuring physiological (plasma cortisol concentration) and behavioural responses after different analgesic protocols with carprofen and lidocaine regional nerve block.

Material and Methods

Farm and animals

All the experiments were done at the same 300 milking-cow dairy farm. At this farm new-born calves are kept in individual hutches, bedded with straw, until weaning. Before weaning they are fed milk at 5% of body-weight in the morning and evening and have free access to hay, calf starter and water. Weaning is done when the calf eats over one kilogram/day of concentrate for three consecutive days. After weaning calves are moved to an open stable and have free access to concentrate, alfafa hay and water.

Experimental Procedures and Design

The study was divided into three separate experiments so as to reduce the individual stress from recurrent handling and blood sampling. In Experiment 1 the

effects of hot-iron disbudding from 1 to 24 hours, was investigated; Experiment 2 concerned the effect of disbudding during the first hour after the procedure; and Experiment 3 the response after a regional nerve block had subsided.

Common procedures

All disbudding was carried out between 10 and 11 a.m. by the same operator, blind to the treatments. The iron was electrically-heated and applied over the horn bud for ~30 seconds for each horn, producing a deep burn of the tissue at the base of the horn. A cold device was applied for the same time to the control calves (sham-disbudded). Disbudding was done a few days after weaning, corresponding to the age of 8 to 10 weeks.

Cornual nerve anaesthesia was achieved by the injection of 5 ml of 2% lidocaine (Anestesin ®, Laboratorio Sorologico, Portugal), without adrenaline, just ventral to the lateral edge of the frontal bone, midway from the base of the horn to the lateral cantus of the eye (Noordsy, 1994; Greene, 2003). In control groups groups, a 0.9% saline solution was administered in the same way. Carprofen (2.5 ml, approx 1.4 mg/kg; Rimadyl®, Pfizer-Animal Health, Dundee, UK) was given i.v. 15 minutes before the procedure was carried out or, in controls, the same dose of a saline solution was given i.v. Animals' approximate weight (80 – 90 kg) was estimated by body size.

Blood sampling (7ml) into a heparinised tube was by left jugular venipuncture. Blood was kept on ice then centrifuged and the plasma frozen (-20C). Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA) at the Faculdade de Medicina Veterinaria.

Behaviour was assessed by an experienced veterinarian blind to the treatments. The frequencies of four pain-related behaviours (ear-flicking, head-shaking, head rubbing with hind foot and quick transitions from standing to lying and back to standing) were recorded by a veterinarian just before each blood sampling. Struggling during the procedure was graded from 0 = no struggling to 5 = severe struggling.

Experiment 1

Twenty-eight female calves, mean age 88 ±17 days, were randomly assigned to four groups: DA_1: disbudded after lidocaine injection (n=7); DAC_1: disbudded after lidocaine and carprofen injection (n=7); D_1: disbudded after treatments with saline (n=7); ND_1: sham-disbudded after treatments with saline (n=8). Blood was collected 5 min before

the procedure and then at 1, 3, 6 and 24 hours after disbudding. Behaviour was assessed for periods of 15 minutes at 15 min, 1, 3, 6 and 24 hours after disbudding.

One calf was eliminated from the DA_1 group because of clinical disease signs shown during the experiment.

Experiment 2

Thirty-seven female calves, mean age 75 ±9 days, were randomly assigned to five groups: DA_2 disbudded after lidocaine (n=7); DAC_2: disbudded after lidocaine and carprofen (n=7); DC_2: disbudded after carprofen (n=8); D_2: disbudded with no treatments (n=7); ND_2: sham-disbudded (n=8). Blood was collected 5 min before the procedure and then at 10, 30 and 50 minutes after disbudding. During the procedure struggling was graded by the observer. Pain-related behaviours were recorded, for periods of 10 minutes, just before each blood sampling.

Experiment 3

Fourteen female calves, mean age 64 ±7 days were randomly assigned to two groups: DA_3: disbudded after lidocaine nerve block (n=8); ND_3: sham-disbudded (n=6). Blood was collected 5 min before the procedure and then at 90, 120 and 150 minutes after disbudding. Behaviour was assessed for periods of 10 minutes at 10 minutes, 80, 110 and 140 minutes after disbudding.

One calf was later eliminated from the ND_3 group because of illness.

Statistical analysis

Analysis was done with the programme SPSS for Windows ®. Distributions of the variables were shown by Levene and Shapiro-Wilks tests to be non-normal, so non-parametric analyses were used. Differences in cortisol levels or pain-related behaviour incidence between the five groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance. Differences in cortisol over time, within the same groups, were tested using the Wilcoxon matched-pairs signed-ranks test. *P*-values less than 0.05 were considered significant.

Results

Experiment 1

There were no differences in age between groups. DA_1 (83 ±15); DAC_1 (96 ±20); D_1 (98 ±15); ND_1 (76 ±11).

Cortisol (Table 6.12.) – No differences were found between base-line plasma cortisol concentrations levels. At 1 hour calves disbudded with no treatment showed higher cortisol than base-line, sham-disbudded and calves treated with regional

anaesthesia and carprofen. Also at 1 h calves treated only with lidocaine showed higher cortisol than sham-disbudded calves. Group treated with lidocaine and carprofen show a higher level of cortisol at 24 hours.

Group	n	Time from dehorning				
		-5 min	+ 1h	+ 3h	+ 6h	+ 24h
DA_1	6	18.21 ±7.81^{aA}	16.94 ±7.58^{abA}	25.17 ±20.05^{aA}	28.19 ±16.61^{aA}	17.11 ±9.05^{aA}
DAC_1	6	20.60 ±11.51^{aA}	13.56 ±8.40^{acA}	19.77 ±16.41^{aA}	16.88 ±17.39^{aA}	38.87 ±20.36^{bB}
D_1	7	15.64 ±9.51^{aA}	33.89 ±15.33^{bB}	20.95 ±25.71^{aAB}	16.51 ±12.18^{aAB}	25.13 ±16.38^{aAB}
ND_1	8	10.64 ±7.56^{aAB}	7.17 ±3.99^{cA}	10.09 ±6.10^{aABC}	12.40 ±6.09^{aBC}	15.66 ±5.98^{aC}

Different lower case superscript letters indicate difference between groups.
Different upper case superscript letters indicate difference across time.

Table 6.12. – Effects on plasma cortisol (mean ±SD) of calves hot-iron disbudded with anaesthesia and analgesia in Experiment 1. DA_1: calves disbudded after treatment with regional lidocaine; DAC_1: calves disbudded after treatment with regional lidocaine and s/c carprofen; D_1: calves disbudded with no treatment; ND_1: calves sham-disbudded.

Behaviour (Table 6.13.) – Immediately after the procedure, animals disbudded with no treatment showed significantly more pain-related behaviours than all other groups. One hour after disbudding both non-treated and treated only with lidocaine groups showed more behaviours compared with the other groups but non-treated ones had more behaviours than ones treated with lidocaine. At 3 hours the lidocaine treated animals continued to show higher incidence of pain-related behaviours compared with treated with carprofen or sham-disbudded but not compared with non-treated calves. At 6 hours, although no statistical significance was found, there were more behaviours in all disbudded groups compared to sham disbudded ones.

Group	n	Time from dehorning				
		+15m	+1 H	+ 3H	+ 6H	+ 24H
DA_1	6	1.50 ±0.84^{a}	1.67 ±1.03^{a}	2.50 ±0.55^{a}	2.83 ±2.40^{a}	0.33 ±0.52^{a}
DAC_1	7	0.57 ±0.53^{a}	0.57 ±0.79^{b}	0.57±1.13^{bc}	1.00 ±1.29^{a}	0.43 ±0.79^{a}
D_1	7	6.14 ±1,35^{b}	4.43 ±1.51^{a}	1.43 ±0.98^{b}	1.86 ±1.57^{a}	0.43 ±0.53^{a}
ND_1	8	0.57 ±0.53^{a}	0.29 ±0.49^{b}	0.14 ±0.38^{c}	0.43 ±0.79^{a}	0.43 ±0.79^{a}

Different lower case superscript letters indicate difference between groups.

Table 6.13. – Effects on incidence of four pain-related behaviors (mean ±SD) in calves hot-iron disbudded with anaesthesia and analgesia in Experiment 1. DA_1: calves disbudded after treatment with regional lidocaine; DAC_1: calves disbudded after treatment with regional lidocaine and s/c carprofen; D_1: calves disbudded with no treatment; ND_1: calves sham-disbudded.

Experiment 2

There were no differences in age (mean days ±SD) between groups: DA_2 (84 ±8); DAC_2 (77 ±4); DC_2 (75 ±2); D_2 (68 ±8); ND_2 (71 ±11).

		Time from dehorning			
Group	n	-5 min	+ 10 min	+ 30 min	+ 50 min
DA_2	7	12.94 ±10.53^{aAC}	44.94 ±15.80^{aB}	15.16 ±8.52^{aA}	8.31 ±6.36^{aC}
DAC_2	7	19.06 ±7.45^{aAB}	32.69 ±17.54^{acA}	12.76 ±3.94^{aBC}	8.10 ±3.45^{aC}
DC_2	8	15.14 ±6.49^{aA}	83.77 ±18.66^{bB}	91.69 ±26.42^{bB}	72.02 ±38.66^{bB}
D_2	7	22.53 ±9.25^{aA}	85.12 ±34.24^{bB}	122.18 ±20.21^{cC}	68.90 ±20.56^{bB}
ND_2	8	19.23 ±9.41^{aA}	14.17 ±4.70^{cA}	14.49 ±8.49^{aA}	11.76 ±7.35^{aA}

Different lower case superscript letters indicate difference between groups.
Different upper case superscript letters indicate difference across time.

Table 6.14. – Effects on plasma cortisol (mean ±SD) of calves hot-iron disbudded with anaesthesia and analgesia in Experiment 2. DA_2: calves disbudded after treatment with regional lidocaine; $DA+C_2$: calves disbudded after treatment with regional lidocaine and s/c carprofen; $D+C_2$: calves disbudded after s/c carprofen; D_2: calves disbudded with no treatment; ND_2: calves sham-disbudded.

Cortisol (Table 6.14.) – At 10 minutes after the procedure all disbudded animals, with the exception of the lidocaine plus carprofen group (DAC_2), had higher cortisol compared with base-line and sham-disbudded, but those blocked by lidocaine (DA_2 and DAC_2) had lower cortisol compared with those not given regional anaesthesia. At 30 minutes non-treated disbudded animals' cortisol peaked at levels higher than all other groups and disbudded with carprofen-only had higher cortisol than those that were given lidocaine. At 50 minutes all those disbudded without anaesthesia (DC_2 and D_2) had higher values than base-line and the other three groups.

Struggling was more severe in the disbudded only (Grade: 3.86 ±0.9) and the disbudded carprofen-only (2.63 ±1.06) calves than the other three groups (Grade > 0.75).

	Time from dehorning		
Group	0 - 10m	+20 - 30m	+40 - 50m
DA_2	1.71 ±1.11[a]	0.71 ±0.76[a]	2.14 ±1.35 [ab]
DAC_2	1.29 ±1,50[a]	1.00 ±1.15[a]	0.86 ±1.21 [ad]
DC_2	4.00 ±1.41[b]	2.88 ±0.99[b]	2.88 ±1.13 [bc]
D_2	6.14 ±2.12[b]	4.86 ±1.46[c]	5.00 ±2.83 [c]
ND_2	1.75 ±1.04[a]	0.88 ±0.64[a]	0.63 ±0.74[d]

Different lower case superscript letters indicate difference between groups.

Table 6.15. – Effects on incidence of four pain-related behaviors (mean ±SD) in calves hot-iron disbudded, with anaesthesia and analgesia in Experiment 2. DA_2: calves disbudded after treatment with regional lidocaine; $DA+C_2$: calves disbudded after treatment with regional lidocaine and s/c carprofen; $D+C_2$: calves disbudded after s/c carprofen; D_2: calves disbudded with no treatment; ND_2: calves sham-disbudded.

Behaviour (Table 6.15) – Immediately after the procedure (< 10min) pain-related behaviour frequency was significantly higher in disbudded animals not given anaesthesia compared with all other groups (P = 0.001). There were no differences between sham-disbudded and those given regional anaesthesia. At 30 min disbudded only calves (D_2) continued to show more behaviours than DA_2, DAC_2, ND_2 (P < 0.001) and DC_2 (P = 0.014). Disbudded plus carprofen-only calves showed more pain-related behaviours than sham-disbudded (P = 0.002) and disbudded plus lidocaine, with or without carprofen (P < 0.002 and P < 0.009, respectively). At 50 min, only calves disbudded plus anaesthesia and carprofen did not show more pain-related behaviours than sham-disbudded calves.

Experiment 3

There were no differences in age between the two groups.

		Time from dehorning				
Group	n	-5 min	+ 90 min	+ 120 min	+ 150 min	Mean post-disbudding
DA_3	8	14.10 ±7.08[aA]	25.56 ±19.76[aA]	18.50 ±13.33[aA]	31.37 ±11.89[aB]	25.15 ±4.58[aB]
ND_3	5	14.65 ±6.14[aA]	15.35 ±3.48[aA]	17.84 ±12.25[aA]	17.71 ±7.86[aA]	16.97 ±2.82[bA]

Different lower case superscript letters indicate difference between groups.
Different upper case superscript letters indicate difference across time.

Table 6.16. – Effects on plasma cortisol (mean ±SD) of calves hot-iron disbudded with anaesthesia in Experiment 3. DA_3: calves disbudded after treatment with regional lidocaine; ND_3: calves sham-disbudded.

Cortisol (Table 6.16) – Disbudded calves showed an overall increase in cortisol concentrations compared with base-line (P = 0.012) and sham-disbudded animals (P =

0.048). Different calves responded at different times (data not shown) and so only at 150 min was a difference found between sham-disbudded and disbudded plus anaesthesia (P = 0.0036). The frequency of pain-related behaviours (Table 6.17.) was higher in disbudded animals at 90 and 120 minutes (both P = 0.024), but not a 150 minutes.

		Time from dehorning			
Group	n	+ 10 min	+90 m	+ 120 min	+ 150 min
DA_3	8	0.75 ±0.71[a]	3.50 ±3.21[a]	3.00 ±2.14[a]	1.75 ±1.49[a]
ND_3	5	1.33 ±0.58[a]	0.33 ±0.58[b]	0[b]	0.67 ±0.58[a]

Different lower case superscript letters indicate difference between groups.

Table 6.17.– Effects on incidence of four pain-related behaviours (mean ±SD) in calves hot-iron disbudded with anaesthesia in Experiment 3. DA_3: calves disbudded after treatment with regional lidocaine; ND_3: calves sham-disbudded.

Discussion

Several studies have confirmed that hot-iron disbudding causes pain in calves for at least 2 hours (Petrie *et al*, 1996; Morisse *et al*, 1995; Graf and Senn, 1999; Faulkner and Weary, 2000; Milligan *et al*, 2004; Vickers *et al*, 2005; Doherty *et al*, 2007). The cortisol results of our Experiment 1 show that distress is present at 1 hour but no difference is evident at 3 hours when compared with sham-disbudded animals. However behaviour analysis shows a high incidence of altered behaviours at 3 hours, suggesting that, although not severe enough to cause a noticeable rise in plasma cortisol, discomfort is present for longer than previously dictated. Although some studies with rats show that mechanical hyperalgesia is still present 2 weeks after full-thickness thermal burns (Summer *et al*, 2007b), we did not find any evidence of pain-related distress in disbudded calves at 6 or 24 hours. This could be due to species differences, a relatively smaller burned area or because we did not look at the more adequate measures to assess hyperalgesia and chronic pain.

Some studies have been contradictory as to the efficacy of regional anaesthesia. Petrie *et al* (1996), using 2% lidocaine, and Doherty *et al* (2007), using 2% and 5% lidocaine, concluded that regional anaesthesia is not very efficacious. In contrast, Grøndahl-Nielsen *et al* (1999) and Graf and Senn (1999) showed that cornual nerve block markedly attenuates behavioural and physiological response for the first two hours after the procedure. However all studies that looked at the struggling during the procedure agree that cornual nerve blocking is efficient in reducing signs of pain. Our results also show a positive effect by reducing the degree of struggling compared with animals disbudded with no anaesthesia. The Experiment 1 cortisol results apparently

indicates that regional anaesthesia is efficient in controlling pain for 24 hours, but analysing the pain-related behaviour incidence we show that pain is present as early as 1 hour and for 3 hours after disbudding. Cortisol levels in Experiment 2 also show that animals treated with regional anaesthesia suffer some distress immediately after the procedure when compared with sham-disbudded, although to a smaller degree than control disbudded ones. This could be due to the handling during disbudding but the fact that the sham-disbudded calves (submitted to the same handling) did not show an increase suggests that some pain is felt even when regional anaesthesia is given.

The results from blood collected after the nerve block supposedly had subsided (Experiment 3) show a rise in cortisol in all treated calves although different calves responded at different times (data not shown). This is probably why no difference between groups is apparent at 90 and 120 min in Experiment 3 or at 3 hours in Experiment 1, when comparing disbudded with sham-disbudded animals, but for overall cortisol response between the two groups in Experiment 3, the difference was evident. Graf and Senn (1999) and Grøndahl-Nielsen *et al* (1999) also showed a delayed increase in cortisol of lidocaine-treated animals at 180 and 210 min post-disbudding. Doherty *et al* (2007) did find a similar increase at 4 hours after blocking with 5% lidocaine but not when using 2% lidocaine. In contrast, behaviour differences are evident at 90 and 120 minutes (Experiment 3) and at 3 hours (Experiment 1) after disbudding suggesting that pain, probably due to extensive inflammation that follows deep thermal burns (Junger *et al*, 2002) is felt by calves when regional anaesthesia subsides. These results also show that the duration of nerve block varies between individuals, perhaps due to anatomical or physiological differences.

The higher level of cortisol at 24 hours in the lidocaine plus carprofen treated group is not easily explained and resulted from very high cortisol in two calves. There were obviously confounding factors that we were not aware of. However, it can be said that the use of both regional anaesthesia and a non-steroidal-anti-inflammatory drug (NSAID) is shown here to efficiently control pain-related distress after hot-iron disbudding. All previous studies using NSAID have used ketoprofen as the analgesic (Milligan *et al*, 2004; Faulkner and Weary, 2000; McMeekan *et al,* 1998). With this study we demonstrated that regional anaesthesia together with carprofen is equally efficient in reducing or eliminating the rise in plasma cortisol and pain-related behaviours from immediately after disbudding to 24 hours after the procedure.

Only two studies have looked at the effect of a NSAID given alone and pre-emptively: McMeekan *et al* (1998) showed that ketoprofen alone had no effect in controlling pain after scoop-dehorning and Stilwell (see Study 6) found the same result when using flunixin-meglumine after paste disbudding. In the present study we showed that carprofen, without regional analgesia, only reduces the intensity of the cortisol and behaviour response at 30 minutes after disbudding when compared with disbudded control animals. Carprofen alone also showed a trend towards the reduction of struggling during the procedure compared with non-treated animals ($P = 0.054$). These results suggest that carprofen alone does have an analgesic effect but not sufficient to eliminate pain caused by hot-iron disbudding.

We conclude that hot-iron disbudding of young calves is a procedure that causes severe pain during the procedure and for, at least, 3 hours. Regional anaesthesia is efficient in reducing struggling and pain signs for the first hour but does not prevent pain-distress when nerve blocking subsides. Carprofen given alone and pre-emptively does not reduce pain significantly although it does reduce the severity of the responses during the first hour. Only the combination of regional anaesthesia, 5ml 2% lidocaine given s/c midway between horn base and lateral eye canthus, with i.v. carprofen resulted in reduced struggling, plasma cortisol and pain-related behaviours during the 24 hours after hot-iron disbudding.

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STUDY 8

Effect of hot-iron disbudding on behaviour and plasma cortisol of calves sedated with xylazine.

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Abstract

Hot-iron disbudding of dairy calves can affect adversely animal welfare. Xylazine is used to reduce struggling during the procedure. We investigated cortisol and behaviour for the first hour after hot-iron disbudding of 41 calves aged 37 ±4 days: disbudded after i/m xylazine (n=10); disbudded after i/m xylazine and cornual nerve blocking with lidocaine (n=10); sham-disbudded after i/m xylazine and lidocaine (n=11); sham-disbudded after i/m saline and lidocaine (n=10). Xylazine-treated groups showed higher cortisol concentrations than saline-treated animals at all times. There were no cortisol inter-group differences in xylazine-treated groups. Xylazine-alone disbudded calves showed an increase in cortisol at +10min compared with base-line. Sham-disbudded calves with xylazine had lower cortisol at +60 min compared with -5, +10 and +25 min. Xylazine-alone disbudded calves struggled more than all other groups. Sham-disbudded with no xylazine struggled more than sham-disbudded with xylazine. Xylazine-alone disbudded calves showed more pain-related behaviours at 10 and 40 min. We conclude that cortisol increases for at least one hour in calves given xylazine even if only sham-disbudded. In this study only struggling and ear flicking could be considered signs of pain in disbudded calves treated with xylazine.

Keywords: hot-iron disbudding, pain, plasma cortisol, behaviour

Introduction – edited and included in Chapter 1.

Objectives

In this study we measured the effect of hot iron disbudding on cortisol and behaviour of calves given xylazine with or without a cornual nerve block. In this way we wanted to assess whether sedation had welfare benefits other than reducing the struggling during the procedure.

Material and Methods

Farm and Animals

The study was carried out in a Portuguese 1,000 cow commercial dairy farm. The calves were kept in groups of 10 in pens floored with wood-shavings. Acid treated milk was permanently available in a large container with several teats for ad libitum drinking. Grass hay, a 18% protein calf-starter and water were also permanently available.

The "Centro de Investigação Interdisciplinar em Sanidade Animal" (CIISA) Committee for post-doc studies, of the Lisbon Faculdade de Medicina Veterinaria, approved all animal use in this project. The disbudding protocol usually carried out at the farm includes the injection of xylazine before disbudding but not local anaesthesia.

Procedures

Forty one female Holstein calves with ages ranging from 37 ±4 days were used in this study. The study was carried out on different days with groups of 5-6 calves. In each of the days the calves were randomly assigned to one of the following groups: hot-iron disbudded after xylazine and saline (**DX**) n=10; hot-iron disbudded after xylazine and lidocaine **(DXL)** n=11; sham-disbudded control after xylazine and lidocaine (**CXL**) n=10. Ten other calves were sham-disbudded on another day after i.m. saline and lidocaine (**CL**) n=10, to avoid interference with sedated animals.

Xylazine (1ml, aprox. 0.2 mg/kg; Vetaxylaze, Dopharma, The Netherlands) or, when applicable, 1ml saline were given intramuscularly 10 min before disbudding. Lidocaine 2%, without adrenaline, (5ml; Anestesin, Laboratorio Sorologico, Portugal) was given s/c, bilateral, on the cornual nerve, mid-way between the base of the horn and the eye lateral cantus just ventral to the frontal bone lateral edge (Nordsy, 1992; Greene, 2003) as soon as the calves went down (aprox. 2 min after xylazine injection). Blood (7 mL) was collected for the first time, by jugular venipucture, 5 min after treatments when all animals that were given xylazine were recumbent and fully sedated. Lidocaine efficacy

was confirmed by no ear flicking after needle pricking the skin around the horn bud just before disbudding.

Disbudding was then performed with a butane heated hot-iron, by placing the device over each horn bud for 20 to 30 seconds. Both sham-disbudded groups were submitted to the same procedure but a cold device was applied to each bud for the same time. Non-sedated calves were forced to lie down and gently restrained for sham-disbudding. The procedures were always performed in the same pen, at the same time of day (10 a.m.) and by the same operator.

After disbudding blood was collected, in the same order as disbudding, at 10, 25, 40 and 60 min by an experienced veterinarian into a heparinised tube. Blood was kept in ice then centrifuged and plasma frozen at -20ºC. Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA) at the Faculdade de Medicina Veterinaria by technicians blind to treatments. The lowest detectable concentration of cortisol was 1.0 nmol/l. The inter-assay coefficients of variation was 9,2% for 1 ng/mL and 3,3% for 5 ng/mL and the inter-assay coefficients of variation was 3,4% (Rodbard, 1974). The between-day differences for plasma cortisol concentrations within groups were not significant so data was pooled.

All behaviour assessment was by the same person, who was blind to treatments with the obvious exception of the non-sedated group. During the procedure each calf reaction was graded by the observer from 0 = no struggling to 5 = severe struggling depending on leg, head and ear movements and also vocalisation. The incidence of five pain-related behaviours (ear-flicking; head-shaking; head rubbing with hind foot; transitions from lying to standing and back to lying; vocalisations) was then recorded for periods of 5 min just before each blood sampling.

Statistical Analysis

Distributions of the variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used (SPSS 15 for Windows ®). Differences, within the same groups, over time were tested using the Wilcoxon matched-pairs signed-ranks test. Differences between groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal–Wallis one-way analysis of variance.

Results

There was no difference in mean age between groups.

The degree of struggling during disbudding is presented in Fig. 6.12. The group disbudded after xylazine only, struggled more than sham-disbudded after xylazine ($P < 0.001$) and that sham-disbudded with no sedative ($P = 0.002$). The sham-disbudded with no sedative showed more struggling than the sham-disbudded after xylazine ($P = 0.011$).

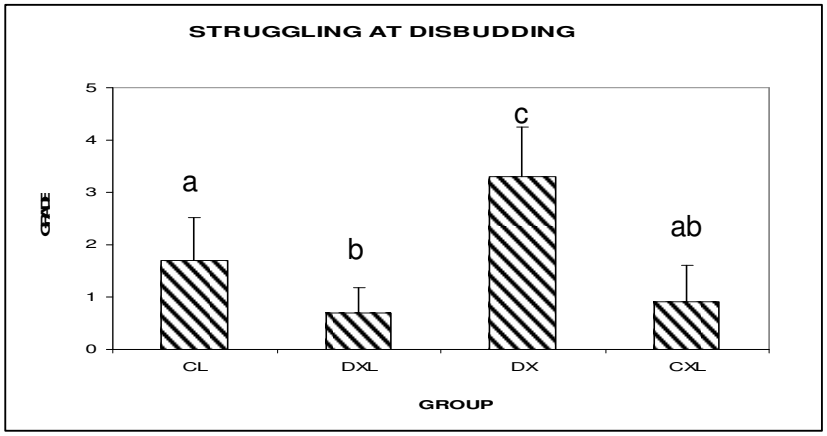

Fig.6.12. - Degree of struggling (mean ±SD) during hot-iron disbudding (scale from 0 = no struggling to 5 = severe struggling). CL (n=10) – sham-disbudding after cornual nerve blocking with lidocaine; CXL (n=10) – sham-disbudding after i/m xylazine and cornual nerve blocking with lidocaine; DX (n=10) – disbudding after i/m xylazine; DXL (n=11) – disbudding after i/m xylazine and cornual nerve blocking with lidocaine.
Different superscript letter indicates differences between groups (P < 0.05).

Plasma cortisol concentrations are presented in Table 6.18. All groups given xylazine showed a higher cortisol level at all times compared with animals not sedated ($P < 0.002$). There were no differences in base-line levels among groups of animals sedated with xylazine. For the xylazine-alone disbudded group cortisol concentrations at 10min were higher than at all other times (always $P < 0.005$). This group also showed lower cortisol at 60 min compared with 25 and 40 min (P = 0.005 and P = 0.009, respectively). Animals disbudded after xylazine and local anesthesia showed a reduction in cortisol at 40min ($P = 0.016$) and 60min ($P = 0.026$) compared with 10min. Sham-disbudded with xylazine showed lower cortisol values at 60 min compared with base-line ($P = 0.007$). Sham-disbudded with no sedative calves did not show differences across time.

Group	n	Time from disbudding				
		-5 min	+10 min	+ 25 min	+ 40 min	+ 60 min
CL	10	16.85 ±10.19^{aA}	18.54 ±11.37^{aA}	16.17 ±6.90^{aA}	10.85 ±3.67^{aA}	10.19 ±4.62^{aA}
CXL	10	60.76 ±24.18^{bAB}	77.78 ±33.29^{bA}	68.29 ±48.77^{bAB}	51.45 ±37.63^{bBC}	33.20 ±28.06^{bC}
DX	10	53.00 ±24.79^{bACD}	94.82 ±19.53^{bB}	76.89 ±21.19^{bA}	54.22 ±23.75^{bC}	37.17 ±21.58^{bD}
DXL	11	53.26 ±41.16^{bAB}	86.34 ±36.75^{bA}	80.79 ±55.25^{bAB}	63.66 ±40.00^{bB}	57.11 ±34.18^{bB}

Different lower case superscript letters indicate difference between groups.
Different upper case superscript letters indicate difference across time.

Table 6.18. – Mean ±SD plasma cortisol concentration (nmol/L) of calves disbudded with hot-iron after xylazine sedation, with or without anaesthesia. CL: calves sham-disbudded after treatment with regional lidocaine; CXL: calves sham-disbudded after treatment with xylazine and regional lidocaine; DX: calves disbudded after xylazine; DXL: calves disbudded after xylazine and regional lidocaine.

Time	Group	Incidence of all behaviours	Specific Behaviour				
			Ear flick	Head shake	Head rub	Transitions	Vocalisation
+10 min							
	CL	0.50 ±0.71^{a}	0.20 ±0.42^{a}	0.20 ±0.42^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
	CXL	0.20 ±0.42^{a}	0.20 ±0.42^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
	DX	2.40 ±1.71^{b}	2.00	0.20 ±0.42^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.20 ±0.42^{a}
	DXL	0.64 ±0.81^{a}	0.45 ±0.52^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.18 ±0.40^{a}
+25 min							
	CL	0.40 ±0.7^{a}	0.10 ±0.32^{a}	0.20 ±0.42^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.10 ±0.32^{a}
	CXL	0.60 ±1.35^{b}	0.20 ±0.42^{ab}	0.10 ±0.32^{a}	0.00 ±0.00^{a}	0.10 ±0.32^{a}	0.20 ±0.63^{a}
	DX	1.90 ±1.60^{b}	1.10 ±1.10^{b}	0.40 ±0.70^{a}	0.30 ±0.67^{a}	0.00 ±0.00^{a}	0.10 ±0.32^{a}
	DXL	0.55 ±0.93^{ab}	0.27 ±0.65^{ab}	0.09 ±0.30^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.18 ±0.40^{a}
+40 min							
	CL	0.5 ±0.71^{a}	0.20 ±0.42^{a}	0.10 ±0.32^{a}	0.00 ±0.00^{a}	0.10 ±0.32^{a}	0.10 ±0.32^{a}
	CXL	1.20 ±1.75^{a}	0.60 ±1.07^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.40 ±0.52^{a}	0.20 ±0.63^{a}
	DX	3.20 ±1.55^{b}	1.00 ±0.94^{a}	0.60 ±0.70^{a}	0.30 ±0.67^{a}	0.70 ±0.82^{a}	0.60 ±0.70^{a}
	DXL	1.00 ±1.67^{a}	0.18 ±0.60^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.36 ±0.67^{a}	0.45 ±0.69^{a}
+60 min							
	CL	0.20 ±0.63^{a}	0.10 ±0.32^{a}	0.10 ±0.32^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.00 ±0.00^{a}
	CXL	2.50 ±2.07^{b}	1.20 ±0.92^{b}	0.40 ±0.84^{a}	0.00 ±0.00^{a}	0.70 ±1.06^{b}	0.20 ±0.42^{a}
	DX	2.80 ±1.55^{b}	1.70 ±1.06^{b}	0.10 ±0.32^{a}	0.40 ±0.70^{a}	0.40 ±0.70^{b}	0.20 ±0.42^{a}
	DXL	2.18 ±0.87^{b}	1.50 ±0.53^{b}	0.00 ±0.00^{a}	0.00 ±0.00^{a}	0.45 ±0.52^{b}	0.36 ±0.50^{a}

Different superscript letters in each period of time indicate difference between groups for which $P < 0.001$

Table 6.19. – Incidence of pain-related behaviours (mean ±SD) of calves for the first hour post-disbudding with hot-iron after xylazine sedation, with or without anaesthesia. CL (n=10) sham-disbudding after cornual nerve blocking with lidocaine; CXL (n=10) sham-disbudding after i/m xylazine and cornual nerve blocking with lidocaine; DX (n=10) disbudding after i/m xylazine;DXL (n=11) disbudding after i/m xylazine and cornual nerve blocking with lidocaine.

The behaviour analysis is presented in Table 6.19. At 10min disbudded calves without anaesthesia DX showed more pain-related behaviours than those disbudded after anaesthesia DXL ($P = 0.008$), sham-disbudded after xylazine CX ($P = 0.001$) and

sham-disbudded with no sedative CL ($P = 0.004$). At all other times the disbudded with xylazine alone DX calves showed more pain-related behaviours than the non-sedated ones CL ($P < 0.001$), but compared with animals disbudded with xylazine plus anesthesia DXL and sham-disbudded with xylazine CXL there were only differences at 40min ($P = 0.005$ and 0.023, respectively). The incidence of pain-related behaviours at 60min was significantly higher in all animals given xylazine (DX, DXL, CXL) compared with CL ($P < 0.001$). Single behaviour analysis (Table 6.19.) showed that the only behaviour that differed between xylazine treated disbudded and xylazine treated sham-disbudded groups was ear-flicking at 10min.

Discussion

The lack of difference in cortisol along time in non-sedated animals shows that restraining and handling had no distress effect on these calves.

The significant increase in plasma cortisol in the xylazine-alone group at 10min, compared with base-line, shows that pain-induced distress is intense even in sedated animals. This was to be expected as xylazine does not have an anaesthetic effect (Flecknell, 2000) and so, for surgical procedures in cattle, it should be supplemented with a local anaesthesia (Greene, 2003). However in our study this increase was temporary and showed no difference compared with the other xylazine treated groups. This may be explained by the "ceiling effect" that occurs when very high levels of cortisol are attained (Mellor *et al*, 2005) and because base-line levels of all xylazine-treated animals were already high compared with the non-sedated group. These results show one disadvantage of using plasma cortisol to distinguish severe degrees of pain or when other factors cause a high base-line cortisol level, as may be the case with xylazine although this effect has not been described

The very high cortisol in all groups given xylazine is an interesting finding. Stafford *et al* (2003) have already shown that plasma cortisol concentration increases in animals given xylazine even before any procedure is carried out. Alpha-adrenergic agonists reduce the tonic activity of the baroreflex, decreasing arterial pressure and causing bradycardia (Campbell *et al*, 1979; Brest *et al*, 1980) and reduce tissue oxygenation (Hodgson *et al*, 2002). This may be a cause of distress to animals. But xylazine also causes muscle relaxation limiting the ability of the animal to react to human proximity and contact. This could mean than stress was induced when sedated calves were approached for blood collection. Although it was impossible to determine in this study

whether it was a physiological or psychological factor that contributed the most to the cortisol response, what these results show is that the HPA-axis is activated in calves heavily xylazine-sedated and recumbent even if no painful procedure is performed, indicating that it is not only a pain-related response.

Stafford *et al* (2003) showed some similar results when studying scoop dehorning and Grondahl-Nielsen *et al* (1999) suggested that lidocaine blocking was more effective than xylazine plus butorphanol in controlling pain because of the lower cortisol in the former treatment-group. However our findings suggest that high levels of cortisol in xylazine sedated animals are not necessarily related to pain and so should be interpreted with caution. Nevertheless, we also found that animals disbudded after being blocked with a local anaesthesia did not show the cortisol increase at 10 min, compared to base-line, which was seen in the xylazine-alone disbudded group. This shows that local anaesthesia does block pain immediately after hot-iron disbudding as was demonstrated in other studies (Doherty *et al*, 2007).

At 60 min cortisol levels were lower than base-line in all xylazine treated groups, although only significant in the sham-disbudded animals. This was to be expected as xylazine has a short half-life in cattle (36 min) and behaviour analysis showed that physical activity started to increase 40 min after xylazine injection. The more rapid decrease of cortisol values in the sham-disbudded animals may be due to the absence of pain-induced distress in this group.

Mish *et al* (2008) state that although sedated calves will not respond to the dehorning procedure they do feel it because xylazine does not possess any anaesthetic activity. In our study we showed that calves treated only with xylazine do respond to the thermo-cautery and that pain can be assessed by the degree of struggling during disbudding. The lower response of disbudded calves that were blocked with lidocaine demonstrates that xylazine alone is not sufficient to control pain caused by the burning. The pain-related signs are not those shown in other studies with calves not sedated (backing, lifting front legs etc…) but are evident to an experienced observer and should not be underestimated. In contrast, there were no differences in the degree of struggling between sham-disbudded animals and those disbudded after lidocaine nerve block. Although some authors (Petrie *et al*, 1996; Vickers *et al*, 2005) did not find lidocaine to have a significant effect on pain control, others did show a benefit (Graf and Senn, 1999; Grondahl-Nielsen *et al*, 1999; Faulkner and Weary, 2002; Doherty *et al*, 2007). The difference may be due to the anaesthesia technique being that s/c injection of 5ml of

lidocaine mid-way between the horn base and the eye seems to give better results than injections of 3 ml (Petrie *et al*, 1996) or the injection at the base of the horn (Vickers *et al*, 2005).

Faulkner and Weary (2000) measured the effect of disbudding on the behaviour of calves given xylazine but only studied the effect from three to 24 hours after the procedure. In our study we looked at behaviours for the first hour after disbudding to try to identify which pain-related behaviour should be used in evaluating early pain in calves submitted to hot-iron dehorning after xylazine injection. Ten minutes after hot-iron disbudding the pain in animals without a block was sufficiently intense to cause a difference in the incidence of behaviours and was also associated with an increase in plasma cortisol concentration. At this time ear-flick was the only behaviour to differ between groups and so should be considered essential to assess pain in xylazine-sedated animals

The characteristic vocalisation associated with xylazine sedation, a low and lingering call lasting for a few seconds, was noted in all groups given xylazine and so should not be used as a sign of pain in animals treated with α2-agonists. The same happened with transitions, calves lying almost immediately after getting up, that occur in all sedated animals when xylazine effect was beginning to subside (over 45 min after injection).

In conclusion we suggest that, although restraining and disbudding is certainly much easier when calves are sedated and recumbent, calves given xylazine but no local anaesthesia are exposed to severe distress. We also suggest that the HPA axis is activated when calves given xylazine are handled, even if no painful procedure is performed. There was no effect of treatment on many of the behavioural measures because the sedation inhibits active movements during the first 30 min and because of the type of behaviours shown by cattle when recovering from the effect of xylazine. Only struggling during the procedure and ear flicking immediately after the disbudding, were useful indicators of the degree of pain caused by hot-iron cauterization in xylazine-sedated calves.

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6.4. Comparing disbudding methods.

Comparing the effect of three different disbudding methods on behaviour and plasma cortisol of calves.

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Summary:

The objective of this paper is to compare the effects of three disbudding methods on plasma cortisol and behaviour of female dairy calves. We compared the results of several studies in which a total of thirty six calves were disbudded with no treatments or sham-disbudded: five were scoop disbudded (S); seven were hot-iron disbudded (HI), eight were caustic paste disbudded (CP); eight were sham-disbudded with iron (ND-HI); and eight were sham-disbudded with paste (ND-CP). In all studies plasma cortisol was measured 5 minutes before the procedure (base-line) and 1, 3, 6 and 24 hours after dehorning. Behaviour was assessed during the procedure and during 15 minute periods 15 min after dehorning and before each blood sampling.

During the procedure the HI group showed more struggling behaviours compared with all other groups. Group S struggled more than CP, ND-HI and ND-CP. There was no difference in cortisol base-line and 24h values between any of the groups. Compared with all other groups, S group had higher cortisol at 1, 3 and 6 hours. At 1 hour CP and HI groups had higher cortisol than both sham control groups and cortisol was significantly higher in CP than in HI. Compared with both sham-dehorned groups, scoop-dehorned animals showed a higher frequency of pain-related behaviours at all times except 24 h and more pain behaviours at 6 hours compared with the other disbudding methods; paste and hot-iron dehorned groups showed more signs than both non-dehorned groups until 3 h.

These results show that: scoop-dehorning causes more pain than any other method until, at least, 6 hours after the procedure; dehorning with an hot-iron elicits more struggling during the procedure; hot-iron and caustic paste disbudding causes pain until 3 h after the procedure but there are no difference in the incidence of pain-related behaviours between the two groups.

Resumo:

O objectivo deste artigo é comparar o efeito de três métodos de descorna sobre os níveis de cortisol e a incidência de comportamentos de dor em vitelas de leite. Comparámos os resultados de três ensaios que incluíram um total de 36 vitelas de leite descornadas sem qualquer tratamento ou em que foi simulada a descorna: descornadas com guilhotina (S, n=5); descornadas com ferro quente (HI, n=7); descornadas com pasta caustica (CP, n=8); simulação de descorna com ferro (ND-HI, n=8); simulação de descorna com pasta (ND-CP, n=8). Em todos os estudos o cortisol plasmático foi medido 15 minutos antes da descorna (níveis basais) e à 1, 3, 6 e 24 horas depois da descorna. O comportamento foi avaliado durante a descorna (debater) e por períodos de 15 minutos aos 15 minutos pós-descorna e antes de cada colheita de sangue.

Durante o procedimento o grupo HI foi o que se debateu mais. O grupo S debateu-se mais do que os grupos simulados e os descornados com pasta. Não houve qualquer diferença entre grupos nos níveis de cortisol antes da descorna e às 24 horas. O grupo S apresentou maiores níveis de cortisol à 1, 3 e 6 horas comparado com todos os outros grupos. Uma hora após descorna os grupos CP e HI mostraram cortisol mais elevado do que os grupos simulados e o grupo CP tinha níveis mais elevados do que HI.

Comparando com os grupos simulados, o grupo S apresentou mais comportamentos de dor a todos os momentos excepto às 24 horas e mais comportamentos de dor às 6 horas quando comparado com os outros dois métodos. Até às 3 horas os grupos CP e HI apresentaram mais comportamentos de dor do que os grupos simulados.

Estes resultados demonstram que a descorna por guilhotina (scoop) causa dor intensa até pelo menos 6 horas pós-descorna; o ferro quente provoca intensa dor durante a própria descorna; a descorna por ferro quente ou pasta causa dor evidente até pelo menos 3 horas após descorna, mas não existem diferenças comportamentais entre estes dois métodos.

Keywords: pain, scoop, hot-iron, caustic paste, dehorning, behaviour, cortisol

Introduction: edited and included in Chapter 1.

Objectives:

The objective of this study was to compare cortisol and behaviour of calves disbudded with scoop, hot-iron or caustic paste, so as to try and grade them according to effects on welfare.

Material and Methods:

Experiment design

Three dairy farms were selected according to the dehorning method used. We tried to replicate the field conditions in a way to conveniently assess the pain and distress experienced by the calves. Handling technique, operator and calf age were routinely used in each farm. All animals were used to human handling and regular presence in the paddocks. The different studies were done for several days but all disbudding, blood sampling and behaviour recording started at 10 a.m. in similar weather conditions – clear sky and mild temperatures. In each farm animals to be disbudded or sham-disbudded were randomly selected and an individual number was sprayed on both flanks. Blood sampling into a heparinised Starsted ® tube (7 ml) took place approximately 5 minutes before the disbudding and then at 1, 3, 6 and 24 hours after the disbudding. Blood was immediately centrifuged. Plasma was then frozen at minus 20ºC. Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA) at the Faculdade de Medicina Veterinaria.

Behavioural assessment was done during the procedure (degree of struggling) or by recording the incidence of five different pain-related behaviours at 15m, 1h, 3h, 6h and 24 hours after the disbudding. The description of the behaviours recorded is in Table 6.20.

Handling and blood collection was as quick and peaceful as possible so as to reduce stress and was done by an experienced veterinarian inside the first 30 seconds after restrain. Table 6.21. presents the number and mean age of animals included in each group.

STRUGGLING AT DEHORNING	DESCRIPTION
Hot-iron and Scoop (occurrence of any of the five behaviours were added)	Lifting front limbs; falling on back limbs; backing; vocalisation; open mouth.
Caustic paste (occurrence of any of the five behaviours were added).	Trying to raise; shaking head; stretching back limbs; vocalisation; open mouth.
BEHAVIOURS AFTER DEHORNING	**DESCRIPTION**
Ear flick	Flicking ears with no apparent reason (e.g. flies),
Head shake	Shaking head.
Head rub	Scratch head against objects or using back leg.
Transitions (lying/raising)	Lying and raising hastily with no resting objective.
Inert Lying	Sternal lying with head on flank and ignoring external stimulus.

Table 6.20. – Description of the behaviours recorded at disbudding and for 24 hours after the procedures.

	DISBUDDING METHOD				
	Scoop (S)	Hot-iron (HI)	Caustic Paste (CP)	Sham-dehorned (ND-HI)	Sham-dehorned (ND-CP)
Animals (n)	5	7	8	8	8
Age in days	120 ±30[a]	98 ±15[b]	25 ±10[c]	76 ±11[b]	31 ±5[c]

Table 6.21. – Number and age (mean ±SD) of calves in each group accordingly to the disbudding method used. Different superscript letters indicate difference between groups for which $P < 0.001$

Each farms' husbandry conditions and disbudding method used were as follows:

Scoop disbudding – This study was done in a single day. Five Holstein-Frisian female calves with mean age 117 ±32 days were kept in concrete floored paddocks. Concentrate, grass hay and water were permanently available. For disbudding the calves were put into a crunch and the head was restrained with a rope. The scoop-dehorner was pushed against the head and rapidly closed to cut off the horn base. The procedure was repeated for the other bud and took no more than 45 seconds in total. Due to farm constraints, there was no sham scoop-disbudded group but two other groups were formed to test regional anaesthesia and analgesia efficacy (see Study 4).

Hot-iron disbudding – this study was repeated with different animals along several days. Fifteen female calves with mean age 98 ±15 days were kept in concrete floored paddocks. They were already weaned and were eating concentrate and alfafa/grass hay.

These animals were randomly allocated to treatment groups with no age differences: HI – hot-iron disbudded with no treatment (n=7) and ND-HI – sham-disbudded (n=8). The disbudding procedure was done with the calves standing and the head restrained by a head-halter. While one person gently pressed the calves against a wall another operator applied the hot-iron to the base of each horn bud for the duration of 30 to 45 s. The sham-disbudding was done in the same way but a cold device was applied to the head. The total procedure took from 90 to 120 seconds and was always done by the same stockman.

Caustic-paste disbudding - this study was repeated with different animals along several days. Sixteen Holstein-Friesian female calves with mean age 25 ±10 days were kept in a large straw-bedded paddock with ad-libitum access to computer-controlled milk distributor. These animals were randomly allocated to treatment groups with no age differences: CP - caustic paste disbudded with no treatment (n=8) and ND-CP – sham-disbudded (n=8). For the disbudding animals were forced to lye down, the hair around the horn bud was clipped and the paste was applied to each horn. Sham-disbudded animals were handled in the same way but an inert gel was applied instead of the paste. The total procedure took no more than 60 seconds.

Two calves were removed from the study (one from group ND-CP and one from group HI) due to respiratory disease.

Statistical analysis

Distributions of these variables were shown by Levene and Shapiro–Wilks tests to be non-normal, so non-parametric analyses were used. Differences between the five groups at each time were determined by the Mann–Whitney U-test following a Kruskal–Wallis one-way analysis of variance. P-values less than 0.05 were considered significant. SPSS® for Windows (version 14) was used for the analysis.

Results

Age

There was a difference in ages between the groups (P =1.11) but not for the two hot-iron groups (HI and ND-HI) and the two caustic paste groups (CP and ND-CP).

Cortisol

Table 6.22. and Fig 6.13. show the plasma cortisol values for the different disbudding groups.

Comparing the values at each moment we found that:

- There are no differences in baseline levels between any of the groups (P = 0.669).

- There were no differences at any time between the two sham-disbudded groups (always P > 0.05). - At 1h after disbudding there were differences between both sham-disbudded groups and groups S (P < 0.004) and CP (P < 0.001). Group HI had higher cortisol than ND-HI (P = 0.002) and showed a trend when compared with group ND-CP (P = 0.051).

- At 1 h scoop disbudded animals showed a difference in cortisol levels from the other CP and HI groups (P = 0.019 and P = 0.003, respectively). At 3 and 6 h this difference was: for CP, P = 0.002 and P = 0.019, respectively; and for HI P = 0.03 and P = 0.01, respectively.

- At 1 h CP showed a higher cortisol levels than HI (P = 0.001).

- There are no differences between groups at 24 hours (P = 0.126).

Group	n	Time from disbudding				
		- 5 min	+ 1 h	+ 3h	+ 6 h	+ 24 h
S	5	14.44 ±6.76 [a]	90.13 ±19.71 [a]	66.62 ±10.54 [a]	47.26 ±16.20 [a]	30.42 ±17.23 [a]
HI	7	15.64 ±9.51 [a]	33.89 ±15.33 [b]	20.95 ±25.71 [b]	16.51 ±12.18 [b]	25.13 ±16.38 [a]
CP	8	16.86 ±11.15 [a]	62.64 ±10.32 [c]	19.44 ±14.14 [b]	16.60 ±18.41 [b]	12.34 ±12.05 [a]
ND-CP	8	13.78 ±9.8 [a]	14.54 ±9.25 [d]	12.32 ±12.32 [b]	20.15 ±13.88 [b]	13.26 ±14.09 [a]
ND-HI	8	10.64 ±7.56 [a]	7.17 ±3.99 [d]	10.09 ±6.10 [b]	12.40 ±6.09 [b]	15.66 ±5.98 [a]

Different superscript letters in each period of time indicate difference between groups for which $P < 0.05$

Table 6.22. – Differences in mean cortisol ± SD (nmol/L) between disbudding methods for the first 24 hours after the procedures.

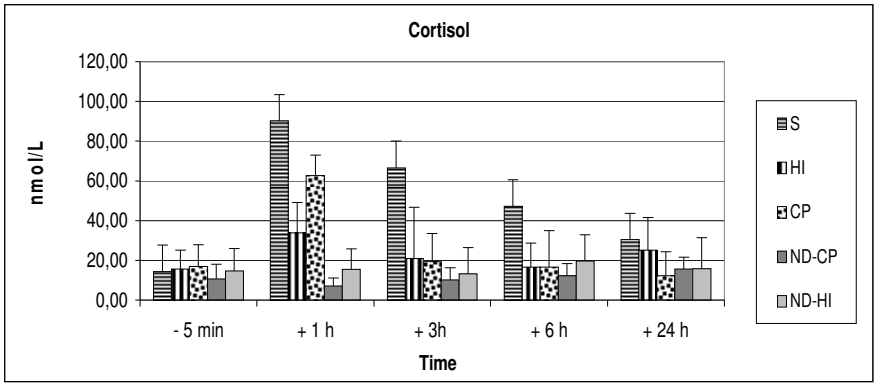

Figure 6.13. – Mean ±SD plasma cortisol of calves disbudded by different methods. S - Scoop; HI - hot-iron; CP - caustic paste; ND-HI – sham-disbudded with iron; ND-CP – sham-disbudded with paste.

Behaviour

Table 6.23. and Fig. 6.14. show the pain-related behaviour incidence for the different groups.

Comparing the values at each moment we found that:

- Struggling was significantly higher in the HI group than in any of the other groups ($P < 0.01$). Group S struggled more than the sham-disbudded and the caustic paste-disbudded animals ($P < 0.05$). There were no differences in struggling between sham-disbudded and paste disbudded animals ($P > 0.491$).

- At 15 min after the procedure sham-disbudded calves showed less pain-related behaviours when compared with S, CP and HI groups ($P < 0.01$, $P < 0.001$ and $P < 0.001$, respectively).

- The S group showed more pain behaviours than sham-disbudded at all times except 24 hours but only at 6 hours when compared with the two other disbudded groups (for CP, $P = 0.03$; for HI, $P = 0.048$).

- The HI group showed more behaviours than the ND-HI until 3 h (at 6h there was a trend with $P = 0.054$) and more behaviours than ND-CP at all times except at 24 h.

- The group CP showed more behaviours than sham-disbudded animals at 15m and 1 h (and at 3h when compared with ND-HI)

- There was only a trend towards difference at 6h ($P = 0.054$) when comparing behaviour incidence between HI and CP groups.

Comparing the incidence of individual behaviours between the different groups (Table 6.23. and Fig. 6.14) we found that:

- A higher number of very active behaviours like head-shaking and head-rubbing in groups S and HI, but a more even distribution in group CP.
- Transitions were very rarely recorded in S and HI animals but were common in CP group.
- Vocalisations were rarely recorded – only during the procedure for two animals in the HI group.
- In the sham-disbudded animals ear-flicking was almost the only behaviour recorded.

- Three animals in the CP, one in the HI and none in the S group showed "inert-lying" behaviour.
- A high number of behaviours (9) were still observed in the S group at 24 h.
- The total behaviours' incidence recorded during the 24 hours was: Scoop: 17.2; Hot-iron: 14.3; Caustic Paste: 12.4; Sham-disbudded: 1.7.

		Time from disbudding						
Group	**Behaviour**	0	+ 15 min	+ 1 h	+ 3 h	+ 6 h	+ 24 h	Total
	Struggling (¥)	2,2[a]						
	Head shake		10	16	5	5	4	40
	Ear flick		4	3	1	7	3	18
S	Head rubbing		7	9	3	6	2	27
n=5	Transitions		1	0	0	0	0	1
	Inert lying		0	0	0	0	0	0
	Total		22	28	9	18	9	86
	Mean		**4.40[a]**	**5.60[a]**	**1.80[a]**	**3.60[a]**	**1.80[a]**	**17.2**
	Struggling (¥)	3,43[b]						
	Head shake		9	8	2	4	0	23
	Ear flick		16	16	3	4	3	42
HI	Head rubbing		18	6	5	3	0	32
n=7	Transitions		0	1	0	1	0	2
	Inert lying		0	0	0	1	0	1
	Total		43	31	10	13	3	100
	Mean		**6.14[a]**	**4.43[a]**	**1.43[a]**	**1.86[b]**	**0.43[a]**	**14.29**
	Struggling (¥)	0,8[c]						
	Head shake		22	8	0	0	0	30
	Ear flick		9	3	6	0	0	18
CP	Head rubbing		13	8	7	2	1	31
n=8	Transitions		7	3	2	2	0	14
	Inert lying		0	1	4	1	0	3
	Total		51	23	19	5	1	99
	Mean		**6.38[a]**	**2.88[a]**	**2.38[a]**	**0.50[bc]**	**0.13[a]**	**12.38**
	Struggling (¥)	1,00[c]						
	Head shake		1	0	1	1	2	5
	Ear flick		3	1	0	2	1	7
ND-HI	Head rubbing		0	0	0	0	0	0
n=8	Transitions		0	0	0	0	0	0
	Inert lying		0	0	0	0	0	0
	Total		4	1	1	3	3	12
	Mean		**0.6[b]**	**0.3[b]**	**0.1[b]**	**0.4[bc]**	**0.4[a]**	**1.7**
	Struggling (¥)	0,80[c]						
	Head shake		0	0	1	0	2	3
	Ear flick		3	0	0	0	1	4
ND-CP	Head rubbing		0	1	0	1	0	2
n=6	Transitions		0	0	1	0	0	1
	Inert lying		0	0	0	0	0	0
	Total		3	1	2	1	3	10
	Mean		**0.5[b]**	**0.2[b]**	**0.3[b*]**	**0.2[c]**	**0.5[a]**	**1.7**

Table 6.23. – Degree of struggling during disbudding and individual and total incidence of pain-related behaviours (mean ±SD) of calves for the first 24 hours post-disbudding with scoop (S), hot-iron (HI), caustic paste (CP) or sham-disbudded (ND-HI and ND-CP).
(¥) Results from adding the occurrence of five struggling behaviours: from 0=no struggling behaviour observed to 5= all struggling behaviours observed.
Different superscript letters in each period of time indicate difference between groups for which $P < 0.05$.

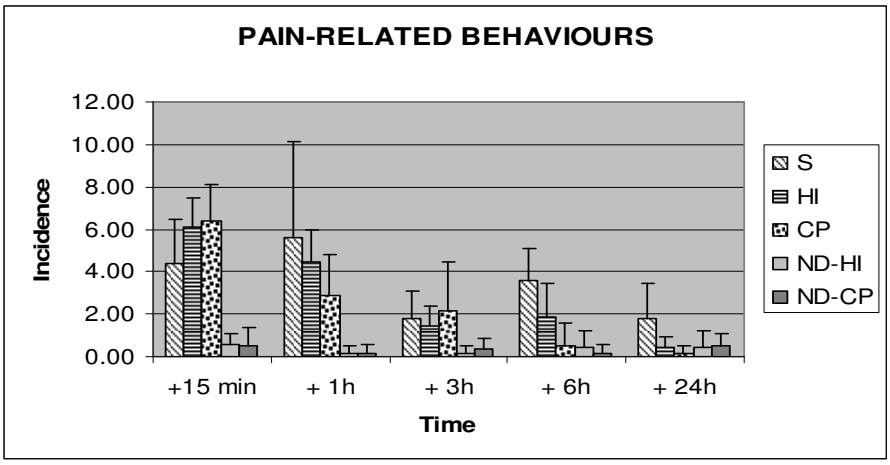

Fig. 6.14. – Mean ±SD incidence of pain-related behaviours of calves disbudded by different methods. S - Scoop; HI - hot-iron; CP - caustic paste; ND-HI – sham-disbudded with iron; ND-CP – sham-disbudded with paste.

Discussion

Although these results were taken from studies at different farms, the weather, time of day, husbandry conditions and study design were very similar. There was a difference in the age of the animals, the scoop-disbudded were the oldest and the paste-disbudded the youngest. However, the fact that there were no differences in baseline cortisol levels between groups and between sham-disbudded groups at all times, suggests that handling, environment and calf's age had no effect on the results. Other studies have compared cortisol and behaviour of calves disbudded at different ages and found no significant differences in the responses (Taschke and Folsch. 1997).

The hot-iron disbudded group showed a very high degree of struggling, average grade of 3.63, and included the only two animals that vocalized in all the studies (both calves with maximum struggling score of 5). These behaviours are a sign of intense distress that was to be expected when an extremely hot device is in contact with live tissue for more than 30 seconds and the animals are forcedly restrained. The fact that relatively few animals vocalize when exposed to intense stress and pain is probably due to cattle bio-adaptation by which signs of vulnerability are hidden from potential predators (Broom, 2001a).

The actual disbudding through amputation is probably equally painful but the procedure is very quick and so the animal does not have to struggle so much to get

away from restraint and from the aggression source. With caustic-paste, the struggling was minimal and not different from sham-disbudded calves. We suggest that there are two reasons for this: because product activation, caustic activity and, consequently, pain takes a few seconds to come to effect; and because younger animals (~1 month) are more used to handling and usually resist less to restrain and human proximity.

The method that showed the uppermost and longest pain-related distress was the scoop-disbudding. The extent and duration of cortisol increase was similar to that found in other studies (Petrie *et al*, 1996; Stafford *et al*, 2003). The cortisol level was higher in the S group compared with all other groups at all times until, at least, 6 hours after the procedure. The cortisol' response level at 1 hour may even have been limited by the "ceiling effect" that is described as the maximum hormonal level possibly attained after a negative experience (Molony and Kent, 1997; Moberg, 2005). This physiological limitation should also be taken in account when reading the results at 6 hours because the decrease may be due to the exhaustion of the system and not necessarily to the reduction in pain, which was seen to be still very severe by the behaviour observations.

The other two disbudding methods only showed higher cortisol at 1 hour, when compared with sham-disbudded, suggesting that pain is limited to the first few hours after the procedure. Morisse *et al* (1995) showed similar results although differences were less. There were also important differences between the caustic-paste and hot-iron groups indicating that the first causes a more intense pain at 1 hour. Similar results were found by Morisse *et al* (1995).

However, the behaviour-incidence analysis show that at 1 and 3 hours all three methods cause similar pain and that at 6 hours the pain is much more severe in the scoop-disbudded than in all the other groups. In contrast with the cortisol results, the comparison of behaviour incidence of sham-disbudded animals with caustic-paste or hot-iron disbudded ones show that the disbudded calves still suffer some pain at 6 hours and that there are no differences between these two disbudded groups. It is worth mentioning that the scoop-disbudded group shows a wavering in the behaviour incidence that is not found in any of the other groups – a decrease at 3 hours compared with 1 hour and a very significant increase at 6 hours. This may have been a consequence of the intensity of pain suffered by these animals that reduced their activity in response to a previous very painful period or because the severe

inflammation created a state of hyperalgesia that resulted in extra pain. Another possibility is that the decrease in behaviour at 3 hours was due to an external factor, not evident to the observer, that distracted the calves.

At 24 hours there were still nine pain-related behaviours recorded in the scoop group compared with three for the hot-iron and sham-disbudded and one for the paste-disbudded. However, the fact that there was a very large variation between individuals meant that this difference was found to be non-significant.

By comparing the total incidence of each behaviour within each group, it was shown that the scoop-disbudding caused almost exclusively three behaviours – head-shaking, ear-flicking and head-rubbing, being the first the most prevalent. The majority of behaviours shown by the hot-iron disbudded calves were also these three but the most prevalent was "ear-flicks". In contrast, the behaviours of the caustic-paste disbudded group were more evenly distributed. The animals disbudded by caustic-paste showed a relatively high incidence of "transitions" compared with the other methods but it is not certain if this was due to the difference in the type of pain or the age of the animals. Other studies (Vickers *et al*, 2005) showed a high incidence of "transitions" after hot-iron disbudding in animals aged 10 to 30 days. However, these animals had been sedated a few hours previously with xylazine and it was not possible to rule out an ongoing response to the sedative.

Very few animals showed the "inert-lying" behaviour and of the four that did show it three were from the caustic-paste group. The animals showing "inert lying" behaviour were also the ones that showed cortisol levels higher than the group average. High cortisol levels have been shown to be related to states of depression (Tse, 2004) and Lane (2006) suggests that a state of helplessness and frustration that may result from a situation in which an animal is not able to escape or cope with pain, causes a state very similar to depression. Very high levels of glucocorticoids have been detected in animals suffering from this kind of frustration (Gregory, 2004; Sumida *et al*, 2004). The inert lying may be the best way these calves have to cope with the pain caused by some methods of disbudding. At this moment we can not say if this is a response to the type of pain or the way very young animals react to severe pain, distress and high cortisol. It is well known that young calves adopt an inert posture when frightened or anxious, for example when left unattended in the field by their dam, and so this behaviour may be a biological response to stress, triggered by high levels of cortisol.

Our results show that comparing different methods is useful even when there are differences in age. We also show that by assessing the physiological (cortisol) and behavioural response it is more likely to understand the differences in intensity and duration of pain-related distress. This is especially true for the methods that cause long lasting pain because cortisol is less reliable for dull and chronic pain (Ley *et al*, 1996; Broom and Johnson, 2000; Mellor *et al*, 2005; Lane, 2006). Thus the fact that cortisol returns to baseline levels should not be seen as a sign of "no pain".

We concluded that amputation-disbudding causes more intense and long-lasting pain when compared with caustic-paste and hot-iron disbudding. Morisse *et al* (1995) and the EFSA report on welfare of calves (2006) assert that caustic paste is more painful than hot-iron disbudding, but Vickers *et al* (2005) suggest that the contrary is true. Our results show that hot-iron disbudding causes very severe distress during the procedure and pain that lasts for at least 3h. Compared with hot-iron, paste disbudding causes less struggling during the procedure, higher cortisol level at 1 hour and same incidence of pain-related behaviours until 3 hours. Although more studies should be made to evaluate differences during the first hour, we suggest that the overall distress does not differ between these two methods.

Our study also shows that the behaviours performed by animals in pain differ with the method and, probably, with the age of the animal. This should be taken in account in the field at the risk of considering a painful procedure as non painful. For example, transitions from standing to lying and back to standing seem to be very frequent after paste disbudding but not after other methods of disbudding. We also concluded that young animals react to distress caused by burns by adopting an apathetic attitude that may prevent the recognition of pain in these animals.

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Chapter 7 – General discussion and conclusions.

7.1. General discussion

7.1.1. How painful are routine procedures?

Our studies have shown that castration and disbudding both cause pain and distress to calves, independently of the method used, but this does not mean that they are equally painful. Only by comparing alternative methods and analgesic protocols is it possible to give advice on the use of certain procedures. For example, dairy calves' scoop dehorning causes severe and prolonged pain and can easily be replaced if good husbandry practices are followed.

One problem in assessing the severity and duration of pain after these procedures is to rely on a single measure to reach a conclusion. By assessing plasma cortisol and pain-related behaviours in all our studies, we managed to reduce the effect of external and individual factors demonstrating that pain lasts longer than would be expected if only one of the measures was used. Even though our assessment of behaviour did increase the reliability of the findings we have to remember that probably many less evident signs of pain went unnoticed. Identifying, measuring and grading these subtle behaviours are exciting challenges for new research.

Additionally, by undertaking the studies under field conditions, we showed what the animal goes through when the procedure is carried out on the farm. In contrast with other studies, in which animals were kept in very controlled environment with very few movements allowed and reduced interference with herdmates, we showed that pain after some mutilations may last longer than previously admitted. This is an important finding because all measures set up to improve welfare should be based on what the animals experience on the farm. Although acute pain may be correctly assessed in close confinement the same may not apply for chronic pain, such as the one that occurs with inflammation. For example, castrated calves may have to fight or be mounted by other animals, may have to move some distance for water or food, sensitized tissues may be harmed by insects or bed material, hot temperature may increase swelling, etc...

7.1.2. Justification for the procedures – the final balance.

It is obvious that the best way to prevent pain is to avoid causing it. Although it is true that some procedures are undoubtedly necessary, inclusively for the benefit of the animal (e.g. amputation of a severely infected claw) there are others which justification has to be carefully scrutinized. For these, expected pain and distress have to be weighed against benefits as well as compared with alternatives. For example, there are arguments in favour and against the two routine procedures studied: Are they indispensable? What are the benefits? What are the alternatives? Only after answering these questions will it be possible to evaluate the validity of the results and the ethical merit of their use.

So let's look in detail to the arguments that support the need for disbudding and castration. When evaluating the welfare impact of a painful procedure we should consider three factors – the intensity of pain, the expected duration of pain and the prevalence of the procedure at farm level. For example, we can have procedures that are very common but cause very little distress (e.g. an intramuscular injection) and, in contrast, very painful interventions (e.g. claw amputation) that are relatively rare. However, we can have very painful conditions that are very prevalent like, for example, sole ulcers in dairy cows.

For the procedures studied we have two different pictures: the very prevalent disbudding causes short duration intense pain while castration causes long lasting severe pain but is not very frequently performed (NOTE: this is the Portuguese condition that is completely different from what happens in the US or Australia where thousands are castrated each month).

Taking this in account we shall now analyze the arguments in favour of castration – it changes meat characteristics, reduces sexual behaviour, reduces aggressiveness and precludes unwanted pregnancies when keeping males and females together. Although in theory castration may prevent injuries and so help to improve the welfare of calves and steers kept in feed-lots it is difficult to support the idea that this procedure is done for the benefit of the animal. So the main reasons for cattle castration are economical – increase daily weight gain by reducing activity and obtaining increase profit by selling meat that is preferred by consumers.

Stafford (2007) states that "*at present* (developing protocols for alleviating on-going pain) *is neither practicable nor economically possible*" acknowledging that cattle must suffer to retain profitability. Our studies have shown that it is practicable to reduce some pain for at least 48 hours, although we have to admit that we do not believe

producers will use long lasting pain control unless forced to do so. So, the answer to reduce long lasting suffering in thousands of calves lays in getting producers to change their production conditions and consumers to change their eating habits. By weighing the advantages and disadvantages of the procedure, we conclude that cattle castration could be avoided or at least reduced if humans put their mind to it.

The reasons for dehorning are economic, safety and welfare related. Bruises and more severe injuries may occur in beef producing animals in feed-lots and during transport and lairage, costing millions to beef producers and causing unnecessary pain to animals (Meischke et al., 1974; Van Donkersgoed, 2001). In dairy farms, horned cows are responsible for increased stress and injuries towards herdmates.

Because many animals are subject to the procedure and acute pain cannot be neglected, alternatives should be investigated. Alternatives to dehorning would be to increase space allowance (e.g. feed-lots and cubicle housing) thus allowing for fearful, timid or inferior ranking individuals to avoid the more aggressive animals. This, unfortunately, does not seem achievable in the near future in intensive production systems.

One other possible alternative to dehorning, and one that is welfare and industry friendly, is to use polled bulls for reproduction. Horns are inherited as an autossomal recessive gene that is common in some beef breeds (Frish et al, 1988) but not in dairy breeds. This means that reducing the need to dehorn feed-lot animals may be possible with breeding strategies but difficult, at the moment, for female dairy calves.

Our studies showed that amputation dehorning is very painful and so, if dehorning is unavoidable, it should be done only in young calves, not because these animals feel less pain but because the methods used are less painful, analgesic protocols are more efficient and treatment is less expensive. This approach should be compulsory in dairy farms and further studied in beef herds (e.g. some beef producing farms have started to disbud very young calves showing that it is also possible in these systems).

7.1.3. Assessing pain – importance of some behaviours.

The apathetic behaviour observed in young ‘paste-disbudded’ calves, also showing high levels of cortisol after being subjected to great stress, deserves further discussion. In her book “The Neuroendocrine Regulation of Behaviour” Schulkin (1999) presents the experiment in which rats were held in the hand of the operator before being placed in the water. Although all rats knew how to swim the ones that were

exposed to the stress of being held did not attempt to swim and drowned. Shulkin goes on to say "*A variety of species will become what is called 'helpless' when they are placed in situations marked by uncontrollable and unpredictable aversive events such as shocks*." and "*After learning that their attempts at adaptive behaviour will not ameliorate the situation, the animals eventually give up*". Other authors have presented this 'helplessness' stating that some animals will stop reacting though they seem anxious and fearful (Maier and Ryan, 1986). This is particularly so given that we know that immobility and/or prostration are sometimes the only responses accompanying pain. (Baars, 2001)

The paste disbudded calves in our studies had, most probably given up reacting against the noxious sensation caused by the chemical burn. The physiological mechanism behind such behaviour is not well understood, but it is probably a way to save energy and useful resources needed for recovery and healing. The fact that some of these calves showed very high plasma cortisol a few hours before adopting this posture may indicate that a hormonal mechanism is involved. Sapolsky *et al* (2000) suggest that the preparative activity of glucocorticoids includes aid in adapting to a chronic stressor. Glucocorticoids are well known for inhibiting glucose transport in various tissues including the brain – inhibition of local cerebra glucose utilization and inhibition of glucose transport to neurons and glia. Furthermore sympathetic activation increases local cerebral glucose utilization within seconds of a stressful stimulus (Bryan, 1990). This reduced glucose availability may be important in young animals that lack some of the homeostasis efficacy of older animals and would justify why we only found this behaviour in the paste disbudded animals (much younger than the hot iron or scoop disbudded ones).

A further explanation to this behaviour could be the effect of endogenous analgesics, namely opioids, produced and released in response to a severe stress. It has been suggested that unresponsive animals may be using endogenous opioids to help them to cope. Zanella *et al* (1991a) showed that there was higher density of mu (υ) receptors in the brain of unresponsive sows. Cronin *et al* (1985) showed that these endogenous substances may be involved in altered behaviour in pigs. This mechanism, however, does not explain why only very young paste disbudded animals showed the inert lying behaviour.

One other important issue to be discussed is the "weight" given to different behaviours. For example, is head shaking more important as a sign of pain than ear

flicking? Is a calf that scratches its head with the hind foot in more pain than one that adopts an "inert lying" position? This question is very complex because choosing one or the other probably depends on many factors other than pain – age, previous experience, presence of other animals, temperature, energy and metabolic status etc... In our studies on disbudding we did find that some types of behaviour are more common after some methods but we could not relate this to differences in pain intensity. By only comparing similar behaviours (ear flicks, head shakes, transitions etc...) there was a risk that individual preferences/abilities would hide important differences. This is the reason why we also compared the total incidence of the pain-related behaviours, giving them equal weights. The soundness of this approach was reinforced by the study (Stilwell, 2006) in which the total incidence of pain-related behaviours was compared to the results of a Visual Analogue Scoring – the calves graded by an experiment observer as "in severe pain" were the ones that also had the highest incidence of pain-related behaviours.

In contrast, some types of behaviour were seldom observed after certain disbudding methods. For example, "transitions" were very frequent after paste disbudding but never seen after scoop disbudding. This may be because animals of different ages react differently but may also be because there are differences in the type of pain caused by chemicals or amputation. If, for example, only transitions were used to evaluate pain, scoop disbudding would be considered a painless procedure! Further studies should be designed so as to try and grade the importance of different behaviours. For instance, studying the incidence of "transitions" in very young calves after being scoop-disbudded, would show if the occurrence of this behaviour is due to the disbudding method or the animal's age.

Finally, a word on vocalization, which some authors consider to be an important sign of stress. Watts and Stockey (2000) say that, under experimental conditions involving pain or social isolation, vocal response is useful as an indicator of welfare and a useful indicator of physiological and psychological functioning. These authors consider vocal responses to be potentially a more revealing source of information about an animal's experience than other measures commonly employed as indicators of pain or distress. This conclusion results from studies with beef cattle during hot-iron branding (Watts and Stookey, 1999).

In our studies of castration and disbudding, vocalization was always assessed during and after the procedures. With the exception of animals that had been sedated with xylazine, very few hot-iron disbudded animals and two clamp-castrated calves, no

other animal subjected to painful procedures exhibited this behaviour. Because some of the xylazine sedated calves vocalized even when not exposed to pain, suggests that this behaviour may be drug-related. In view of this low incidence we have to conclude that vocalization is not a very useful measure of acute or chronic pain and, as suggested by Watts *et al.* (2001), too much individual variability in vocal response may complicate a clear evaluation concerning pain and welfare.

7.1.4. Assessing pain – cortisol.

Although plasma cortisol has been widely used to assess pain in farm animals, it is still seen with scepticism because increases may occur due to many other factors. However, our studies showed that when these secondary causes are carefully controlled (e.g. immediate blood collection after restraining; calm handling; peaceful surroundings; mild temperatures; time of day) blood cortisol is relatively low and unvarying in Holstein-Frisian calves not submitted to a painful experience. This means that cortisol may, in well designed experiments, be considered a very useful indicator of distress caused by acute pain.

Our studies also showed that:

- Drugs may affect the results, as was the case with xylazine, although it is not clear if the mechanism is psychological or pharmacological.
- Although there seems to be a relation between cortisol level and pain severity, a ceiling effect may be reached. This means that cortisol may not be useful in distinguishing animals in very severe pain.
- Individuals show quantitive differences in their response to challenge and this may be due to many factors: temperament, fearfulness, age, breed, previous experience, etc. For example, when faced with the same challenge fearful animals show more pronounced physiological (and behavioural) responses than less fearful ones (Boissy and Bouissou, 1995). We have shown that variation amongst individuals may be considerable, leading to potential neglect of in-pain animals, especially if only group means are used to assess welfare.
- Adrenocortical and behavioural responses of calves are closely connected. By analysing the incidence of pain-related behaviours and the level of cortisol it was possible to identify a relationship that was previously referred to by other works that studied fear in calves (Van

Reenen *et al*, 2001). This may indicate that there is a common underlying biological process behind behavioural and HPA axis response.

7.1.5. More than nociception.

Our studies, although not aimed at finding evidence of emotion in animals, did show that pain is not restricted to the nociception element. This was particularly evident by the behaviours shown by animals subjected to severe pain and the reduction in activity and appetite in castrated calves. As Broom and Fraser (2007) highlight:

It would seem that the distinction between nociception and pain is a relic of attempts to emphasise differences between humans and other animals. The use of the term nociception, which separates part of the pain system from other parts when the system should be considered as a whole, should be discontinued.

In the introduction it is explained that many authors consider that cortisol is widely used to quantify response magnitude and duration to acute painful procedures and these seem to correspond to the predicted noxiousness of the experience. Other authors state that cortisol concentrations closely follow the time course of changes in posture and activity after castration and tail docking (Kent and Mollony., 1993). Our results show that this may not always be the case and that differences might be significant. If cortisol alone was used for evaluating pain after hot-iron or paste disbudding we might be tempted to say that they cause very short-term pain (~1 h) but if we take in account the behaviour we find that some discomfort is still present at 3 and even at 6 hours. This clearly represents a state of continuous distress that affects the individual, resulting in a variety of subjective feelings like anxiety, apprehension, nervousness, fear, frustration, discomfort, soreness and irritation, inactivity etc... Although welfare should not be defined solely in terms of subjective experiences (Broom, 1991) these are obviously important when addressing painful procedures.

The occurrence of suffering depends on several factors including the type, severity and duration of the noxious stimuli but also of: other concurrent stressful events, environment, previous experiences and species, breed or individual differences. Age is also frequently listed as an important factor. Until very recently it was thought that pain and suffering were negligible in babies, resulting in newborns being subjected to painful procedures without the use of efficient anaesthesia. This is still a widespread conception in veterinary medicine and animal production. Even legislation, which

allows for some procedures to be performed by lay people or with reduced pain management, usually relays the message that young animals are more "resilient" to pain. But is this because there is less tissue damage in smaller bodies, because nociceptors are less able or because pain processing is less efficient in young animals? Unfortunately not many studies have looked at differences between ages in the capacity to suffer after routine procedures. Taschke and Folsch (1997) examined stress after hot-iron disbudding in newborn or 4 month old calves and found that age had no effect on the incidence of signs of pain or cortisol levels. Our studies show that pain can be very severe in disbudded young calves and that suffering is present at all ages, from 15 days old calves, submitted to paste disbudding, to 6 month old castrated calves. As was shown, the differences are related more to the way each animal (or age group) tries to cope with the discomfort than to the level of pain or suffering.

7.1.6. Individual welfare.

The welfare of an individual animal has been defined as "*its state as regards to its attempts to cope with its environment*" (Broom, 1988). Therefore if the **individual** is not able to adapt or respond to an environmental challenge its welfare is probably poor.

The statistical analysis that is used on behavioural data is designed, primarily, to find inferences about populations rather than individuals. In the book 'Measuring Behaviour', Martin and Bateson (2007) say: "*…a common aim (of statistical analysis) is to iron out the troublesome effects of individual differences in behaviour and to emphasise what members of a population have in common rather than how they differ*". This statement is of particular importance when assessing welfare because the poor welfare of an individual can be hidden by the coping ability of some others. This was particularly evident in our studies on paste-disbudding in which several young calves adopted an apathetic attitude by just lying down after moments of intense demonstration of pain-related behaviours. Not taking into account the significance of this or allowing its significance to be concealed by the actions of the rest of the group would mean that an important welfare issue might be ignored. The same could be said of individual animals that demonstrate diverse pain-related behaviours and very high plasma cortisol levels when the mean measurements of the group do not differ from animals that have not been exposed to a painful procedure. This may result in the approval of certain procedures as causing short term pain or considering an analgesic protocol as efficient. That is one of the reasons why we reinforce the idea presented by Molony and Kent

(1997) in which animals should be given the benefit of doubt by overestimating the intensity of pain, so as to avoid missing out the ones that are really in pain.

7.2. General conclusions

- Plasma cortisol is a reliable measure of distress if blood is collected soon after restraining and handling calves.
- Burdizzo castration causes prolonged pain (for at least 48 h) as suggested by cortisol levels, pain behaviours and reluctance to move and feed. Flunixin-meglumine only reduces pain up to about 24 h but carprofen is still effective at 48 h.
- Surgical castration causes intense and prolonged pain (for at least 6 h) even when given regional anaesthesia and xylazine. One longitudinal incision (over the raffe) causes more long term pain than two longitudinal incisions over each testicle, although the difference only becomes noticeable at 3 hours after the procedure.
- Scrotum thickness using a cutimeter is a reliable measure of inflammation and pain after surgical castration.
- All disbudding methods cause acute pain.
- The actual procedure of hot-iron burning for 30s is very distressing for calves but pain and struggling are efficiently reduced by blocking the cornual nerve with 2% lidocaine (5 ml each side).
- Scoop disbudding should be avoided because it causes severe pain for at least 6 h and might cause some discomfort as long as 24 h after the procedure. Regional anaesthesia with lidocaine only delays the onset of severe pain. The association of lidocaine with an analgesic reduces pain for 6 h but further research is needed to assess pain after this time.
- Hot-iron and caustic paste disbudding cause pain for at least 3 h but with no significant difference between the two methods. Associating regional anaesthesia to a NSAID (flunixin-meglumine or carprofen) does prevent pain as assessed by plasma cortisol and incidence of pain-related behaviours.
- Xylazine causes a significant increase in cortisol levels of calves even when they are not exposed to painful experiences. This may indicate that it is stressful to approach or handle calves while sedated.

- Using cortisol assessment to evaluate pain in animals sedated with xylazine is of little use.
- Behaviour assessment indicates that hot-iron disbudding causes severe pain in calves treated with xylazine alone. Xylazine sedated calves should never be disbudded without a cornual nerve block.
- Other more reliable methods of evaluating pain of calves sedated with xylazine are necessary.
- Behaviours like head shaking/head rubbing/ear flicking are important in the assessment of pain following any of the disbudding methods.
- Transition from standing to lying is an important pain-related behaviour for paste disbudded calves but not for the other methods. This may be linked to age and not to the disbudding method. Further research is needed to clarify this question.
- Inert lying, or a state of apathy, was recorded in calves after paste disbudding, but it was not clear if the higher incidence of this behaviour was due to the disbudding method or the younger age of the animals in this group.
- Inert lying should be used to assess pain in young calves.

Final words

There is enough evidence that routine procedures, like disbudding and castration, cause acute and chronic pain. It was also established that there are methods to reduce suffering in animals subjected to these procedures although legal constrains, cost and availability of drugs may reduce the ability to intervene.

Farmers and stockpersons, if shown how, are willing to adopt some pain control measures. One rewarding consequence of our studies was the fact that farmers and stockpersons were impressed by the efficacy and simplicity of some treatments, became more aware of subtle signs of pain and were more open to the performance of control measures.

Justification for castration has to be strong – the balance between the pain caused and the economic benefits should be carefully pondered.

Finally a word on the justification to continue and improve the studies on farm animal welfare in general and pain in particular. There is one statement that perhaps substantiates the work of those, correctly named 'welfarists', that wish to improve domestic animals' welfare – *"The most crucial limitation to the moral philosophy approach to animal welfare is the fact that what matters to the animal is not what we think or feel but what we do"* (Webster, 2005). This is exactly the argument we put forward to those that consider themselves as the sole upholders of concern for animals and vehemently condemn all and any use of animals. We hope that these studies will contribute to the work of those who wish to do something for the welfare of animals.

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