EVALUATION OF STRATEGIES FOR ALLEVIATING DEHORNING DISTRESS IN CALVES.

A Thesis presented in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in Physiology at Massey University

Cheryl Maree McMeekan

March 1997

Acknowledgements

Many people have contributed towards the making of this thesis, all of whom I am grateful to.

Particular thanks is directed towards my supervisors, Professor David Mellor, Dr. Kevin Stafford and Professor Neville Gregory, who through their many helpful discussions, guidance and unfailing encouragement, provided me with the opportunity to develop a thrill for scientific research and learning.

Dr. Robert Bruce for his ever-reliable and cheerful help during trial work.

Neil Ward for his valuable technical advice and hours of effort prior to and throughout the trial days. Also Andrew Dinniss, Brett Guthrie, Linda Sharp, Shauna Sylvester and Vanessa Tilson for their willing help with the trial work.

The farm managers of Massey University Dairy No. 1, Andrew Jull and No. 4, Sean Wilson, for their kind assistance in supplying trial calves and facilities.

The Department of Physiology and Anatomy for providing me the use of the R.I.A laboratory, and Jane Candy for her assistance while I was working in it.

A special thank you to the postgraduates, Andrew Dinnis, Nadine Gibbs, Kate Littin, Rebecca Osborne, Jo Robins, Chris Rogers, David Simcock, Mark Simpson, Mhairi Sutherland, Shauna Sylvester, Sarah Todd and Ricci Wesselink, for their many enthusiastic physiological and philosophical discussions throughout this study, and encouraging words particularly during the final stages of writing.

Finally I thank Ministry of Agriculture (MAF Policy) who financed this study, and for providing the all important student stipend.

Abstract

Animal welfare has been the focus of greater public attention in recent decades, increasing the demand for scientific enquiry into the effects of particular agricultural practices on the well-being of farm animals. The present study monitored the behavioural and plasma cortisol responses of 3-4 month-old calves to assess the distress caused by scoop dehorning during the first 9 hours after horn removal, and the extent that this distress may be reduced by minimising the scoop wound depth, or by giving prior injections of local anaesthetic and/or a non-steroidal anti-inflammatory drug (NSAID).

At the investigated scoop wound depths, the cortisol response which followed dehorning (consisting of an initial peak, followed by a decline at 2 hours to plateau values which did not return to control levels until about 7 hours after treatment), did not differ significantly between deep scoop and shallow scoop dehorning. However, NSAID administration abolished the plateau cortisol phase. Local anaesthetic plus the NSAID abolished both the initial cortisol peak and following plateau response, such that the total integrated cortisol response was not significantly different from control calves. Likewise the behaviour expressed by scoop dehorned calves was most similar to that of pain-free control calves, when both local anaesthetic and the NSAID was given prior to horn removal, compared to either drug alone, or neither. This infers that the distress caused by scoop dehorning in calves has two main components: an initial amputation pain and a more slowly developing inflammation pain, the former alleviated by local anaesthetic and the latter by NSAID.

Administration of local anaesthetic while abolishing the cortisol response during its nerve-blockade action, did not significantly reduce the overall cortisol response due to a marked rise after nerve-blockade effects ended. This suggests scoop dehorned calves despite being given local anaesthetic, still experience notable pain in the hours following scoop dehorning which is likely to be inflammation-induced.

The results of this study suggest little benefit in implementing a shallow scoop strategy in order to reduce post-dehorning pain-induced distress. Rather, administration of an anti-inflammatory analgesic in addition to local anaesthetic prior to scoop dehorning is likely to offer improved pain relief in calves undergoing this amputation procedure.

Table of contents

List of figures

List of tables

CHAPTER ONE: General Introduction

1.1 Animal Welfare

Animals have been described as 'highly dynamic information-processing organisms that continuously try to adapt to environmental conditions using behavioural and physiological mechanisms' (Wiepkema and Koolhaas, 1993). Successful adaptation to one's environment is essential for individual survival, with genetics and prior experience being the prime determinants of the adaptive strategies applied and their subsequent degree of success. These points though few in number, have immense implications for animals, particularly those under human care including farm, companion, work, sport, research, circus and zoo animals. Not only are we continually influencing the genetics and life experiences of animals, and hence influencing their adaptative capabilities, we also have considerable control over the environmental challenges to which they are exposed, and in response to which their adaptive attempts must be made. We are thus in essence influencing the whole biological integrity of animals, and in doing so determining their duration and quality of life.

This is of concern in present day western society for economic, ethical, social and political reasons. With regard to the agricultural arena there has been increased public attention in the way farm animals are treated following publication of such books as 'Animal Machines' (Harrison, 1964), 'Animal Liberation' (Singer, 1975) and 'The Case for Animal Rights' (Regan, 1983). Due to the advances in electronic communication there is also enhanced public awareness of poor animal welfare cases when they happen. This in turn has created legal and economic incentives for the agricultural community to adhere to certain welfare standards through consumer demand, particularly the demands of those in overseas trading countries, with whom the threat of non-tariff trade barriers are a reality (Baddeley, 1992; Stafford, 1994). For example, there is at present a growing concern in the United Kingdom over New Zealand's live-sheep exports to the Middle East, a concern which may be expressed in the near future by their hesitancy in importing other New Zealand branded agricultural products. In recent years there has also been an enhanced understanding of how poor welfare may cause reduced production and thus economic loss to the individual farmer (Cook *et al.*, 1992). In the midst of such growing financial and political incentives, there is the continual and genuine

concern that most farmers have towards the animals with which they work day in and day out.

The aim of this thesis was to examine one aspect of possible farm animal welfare compromise - the distress caused to calves by scoop dehorning. Emphasis was placed on the extent that this distress may be alleviated by minimising the scoop wound depth, or by administering local anaesthetic and/or a non-steroidal anti-inflammatory drug prior to horn removal.

1.11 What is Animal Welfare?

'Welfare' is a common word of everyday speech that means different things to different groups of people (Duncan and Poole, 1990). The Chambers Concise Dictionary defines 'welfare' as 'the state of faring or doing well, freedom from calamity, enjoyment of health, prosperity etc'. Over the years there has been attempts to apply a more precise working definition so that the concept of 'welfare' could be more useful in the assessment of animal health. These include:

- 'Welfare is a wide term that embraces both the physical and mental well-being of the animal' (Brambell Committee, 1965: cited in Duncan and Dawkins, 1983);
- 'Welfare involves existence in reasonable harmony with the environment, both from a physiological and ethological point of view' (van Putten, 1973: cited in Hutson, 1994);
- 'Welfare is a state of complete mental and physical health, where the animal is in harmony with its environment' (Hughes, 1976: cited in Duncan and Dawkins, 1983);
- 'The welfare of managed animals relates to the degree to which they can adapt without suffering to the environments designated by man. So long as a species remains within the limits of the environmental range to which it can adapt, its well-being is assured' (Carpenter, 1980: cited in Duncan and Dawkins, 1983);
- 'The welfare of an animal is its state as regards to its attempts to cope with its environment' (Broom, 1986);
- 'Good welfare is the state of being manifest in an animal when its nutritional, environmental, health, behavioural and mental needs are met' (Mellor and Reid, 1993).

There appears to be general agreement that animal welfare encompasses 'physical and mental health' and 'harmony with or adaptation to the environment'. To say then that an animal's

welfare is dependent upon how well matched it is (both physically and psychologically) with its present environment, is not a novel concept. However, it is useful in that we can easily appreciate how an animal's compatibility with its environment will vary in degree, and thus accordingly so will its welfare. Welfare is not a state within an animal which is either present or not present, rather it is a continuum; there is good welfare and poor welfare, and all states in between on what Mellor and Reid (1993) called the welfare-suffering continuum.

Accepting this, there is still the question of what standards should be applied in order to judge where upon this welfare-suffering continuum an animal may reside when exposed to environmental challenges. The U.K Farm Animal Welfare Council (FAWC) formulated a set of five freedoms, most recently revised in 1993 (Webster, 1994), which focused on the major needs of animals. They provide a broad basis for assessing the extent of an animal's welfare impairment by considering how well these basic needs are being met. The five freedoms are:

1 *freedom from thirst, hunger and malnutrition* - achieved by ready access to fresh water and a diet to maintain full health and vigor.
2 *freedom from discomfort* - achieved by providing a suitable environment including shelter and a comfortable resting area
3 *freedom from pain, injury and disease* - achieved by prevention or rapid diagnosis and treatment.
4 *freedom to express normal behaviour* - achieved by providing sufficient space, proper facilities and company of the animal's own kind.
5 *freedom from fear and distress* - achieved by ensuring conditions which avoid mental suffering.

In very general terms if all five freedoms are met then it is assumed that the animal in question is in a good state of welfare. As each of these freedoms (some will have greater significance than others) becomes compromised, an incompatibility between the animal and the environment develops, resulting in the expression of particular overt behavioural and physiological responses by the animal in an attempt to resolve this mismatch, responses which may indicate reduced welfare. However, it is important to note that in some instances the above freedoms may conflict. For example, freedom from fear may be compromised when stock are moved from one paddock to the next in order that freedom from hunger be

met. Also absolute freedom from all of the above states may not necessarily correspond to optimal animal welfare. It may instead promote boredom and deprive an animal of important stimulating events necessary for the expression of its full behaviour repertoire. Therefore while the five freedoms are valuable in that they allow for a comprehensive examination of the areas of potential welfare compromise, it is helpful to acknowledge that *absolute* freedom from all the above states is unrealistic and in some cases may even be undesirable.

In the event of an animal-environment incompatibility, whether the reduced animal welfare which may follow equates only to that animal's conscious perception of its compromised state or to its biological fitness whether conscious or not, has been a point of controversy within the scientific literature. Impaired animal well-being has been associated with the presence of an unpleasant mental state, requiring an animal to be both sentient and conscious in order for it to have poor welfare (Dawkins, 1990; Sandoe and Simonsen, 1992; Mellor and Reid, 1993). Conversely, animal welfare has been defined more broadly to incorporate a general state of biological fitness (Barnett and Hemsworth, 1990; Broom, 1991). Accordingly, a diseased or injured animal may have poor welfare, regardless of whether it is conscious or unconscious, since its ability to survive and reproduce within its own environment is diminished. From a further evolutionary perspective animal welfare has been represented in terms of impairment of an animal's innate motivational priorities (Barnard and Hurst, 1996), or conflict between an animal's innate/conditioned expectations of environmental events and actuality (Wiepkema and Koolhaas, 1993).

Regardless of whether or not a definition of 'animal welfare' should be confined to unpleasant emotional states, suppression of innate behavioural motivations, or be expanded to include all impaired biological functioning, few would argue against the conclusion that when intense and/or prolonged distress, pain or suffering is present within an animal then its welfare is being jeopardised. Since any welfare impairment in dehorned calves is likely to be due to pain of horn removal and psychological distress of handling, a brief discussion on what the terms 'pain' and 'distress' mean in this context is warranted.

1.12 Pain

The pain pathway involves activation of high threshold receptors in the periphery (nociceptors) which feed in complex ways to a series of ascending pathways that carry information from the spinal cord to the brain (Livingston *et al.*, 1992). If this pathway is disrupted in some way, such that higher centres of the brain do not receive this information then pain is not perceived, even in the presence of tissue injury. Pain thus consists of a cognitive component as well as the usually present initiating nociception. Pain has been defined by the International Association for the Study of Pain as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage' (Headley, 1993).

Pain due to its personal subjective nature cannot be readily measured in a quantitative manner to allow accurate comparisons between individuals, be they of our own species or others. Although we may never know how bad an animal is actually feeling when in a particular injured or diseased state, we do know that it is likely to feel something. Similar anatomical structures and neurophysiological mechanisms leading to pain perception, and learned avoidance behaviour to stimuli known to cause pain in humans are evidence of this (Bateson, 1991). It is also apparent that in the event of tissue injury the pain which follows is likely to be different from the pain commonly experienced by animals in their everyday lives associated with little or no tissue damage and which serves as a protective function (Woolf and Chong, 1993). Pain following injury can be characterised as a multifactorial event. In the context of tissue amputation it may involve the following components:

1 - initial acute pain of amputation caused by:
    - direct severing of nerves.

2 - longer-term (possibly chronic) post-amputation pain caused by: - central nervous system function which has been modified by the initial afferent pain fiber barrage, central sensitisation.
    - ongoing sensory signals generated from damaged tissue, peripheral pain, induced by inflammatory mediators (inflammatory pain) and neuroma development (neuropathic pain);

This then suggests that administering a number of different analgesic/anaesthetic regimes within the same animal, each orientated towards alleviating one or more of the above components will offer the best pain relief. This was assessed in the present study by giving scoop dehorned calves prior injections of:

a) a local anaesthetic, expected to inhibit propagation of action potentials along afferent nerves (Hall and Clarke, 1991), thereby reducing the initial pain and the possible later development of central sensitisation;

b) a non-steroidal anti-inflammatory drug, expected to reduce peripheral sensitisation of nociceptors via its inhibitory action on the synthesis of the inflammatory mediator prostaglandin (Goldstein, 1992).

1.13 Distress

In this study 'distress' is taken to mean a broad term which incorporates all unpleasant emotional experiences of physical and/or psychological origin, and is characterised by marked changes in an animal's physiology and behaviour. Pain is a form of distress. However in the absence of pain, distress may still be experienced as can occur during social isolation (van Adrichem and Vogt, 1993; Minton, 1994), confinement with unfamiliar/dominant co-specifics (Dantzer and Mormede, 1983), or exposure to novel experiences such as transport and human handling (Mitchell *et al.*, 1988). If an injured animal which is already experiencing pain is additionally exposed to such psychological stressors, its pain may be exacerbated as a result of interaction between the sympathetic nervous system and C-afferent fibers (Jänig, 1992). In humans this is commonly experienced when one fearfully visits the dentist. Conversely, the above events could reduce pain perception by effectively taking the animal's mind off its physical discomfort in the face of an added challenge.

In the context of the present study, measurement of calf distress following scoop dehorning will thus not relate to the pain of horn amputation *per se*. Rather it will measure the distress experienced by dehorned calves perceiving pain in the midst of other possible modulating psychological factors, such as confinement with co-specifics and close human contact. Although initially this may appear to cause interpretative difficulties, these novel experiences at least resemble that which would occur in normal dehorning practice.

1.2 Measuring Animal Welfare

Environmental conditions which to us may seem relatively benign, can be challenging and indeed distressful for other species, due to inter-species variation in behavioural needs and senses. Therefore, it is not the actual event but the animal's individual psychological interpretation of that event which determines whether or not adversity is experienced. This then implies that in order to assess conditions of distress, pain and suffering, we need to measure the subjective state of animals. That is, determine what animals are actually feeling. However, knowing the private experiences of another individual is difficult (Dawkins, 1980; Curtis and Stricklin, 1991). The best that can be achieved at present is to accumulate evidence in an indirect manner by monitoring the relative responses of animals following a defined event and then carefully extrapolate these responses back to the animals subjective state.

In the present study behavioural and plasma cortisol responses were measured in calves following scoop dehorning, in an attempt to judge the degree of distress caused by this procedure in the presence and absence of anaesthesia and/or analgesia. Since interpreting responses of animals in terms of their subjective state is perhaps the most difficult component of animal welfare assessment, a brief discussion on the validity of using changes in behaviour and plasma cortisol as an index of distress is appropriate.

1.21 Plasma cortisol

The hypothalamic-pituitary-adrenal (HPA) axis, a functional component of the neuro-endoncrine system, is the physiological pathway most classically associated with distress. Hans Seyle was the first to demonstrate that a wide variety of stressors such as heat, cold or tissue damage are able to activate this system (Seyle, 1936), causing the hypothalamus to secrete corticotropin releasing factor (CRF) which via the hypothalamic-pituitary portal blood vessels stimulates the release of adreno-cortiotropic hormone (ACTH) from the anterior pituitary into the circulation. ACTH in turn causes the release of cortisol from the adrenal cortex within minutes of the initial insult (Axelrod and Reisine, 1984). It was later concluded that emotional arousal elicited within the animal in response to stressors results in HPA axis activation, rather than application of the stressor itself (Mason, 1968; Burchfield, 1979). The limbic system, thought to be involved in emotional processing

and which has input to the hypothalamus, is the likely neural connective pathway between conscious perception and subsequent neuroendocrine response (Beaulieu *et al.*, 1986).

In recent years changes in cortisol concentrations have been used to assess the aversiveness of a wide variety of husbandry procedures in stock, including handling and restraint (Mellor and Pearson, 1975; Herd, 1989; Zavy *et al.*, 1992), transport (Crookshank *et al.*, 1979; Kent and Ewbank, 1983, 1986a,b), castration and/or tailing of lambs (Shutt *et al.*, 1988; Mellor and Murray, 1989; Mellor *et al.*, 1991; Lester *et al.*, 1991a, b, 1996; Wood *et al.*, 1991; Kent *et al.*, 1993; Kent *et al.*, 1995), castration of kids (Mellor *et al.*, 1991) and castration, dehorning or tail docking of calves (Laden *et al.*, 1985; Boandl *et al.*, 1989; Cohen *et al.*, 1990; Sylvester *et al.*, 1993; Robertson *et al.*, 1994; Morisse *et* al., 1995; Taschke and Folsch, 1995; Petrie *et al.*, 1996a, b; McMeekan *et al.*, 1997).

However using cortisol to assess husbandry distress is not void of interpretative difficulties. Events such as coitus, voluntary exercise and nursing behaviour, which presumably are not unpleasant have been cited to cause HPA axis activation (Rushen, 1986; Rushen and de Passille, 1992). It is thus not intended here that any increase in cortisol immediately implies a distressed state. Rather, if an event to which an animal is exposed, be it positive or negative, results in a significant elevation of cortisol then this may be used as a guide to assessing the physical and/or psychological intensity of that event. Since tissue amputation is unlikely to be a pleasant experience for animals, marked HPA axis activation depicting this intensity is thus assumed to represent pain-induced distress. This is supported by the fact that the marked cortisol concentration elevations observed immediately after calf dehorning and lamb castration/tailing are abolished in the presence of local anaesthesia (Wood *et al.*, 1991; Petrie *et al.*, 1996a; Dinniss *et al.*, 1997; SP Sylvester, DJ Mellor, KJ Stafford, RA Bruce, RN Ward, unpublished data). With regard to chronic pain however, cortisol may be a less useful measure (Livingston, 1994).

When using cortisol as an index of pain-induced distress it is important to be aware of the possibility that a specific pain stimulus may cause sufficiently intense distress as to induce maximal HPA axis activation. Any further increase in pain will not then elicit greater cortisol secretion. This may explain the reported insensitivity of cortisol concentrations to variations in the magnitude of painful electric shocks, a criticism of its use in distress

measurement (Rushen, 1986; Rushen and de Passille, 1992). However, injection of large doses of exogenous ACTH to achieve maximal cortisol levels within an animal can be used as a reference by allowing such an occurrence to be recognised. In addition it is acknowledged that changes in behaviour (Morisse *et al.*, 1995; Taschke and Folsch, 1995; Lester *et al.*, 1996), heart rate and plasma catecholamine concentrations (Friend, 1980; Mason and Mendl, 1993) may better reflect immediate pain in animals due to cortisol's own inherent secretory delay in response to stressors. This has particular relevance to the results presented in Chapter 3, where it is discussed further.

It is also important to note that although HPA axis activation is associated with distress, this does not imply that increased cortisol concentrations are inherently 'bad'. Cortisol has numerous physiological functions, thereby aiding adaptation to a stressor. These include the modulation of blood glucose levels, inflammation and catecholamine action. Adrenalectomised animals cannot survive even mild physical exertion without glucocorticoid therapy (Friend, 1980).

As an end note it should be asked how intense and for what duration does a cortisol elevation have to be in order that it be considered significant enough as to signify distress? Some have attempted to set a cut-off point with a 40% increase suggested to denote the beginnings of distress (Barnett and Hemsworth, 1990). However, this is simply guess work and in reality there is no ready answer to this question. A more meaningful approach and the one undertaken in the present study is to use cortisol responses as an index of distress in a relative context. That is, if an animal subjected to a putatively noxious stimulus has a significantly higher cortisol response relative to an animal not exposed to that stimulus (all other factors controlled for) then it is assumed that the former animal perceived that stimulus as distressful. Either response in isolation has little relevance in terms of assessing an animal's well-being.

1.22 Behaviour

Animals are motivated to avoid stimuli which give rise to negative emotional states such as pain, and may increase or decrease their behavioural activities in accordance with this objective (Kitchen *et al.*, 1987; Lawrence, 1991). Indeed it is often a change in behaviour

from normal that gives the first indication that an animal's welfare may be at risk (Matthews, 1992). It is for these reasons that behavioural observation is used as a guide for the welfare assessment of animals during and following exposure to certain husbandry procedures. Branding (Lay *et al.*, 1992), castration (Robertson *et al.*, 1994), docking (Petrie *et al.*, 1996b) and dehorning (Morisse *et al.*, 1995; Taschke and Folsch, 1995) have been associated with disruption of normal daily activities and frequent repetition of certain behavioural characteristics.

Behavioural observations are a valuable measure of pain for they involve minimal interference to the animal (Duncan and Poole, 1990). However, there are interpretative difficulties, particularly when the behaviours examined are in response to a husbandry procedure which involves more than one type of method. For instance, do lambs behaving in a characteristic subdued manner following knife docking experience less pain than those who typically express overt behaviour after ring docking (Lester *et al.*, 1996), or are the former simply too sore to move? In the present study all calves were dehorned by the one scoop amputation method, and so this potential problem was avoided.

There is substantial variation in behavioural responses to pain between species. This is of particular relevance to this thesis since it is generally acknowledged that herbivores respond less dramatically to painful stimuli (BVA Animal Welfare Foundation, 1985; Rollin, 1985). However, such limited behavioural responses to pain do not necessarily imply that the pain experienced is slight. Other innate behavioural motivations may take precedence (eg, grazing) in the presence of pain perception. This was kept in mind when interpreting the behavioural responses of calves following dehorning in the present study.

1.3 Outline of thesis

This thesis is divided into six chapters, consisting of a general introduction, four experimental studies and a general discussion. Each experimental chapter contains its own brief summary, an introduction, and sections devoted to materials and methods, results and discussion.

The first three experimental studies (described Chapter's 2, 3 and 4) assessed dehorning distress in calves from a physiological perspective, and the fourth (Chapter 5) from a behavioural perspective.

The same method of dehorning (scoop amputation) was used in all four studies.

Two main questions were addressed:

1. Does the depth of the scoop wound influence the degree of distress experienced by calves following horn removal (Chapter 2)?

2. To what extent does a long-acting local anaesthetic and/or a non-steroidal anti-inflammatory drug alleviate the pain-induced distress experienced by calves in response to scoop dehorning (Chapter 3, 4 and 5)?

CHAPTER TWO: Effects of shallow scoop and deep scoop dehorning on plasma cortisol concentrations in calves.

2.1 Chapter Summary

Scoop dehorning is one method of horn amputation. Plasma cortisol levels were used to investigate the effects of wound depth caused by deep scoop and shallow scoop dehorning on the acute pain-induced distress experienced by 3-4 month-old calves during the first 9 hours following horn amputation. Deep scoop and shallow scoop dehorning caused a prolonged cortisol elevation which returned to control values within 6 hours and 8 hours respectively. There were no significant differences between deep and shallow dehorning with regard to mean plasma cortisol concentrations and integrated cortisol responses during the 9 hours after dehorning. Linear regression analysis revealed no significant correlation between wound depth and integrated cortisol response. Hence, at the investigated scoop wound depths, there was no detectable benefit in implementing a shallow scoop strategy in order to reduce acute pain-induced distress caused by scoop dehorning in 3-4-month-old calves.

2.2 Introduction

Dehorning of cattle is a routine husbandry procedure developed to decrease injury to herd-mates and stockhandlers, particularly during yarding and transport. Presently in New Zealand cattle up to 20 months of age may be dehorned without prior administration of local anaesthetic (AWAC, 1994).

A variety of techniques involving either direct horn amputation or ablation of horn germinal tissue are used to disbud and dehorn cattle. The extent of pain-induced distress evoked within cattle by these techniques has been quantified using hypothalamic-pituitary-adrenal axis activation as an index. Dehorning by caustic stick (potassium hydroxide), cauterising iron, embryotomy wire, guillotine, saw and scoop were shown to be distressful to cattle as indicated by changes in their plasma cortisol concentrations (Laden *et al.*, 1985; Boandl *et al.*, 1989; Sylvester *et al.*, 1993; Morisse *et al.*, 1995; Petrie *et al.*, 1996a;).

However, calf distress responses to these dehorning methods may vary, both between and within each method. Cortisol responses following scoop dehorning were lower in lighter weight calves (Petrie, 1994). Since lighter weight calves are likely to have smaller diameter horns (Taschke and Folsch, 1995), and the depth of the wound caused by scoop dehorning is influenced by the size of the horn bud, it is possible that the smaller cortisol distress response may be associated with a shallow amputation wound (Petrie, 1994). In addition guillotine dehorning, a method involving amputation of the horn bud at the skin-horn junction, has been shown to elicit a marginally smaller cortisol distress response compared to that caused by the more invasive gouge-type wounds characteristic of scoop dehorning, suggesting a wound depth effect (SP Sylvester, DJ Mellor, KJ Stafford, RA Bruce, RN Ward, unpublished data).

The present study was designed to investigate whether or not the depth of scoop wounds influences the magnitude and/or duration of the plasma cortisol response during the first 9 hours after dehorning, in order to assess whether or not using a shallow scoop strategy would reduce post-dehorning pain-induced distress. To this end calves were dehorned using scoops which caused shallow or deep wounds, and the cortisol responses have been compared.

2.3 Materials and Methods

2.31 Animals

Thirty female Friesian calves, 14-16 weeks of age, weighing between 91 and 120 kg (mean 107 kg) and scheduled for dehorning according to normal farm practice were used. At 1700 hours on the day before the study the calves were housed indoors, allocated randomly to one of three groups ensuring that any difference in horn bud sizes were evenly represented in each group and spray painted with an identification number. Free access to water was provided over-night, but food was withheld. The following morning at 0500 hours equal numbers of calves from each of the three groups were moved quietly into two trial pens (11 m^2). Blood sampling commenced at 0700 hours.

2.32 Blood sampling

Blood samples (10 ml) were taken by venepuncture from either jugular vein at 0.25 hour (-0.25 hour) before treatment and at 0.25,

0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 8.0 and 9.0 hours after treatment. On each occasion the calf was restrained firmly but gently against a wall of the pen by two people while a third person took the blood sample. The whole procedure from restraint to completion of sampling usually took less than 15 seconds. The order of bleeding the calves in each pen was the same on each occasion.

2.33 Treatments

There were three treatments, each conducted while the calf was manually restrained against a pen wall by two people. Treatments took no longer than 15 seconds to perform. All amputated horns were retained, weighed immediately and then stored in 10% formulin isotonic saline solution for later measurement of scoop wound area and depth. No local anaesthetic was used in any of the treatments.

Control group

The horn buds and adjacent skin were handled firmly but not amputated.

Shallow scoop group

The horn buds were amputated with a modified standard dehorning scoop (Figure 2.1). The scoop's (Barnes Dehorners, Stones, U.S.A) two interlocking semicircular blades each of original diameter 56 mm × 38 mm were reshaped to 50 mm × 44 mm (Figure 2.2). This rounder shape helped to ensure that when the blades were closed over the horn bud, by separating the handles and pushing down towards the calf's head (Petrie *et al.*, 1996a), a more shallow gouge resulted even when the scoop was placed over larger diameter horn buds. The shearing action of the blades causing horn bud amputation was a single motion, taking less than one second to perform (Figure 2.3).

Deep scoop group

The horn buds were removed with a standard dehorning scoop (Barnes Dehorners, Stones, U.S.A), consisting of two interlocking semicircular blades each of diameter dimensions 56 mm × 38 mm (Figure 2.2). The resulting scoop wound was deeper than that produced by the shallow scoop, due to the cutting action of the more oval-shaped semicircular blades. This instrument is used in conventional scoop dehorning practice.

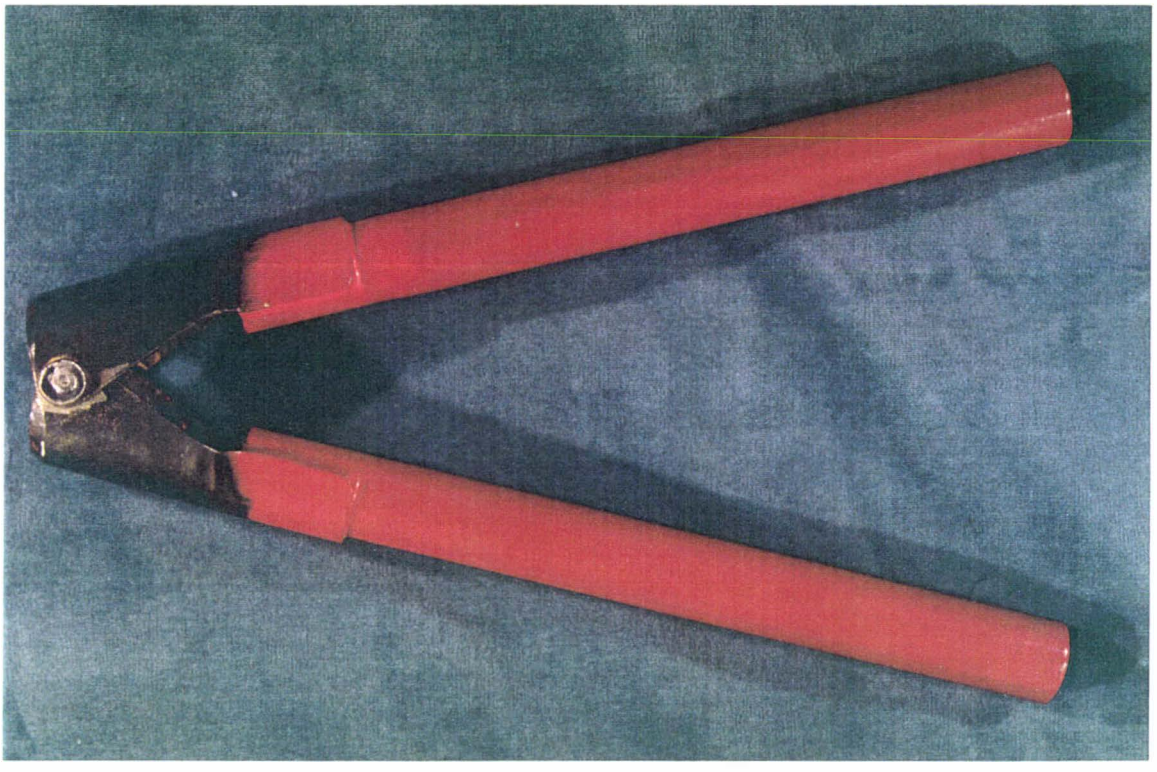


Fig 2.1: Shallow scoop (Barnes dehorners, U.S.A.) used to disbud the horns of calves.

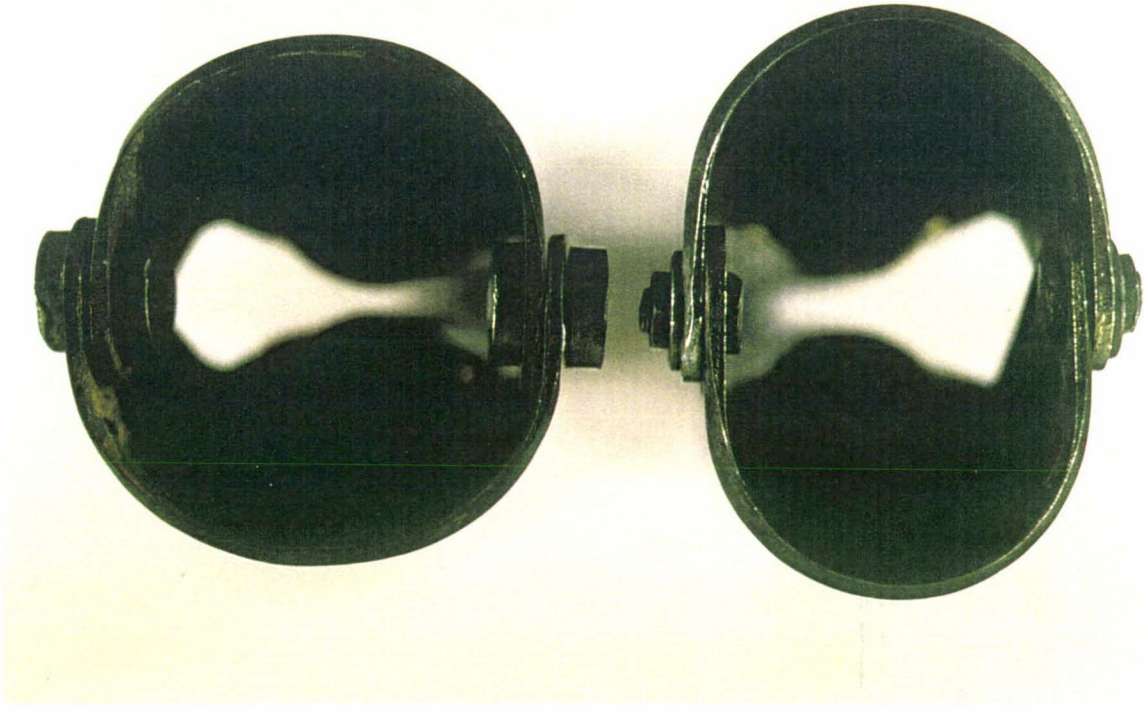


Fig 2.2: End view of shallow scoop (left) and deep scoop (right) blades.

Fig 2.3: Horn amputation using the scoop.

2.34 Plasma cortisol assay

Blood samples were collected in heparinised vacutainers, chilled immediately, then centrifuged and the plasma was removed and stored at -20 °C until required. Plasma cortisol concentrations were determined by using a non-extraction tritium radio-immuno-assay method (Endocrine Sciences, 4301 Lost Hills Rd, CA 91301); the lowest detectable concentration was 0.1 ng/ml and the intra-assay and inter-assay coefficients of variation were 6.8% and 9.9%, respectively.

2.35 Integrated cortisol results

The integrated cortisol responses were calculated to give a single measure of the magnitude and the duration of any increase in plasma cortisol concentration after a treatment. The integrated cortisol response is defined as the area between a horizontal line drawn through the pretreatment concentration (at -0.25 hour) and the cortisol response curve during defined periods after treatment when the concentrations were greater than the pretreatment value (Mellor and Murray, 1989).

2.36 Statistical analysis

Where applicable the cortisol results have been expressed as the mean ± standard error of the mean (SEM). Except where otherwise stated all cortisol results refer to plasma cortisol concentrations adjusted by subtracting the pretreatment value. Hence the cortisol response curve is expressed as the change from the -0.25 hour plasma cortisol concentration. Significant differences between mean concentrations and between integrated cortisol responses were determined using Student's t test assuming unequal variation (Microsoft Excel V 5.0, Microsoft Corporation, U.S.A). Possible correlations between integrated cortisol responses and scoop wound depth (distance measured from skin-horn junction to the bottom of the amputated bone), scoop wound area (area of the amputated horn base consisting of both bone and skin), bone length (distance from the top of the bone present within the horn bud to the bottom), bone area (area of the base of the amputated bone) and weight of horn bud were analysed by linear regression (Microsoft Excel V 5.0).

2.4 Results

Difficulty was experienced in consistently producing the intended deep or shallow wounds in four of the twenty dehorned calves due to head movement of these animals, but amputated tissue sizes were similar for both horns in each calf. After analysis of horn bud scoop wound depths three calves that were intended to have deep scoop wounds and one intended for shallow scoop dehorning were reallocated to the shallow scoop and deep scoop groups, respectively (Table 2.1). All five horn parameters measured were significantly different between the deep scoop and shallow scoop dehorning groups (Table 2.1).

There were no significant between-group differences in the mean plasma cortisol concentrations at the -0.25 hour pretreatment sample (Table 2.2) and the bleeding order of the calves had no significant effect on plasma cortisol concentration (Figure 2.4).

Control

Restraint and blood sampling were followed by a significant rise in plasma cortisol concentration (5 ng/ml; $P<0.05$) which returned to starting values within 30 minutes of treatment. Thereafter, no significant changes in plasma cortisol concentrations occurred (Figure 2.5).

Shallow scoop

Shallow scoop dehorning caused a marked rise in mean cortisol concentration (29 ng/ml), followed by a fall to plateau values which were sustained until approximately 4.5 hours after treatment. The mean cortisol concentration had returned to control values by 8 hours (Figure 2.5). When evaluated in individual calves cortisol concentration returned to pretreatment values at 7.8 ± 0.38 hours and returned to corresponding control values at 7.5 ± 0.45 hours.

Deep scoop

Deep scoop dehorning caused a marked transient rise in mean cortisol concentration of 28 ng/ml and plateau values were sustained subsequently until approximately 4.5 hours after treatment. Cortisol concentrations returned to control values by 6 hours (Figure 2.5). When evaluated individually cortisol concentration returned to pretreatment values at 7.3 ± 0.51 hours and returned to corresponding control values at 7.3 ± 0.52 hours.
There were no significant differences between the shallow scoop and deep scoop groups in their plasma cortisol concentrations at any

time after dehorning, and nor did the duration of the cortisol responses differ significantly between the two scoop groups.

2.41 Integrated cortisol responses

The integrated cortisol responses were calculated for three periods: from 0.25 hour before until 9 hours after treatment, from 0.25 hour before until 1.5 hours after treatment, and from 1.5 hours to 9 hours after treatment (Table 2.2).

At all three time periods the integrated cortisol responses caused by both shallow and deep scoop dehorning were significantly greater than that elicited by control handling. There were no significant difference between integrated cortisol responses of the shallow scoop and deep scoop groups for any time period.

2.42 Linear regressions

There were no significant correlations for any of the three time periods between the integrated cortisol responses and the scoop wound depth, total scoop wound area, bone area, bone height or weight of the horn buds removed (correlation coefficients, r= -0.37 to +0.06: Table 2.3). Likewise, no significant correlation was found between these wound indices and the time taken for plasma cortisol concentrations to return to pretreatment values or mean control values following dehorning (r= -0.40 to + -0.11).

2.5 DISCUSSION

Using cortisol as an index of distress, this study supports the previous findings that scoop dehorning in the absence of local anaesthesia and/or analgesia is a markedly distressing experience for calves for a period of at least 6-8 hours following the amputation procedure (Petrie *et al.*, 1996a; Sylvester *et al.*, 1993). However, there was little evidence to suggest that the depth of scoop injury significantly influenced the magnitude and/or duration of the post-dehorning distress response. There were no significant differences between the mean plasma cortisol concentrations following shallow scoop and deep scoop dehorning, nor did the corresponding integrated cortisol responses differ significantly.

Table 2.1: The mean (± SEM) dimensions of amputated horn buds for the dehorning treatment groups.

Treatment	n[a]	mean horn weight (g)	mean scoop depth (mm)	mean scoop[b] area (mm^2)	mean bone area (mm^2)	mean bone[c] height (mm)
Shallow scoop	12	3.9± 0.37	1.1 ± 0.2	479 ± 38	133 ± 12	6.4 ± 0.66
Deep scoop[d]	8	7.5± 0.27**	3.0 ± 0.2**	703 ± 21**	240 ± 11**	11.8 ± 0.69**

[a] The number of horn bud pairs.

[b] Total scoop area consisting of both bone and skin.

[c] The height from top of the cornual process of the frontal bone present within the horn bud to the bottom of this amputated bone.

[d] ** Significantly different from corresponding shallow scoop values ($P<0.01$).

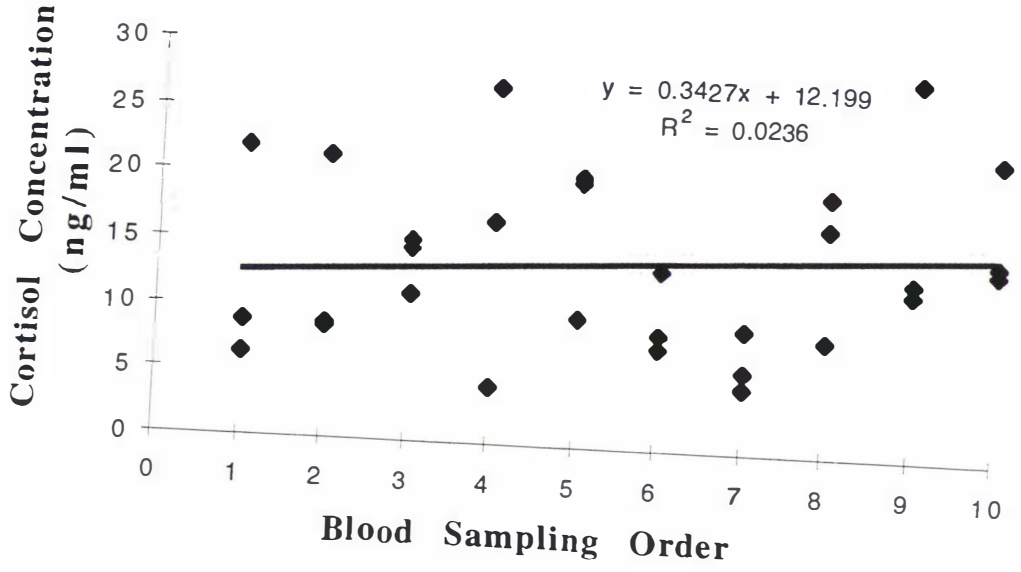


Fig 2.4: Relationship between plasma cortisol concentration and the sample order for the pretreatment blood sample.

Table 2.2: The pretreatment plasma cortisol concentrations (mean ± SEM) for each group and the integrated cortisol responses (mean ± SEM) for the complete recording period of 9 hrs, the first 1.5 hours and final 7.5 hours after treatment.

Group	n	Pretreatment plasma cortisol concentration (ng/ml)	Integrated cortisol response (ng/ml.min)		
			over total 9 hour period	over first 1.5 hours	over final 7.5 hours
Control	10	13.7 ± 2.2	1350 ± 353	334 ± 72	1016 ± 326
Shallow scoop	12	13.3 ± 1.6	8975 ± 1169*	2135 ± 202*	6839 ± 982*
Deep scoop	8	15.7 ± 2.8	8358 ± 989*	2132 ± 281*	6226 ± 807*

* significantly different ($p<0.01$) from corresponding control calves.

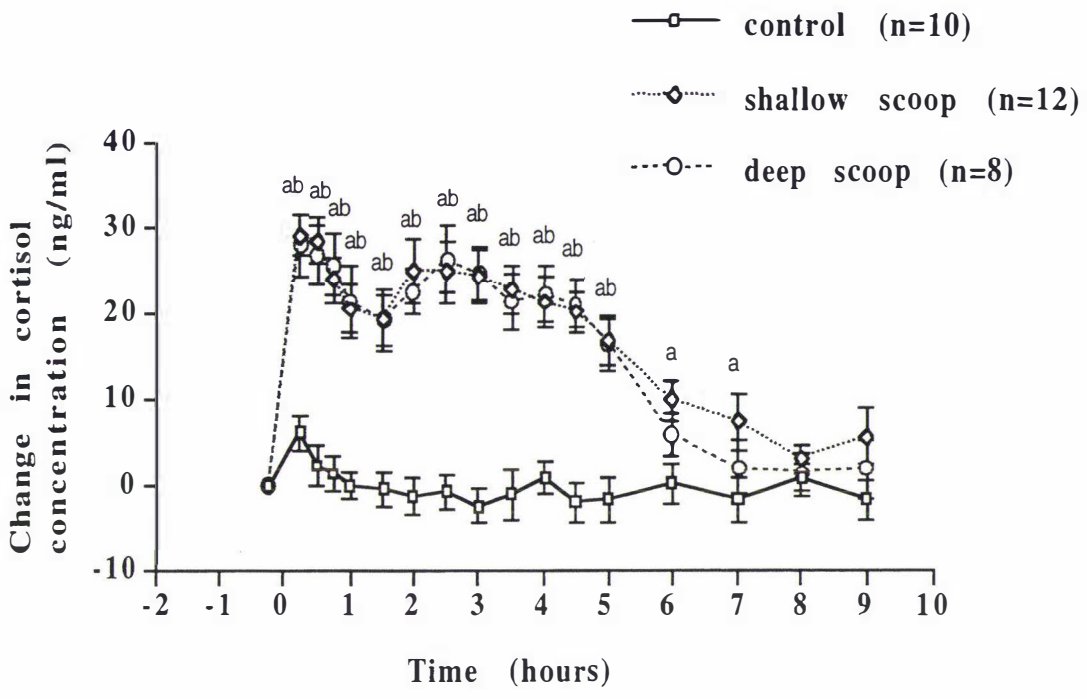


Fig 2.5: Changes in plasma cortisol concentration (Mean ± SEM) in calves in response to shallow and deep scoop dehorning. Time 0 represents the time of dehorning. [a = shallow scoop significantly different from control; b = deep scoop significantly different from control].

Table 2.3: Linear regression analysis of integrated cortisol response at three different time periods versus horn bud parameter measurements. All values represent R.

Integrated cortisol response	**Horn Parameters**				
	horn weight	scoop depth	scoop area	bone area	bone height
over initial 1.5 hours	-0.18	+0.06	-0.17	-0.14	-0.20
over final 7.5 hours	-0.36	-0.06	-0.37	-0.32	-0.32
over total 9 hours	-0.33	-0.04	-0.35	-0.29	-0.31

Likewise, regression analysis revealed no significant correlation between the various indices of wound depth and the integrated cortisol response. The only significant difference was observed late in the cortisol response, 6-8 hours after dehorning. The mean cortisol concentrations in shallow scoop calves remained significantly higher than in control calves until 8 hours after dehorning, compared to 6 hours after dehorning in deep scoop calves (Figure 2.5). However, this is not likely to be biologically significant because an analysis of individual cortisol results revealed no significant difference between groups in the duration of the cortisol responses.

In order to support the conclusion that depth of scoop wound has no apparent effect on the acute pain-induced distress experienced by calves following horn amputation it is necessary to exclude the following possibility; that shallow scoop dehorning may have elicited a maximal cortisol response such that any greater perceived pain with deep scoop dehorning would not be paralleled by a corresponding increase in cortisol secretion. It is known that although the initial rapid rise to peak concentrations does apparently reflect a maximal adrenocortical response, the subsequent decrease and the lower plateau values represent submaximal cortisol secretory rates (Petrie *et al.*, 1996a). Consequently, during the plateau and subsequent stages of the cortisol responses observed here (Figure 2.5), there was scope for higher plasma concentrations to have been elicited in the deep scoop calves had the procedure been significantly more noxious than the shallow scoop method. The similar cortisol responses in both groups therefore do indicate an absence of a difference in the pain-induced distress caused by the two methods.

It should also be noted that it is possible the difference in the wound depths caused by shallow and deep scoop dehorning in this trial, while significant, may not have been large enough to cause post-dehorning distress of different magnitudes. However, further increasing the wound depth would increase the risk of skull perforation and hence possible development of painful sinusitis during the days following dehorning. Skull perforation is also dependent upon calf age, since an extension of the frontal sinus invades the horn bud cornual process when the animal is approximately six months old (Dyce, *et al*, 1987). Thus, in spite of the fact that there were no detectable differences in the overall cortisol responses to shallow and deep scoop dehorning, minimising the wound depth to avoid skull perforation would be desirable,

particularly in older calves. Of course scoop wounds should not be so shallow that horn regrowth occurs. There was no evidence of horn regrowth in any of the calves involved in this study.

Finally, if the pain-induced distress experienced by calves during and immediately following dehorning is to be reduced, it is evident that other dehorning methods as well as possible anaesthetic and/or analgesic administrative strategies are needed. Some of these have been investigated during work described in Chapters 3 and 4.

CHAPTER THREE: Effect of long-acting local anaesthetic on the acute cortisol responses to scoop dehorning in calves.

3.1 Chapter Summary

Changes in cortisol concentrations were used to monitor the pain-induced distress in 3-4 month-old Friesian calves during the 9.5 hours following scoop dehorning in the presence or absence of long-acting local anaesthetic. Local anaesthetic abolished the otherwise marked cortisol elevation which follows scoop dehorning during its 4 hour nerve-blockade action. However, administration of local anaesthetic did not significantly reduce the overall cortisol response, due to a delayed appearance of a marked cortisol rise when the nerve-blockade effects wore off. Extending the duration of the nerve-blockade analgesia to cover the usual 7-8 hour acute distress response which follows scoop dehorning, did not completely abolish that response. There was no detectable difference in the cortisol response of those calves administered local anaesthetic immediately before versus 20 minutes before scoop dehorning. However, allowing time for the local anaesthetic to act before dehorning is still recommended, as it is questionable whether the plasma cortisol response is rapid enough to reflect immediate pain-induced distress.

3.2 Introduction

Cattle dehorning is a routine husbandry procedure. Its purpose is to reduce injury to herd-mates and stock handlers particularly during yarding and transport. It is widely recognised as a necessary procedure (Armstrong, 1985; Stafford and Mellor, 1993). However, it is also clear that immediate and marked pain is experienced by cattle following dehorning, whether it be done by amputation (Carter *et al.*, 1983; Sylvester., 1993; Petrie *et al.*, 1996a; McMeekan *et al.*, 1997 [Chapter 2]) or germinal tissue destruction by cautery (Laden *et al.*, 1985; Boandl *et al.*, 1989; Morisse *et al.*, 1995; Taschke and Folsch, 1995; Petrie *et al.*, 1996a).

The alleviation of dehorning pain in calves has received increased attention in recent years. Cauterisation of horn bud germinal tissue by a gas-heated disbudding iron evoked a smaller cortisol distress response compared to direct scoop amputation in 6-8 week old

calves (Petrie *et al.*, 1996a). However, cautery dehorning is suitable only for calves less than 2 months of age. In older animals scoop dehorning is a common method of horn amputation. Minimising the scoop wound depth by deliberately scooping shallow was found to be ineffective in reducing post-dehorning distress (McMeekan *et al.*, 1997 [Chapter 2]). Administration of short-acting local anaesthetic (lignocaine) in scoop-dehorned calves appears useful as it abolished the initial post-dehorning pain, but it is evident that pain is perceived about 2 hours after dehorning when the nerve-block analgesic effect wears off (Petrie *et al.*, 1996a; SP Sylvester, DJ Mellor, KJ Stafford, RA Bruce, RN Ward, unpublished data).

The therapeutic benefit of administering long-acting local anaesthetic prior to dehorning calves with a scoop has not been evaluated. However, it is known that when the duration of local anaesthetic action exceeds the duration of castration and tailing distress in lambs the usual behavioural and cortisol responses are abolished (Wood *et al.*, 1991). On a similar basis it may be possible to alleviate the acute distress response which follows scoop dehorning in calves, a response consistently observed to last 7-8 hours (Sylvester *et al.*, 1993; Petrie *et al.*, 1996a; McMeekan *et al.*, 1997 [Chapter 2]), by extending the duration of nerve-block analgesia in these animals. This reasoning was examined in the present study by administering long-acting local anaesthetic (Bupivicaine hydrochloride) to calves in two ways. It was given once prior to scoop dehorning or prior to and again at 4 hours after dehorning, such that the anticipated period of nerve-blockade analgesia was extended to approximately 4 hours and 8 hours, respectively (Link and Smith, 1956).

In previous studies local anaesthetic was administered 20 minutes before dehorning to alleviate the painful procedure of horn amputation (Sylvester *et al.*, 1993; Petrie *et al.*, 1996a). This allowed sufficient time for the local anaesthetic to take effect. However, whether calves experience greater pain when dehorning is carried out immediately after local anaesthetic administration is unclear. In the present study bupivicaine was injected either immediately before or 20 minutes before scoop dehorning to allow this to be assessed.

The pain-induced distress associated with horn amputation and the degree to which it may be alleviated by local anaesthetic administration was quantified using changes in plasma cortisol concentration as an index. Cortisol has been used as a physiological

parameter to assess the aversiveness of a wide variety of husbandry procedures in stock, including handling and restraint (Mellor and Pearson, 1975; Herd, 1989; Zavy *et al.*, 1992), transport (Crookshank *et al.*, 1979; Kent and Ewbank, 1983, 1986a,b), castration and/or tailing of lambs (Shutt *et al.*, 1988; Mellor and Murray, 1989; Mellor *et al.*, 1991; Lester *et al.*, 1991a, b, 1996; Wood *et al.*, 1991; Kent *et al.*, 1993; Kent *et al.*, 1995), castration of kids (Mellor *et al.*, 1991) and castration, dehorning or tail docking of calves (Laden *et al.*, 1985; Boandl *et al.*, 1989; Cohen *et al.*, 1990; Sylvester *et al.*, 1993; Robertson *et al.*, 1994; Morisse *et* al., 1995; Taschke and Folsch, 1995; Petrie *et al.*, 1996a, b; McMeekan *et al.*, 1997).

However, during times of trauma-induced distress it is possible that inflammatory mediators released from the damaged tissue may have a direct stimulatory action on the hypothalamic-pituitary-adrenal axis, and cause increased cortisol release independently of any noxious sensations (Kent *et al.*, 1993). It has been suggested that such a mechanism might contribute to the elevation in cortisol concentration which occurs up to 7.5 hours after scoop dehorning in calves (Petrie *et al.*, 1996a). In the present study administration of long-acting local anaesthetic in calves prior to scoop dehorning helped to assess this possibility. It was assumed that any increase in cortisol secretion observed during the period of nerve-block analgesia would be due to a mechanism acting independently of any noxious sensory input from the damaged area. The absence of a cortisol response would not only indicate relief from pain-induced distress, but also absence of adrenocortical activation during an inflammatory response.

3.3 Materials and Methods

3.31 Animals

Seventy female Friesian calves, 3-4 months of age, weighing between 62 and 110 kg (mean 86 kg) were used in this study, which was conducted over four trial days. At 1700 hours on the evening before each trial day 17 or 18 calves were housed in an open-fronted shed with a sawdust floor, allocated to one of seven treatment groups and spray painted with an identification number. Equal numbers from each treatment group were held in two pens overnight, together with calves involved in a separate study which was run simultaneously (Chapter 4), such that each of the two pens

contained 16 or 17 calves in total. Calves were given free access to water over-night. At 0500 hours on the morning of each trial day, the mobs were moved quietly into two smaller pens located in the same open-sided shed (10.4 m^2). Blood sampling commenced at 0700 hours.

3.32 Blood sampling

Blood samples (10 ml) were taken by venepuncture from either jugular vein at 0.33 hour before treatment (-0.33 hour) and at 0.0, 0.33, 0.66, 1.0, 1.33, 1.83, 2.33, 2.83, 3.33, 3.83, 4.33, 4.83, 5.33, 6.33, 7.33, 8.33 and 9.33 hours after treatment. On each occasion the calf was restrained firmly but gently against a wall of the pen by two people while a third took the blood sample (Figure 3.1). The whole procedure from restraint to completion of sampling usually took less than 15 seconds. The order of bleeding the calves in each pen was the same on each occasion.

3.33 Treatments

There were seven treatments, each conducted while the calf was manually restrained against the pen wall by two people. The injection and amputation procedures took no longer than 30 seconds to perform on each occasion.

Control (='Control')
The horn buds and adjacent skin were massaged but not amputated.

Bupivicaine (4 hours) control (='LA4 control')
Local anaesthetic (6 ml of 0.25% Bupivacaine hydrochloride; Marcain Astra Pharmaceutical Pty Ltd, Australia) was injected around each cornual nerve, midway along the lateral edge of the frontal bone crest (Weaver, 1986), 20 minutes prior to calves having their horn buds massaged but not amputated (Figure 3.2). The duration of local anaesthetic action was approximately 4 hours (Link and Smith, 1956).

Bupivicaine (8 hours) control (='LA8 control')
Calves were administered local anaesthetic as above. A second equivalent dose was injected 4 hours after the horns were massaged but not amputated, such that the duration of local anaesthetic action was 7-8 hours.

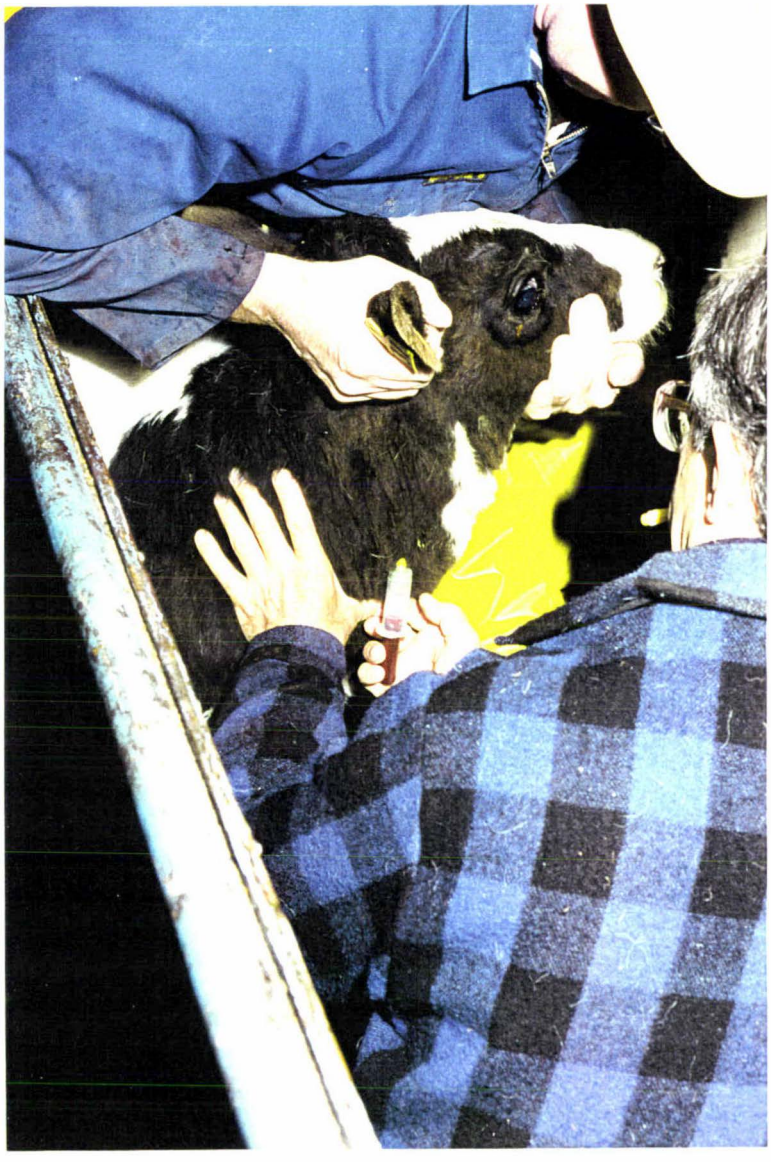


Fig 3.1: The blood sampling procedure.

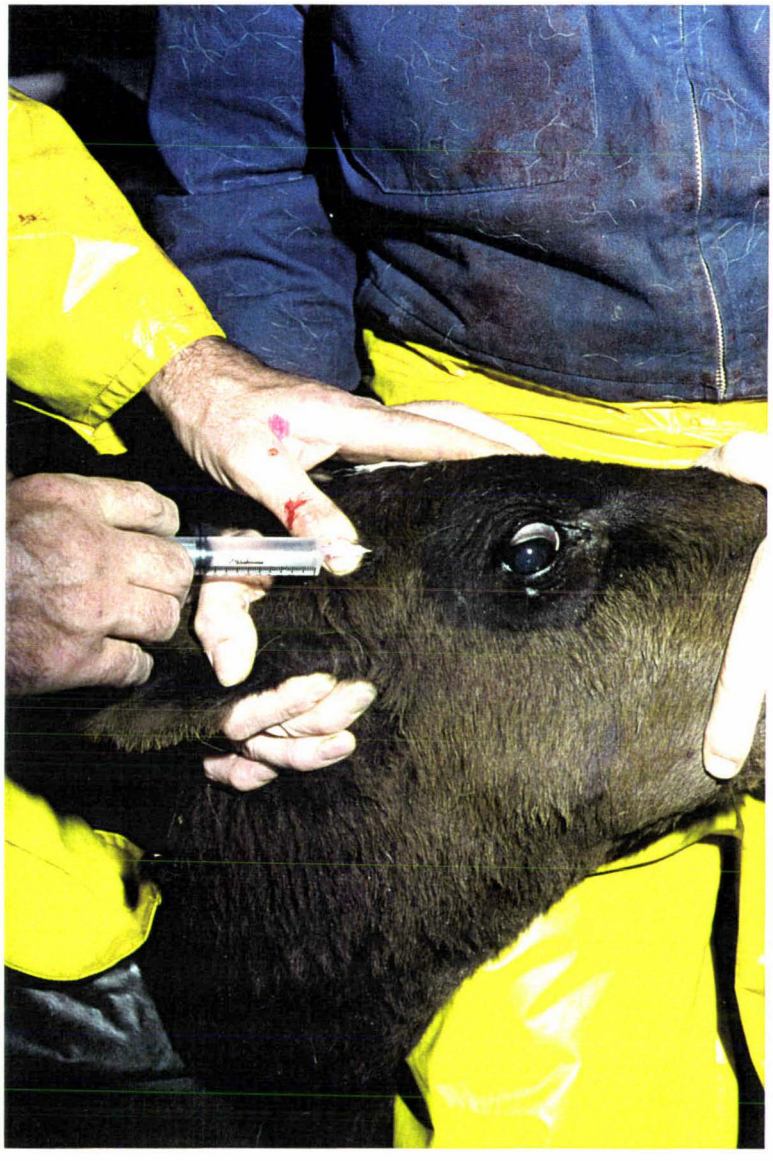


Fig 3.2: Injection of local anaesthetic.

Scoop (= 'Scoop')
The horns were removed with a standard dehorning scoop (Barnes Dehorners, Stones, USA) consisting of two interlocking semicircular blades each of diameter 56 mm × 38 mm attached to handles. The act of closing the blades, by separating the handles and pushing down towards the calf's head, amputated the horn, adjacent skin and some underlying bone (Petrie *et al.*, 1996a).

Bupivicaine (4 hrs) + scoop (='LA4 + scoop')
Local anaesthetic was administered 20 minutes before the horns were removed with the scoop as described above. Duration of nerve-block analgesia was approximately 4 hours.

Bupivicaine (4 hrs) + scoop immediately (='LA4$_{[t=0]}$ + scoop')
Local anaesthetic was administered as described for LA4 control calves immediately prior to the horns being removed with the scoop as described for the Scoop calves. Duration of nerve block analgesia was approximately 4 hours.

Bupivicaine (8 hours) + scoop (='LA8 + scoop')
Local anaesthetic was administered 20 minutes before the horn buds were removed with the scoop and again 4 hours after dehorning, such that the total duration of nerve-block analgesia was 7-8 hours.

3.34 Plasma cortisol assay

Blood samples were collected in heparinised vacutainers, chilled immediately, then centrifuged and the plasma was removed and stored at -20 °C until required. Plasma cortisol concentrations were determined by using a non-extraction tritium radio-immuno-assay method (Endocrine Sciences, 4301 Lost Hills Rd, CA 91301); the lowest detectable concentration was 0.1 ng/ml and the intra-assay and inter-assay coefficients of variation were 7.6% and 9.5%, respectively.

3.35 Integrated cortisol responses

The integrated cortisol responses were calculated to give a single measure of the magnitude and the duration of any increase in plasma cortisol concentration after treatment. The integrated cortisol response is defined as the area between a horizontal line

drawn through the pretreatment concentration (at -0.33 hour) and the cortisol response curve during defined periods after treatment when the concentrations were greater than the pretreatment value (Mellor and Murray 1989).

3.36 Statistical analysis

The pretreatment cortisol results have been expressed as the mean ± standard error of the mean (SEM), and all other results are expressed as the change from these levels. Hence the cortisol curves are expressed as the change from the -0.33 hour plasma cortisol concentration. Significant differences between mean concentrations and between integrated cortisol responses were determined using Student's t-test assuming unequal variation (Microsoft Excel V 5.0, Microsoft Corporation, U.S.A). Students t-test: paired two sample for mean (Microsoft Excel V 5.0) was used to determine whether there were significant changes with time after each treatment.

3.4 Results

Thirteen control animals from trial days one and two were reused on the fourth day, due to low availability of calves. The mean pretreatment plasma cortisol concentration of these calves when used the second time was not significantly different from the mean pretreatment value of all other calves involved in this study. However, the mean pretreatment cortisol concentrations in first trial day calves (21 ng/ml) was significantly higher than that of third (13 ng/ml) and fourth (15 ng/ml) trial day calves ($p<0.05$). These differences were small in comparison with the responses observed in calves dehorned in the absence of an anaesthetic or an anti-inflammatory drug. Blood sampling order was found to have a small but significant effect on cortisol pretreatment values ($r = 0.34$; $p<0.05$), and hence was of little probable biological relevance (Figure 3.3).

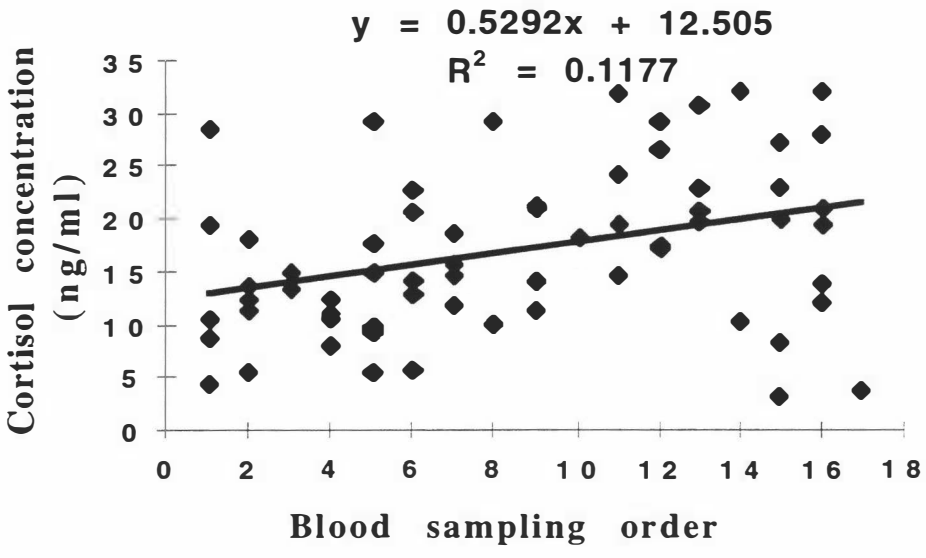


Fig 3.3: Relationship between plasma cortisol concentration and the sample order for the pretreatment blood sample.

Despite the above observations no significant between-group differences were found in the mean pretreatment cortisol concentrations, due to calves given different treatments being evenly distributed throughout the blood sampling order and between all four trial days (Table 3.1).

Control

Restraint and massage of horn buds elicited a small significant rise of 6 ng/ml in the mean plasma cortisol concentrations which returned to pretreatment values 40 minutes after treatment. There was no other significant change in plasma cortisol concentration throughout the 9.5 hour sampling period, except for a fall of 4 to 6 ng/ml below pretreatment values at 2.83, 3.33, 3.83, 4.33, 6.33 and 8.33 hours ($p<0.05$).

LA4 control

A significant elevation in the mean cortisol concentration to 9 ng/ml ($p<0.05$) above pretreatment level occurred 20 minutes after local anaesthetic administration (0 hour), and to 5 ng/ml 1.33 hours later (Figure 3.5). At all other sample times the mean cortisol concentration did not deviate significantly from pretreatment levels.

There was no significant difference between the mean cortisol concentrations of control and LA4 control calves (Figure 3.4).

LA8 control

The mean cortisol concentration increased significantly to 7 ng/ml ($p<0.05$) above the pretreatment value 20 minutes after local anaesthetic administration (0 hour). Thereafter, there was no significant deviation except at 2.83, 3.33, 3.83, 4.83 and 8.33 hours when mean cortisol levels fell to 3-6 ng/ml below the pretreatment value (Figure 3.6).

There was no significant difference between the mean cortisol concentrations of control and LA8 control calves (Figure 3.4).

Scoop

Scoop dehorning caused a marked significant rise in mean cortisol concentration within the first 20 minutes after horn amputation ($p<0.01$), which peaked at 28 ng/ml above the pretreatment level. This was followed by a fall to plateau values which were maintained between 1.83 and 4.33 hours (Figure 3.5). The mean

Treatment	n	Plasma cortisol concentration (ng/ml)
Control	10	18.5 ± 2.6
LA4 control	10	15.3 ± 3.0
LA8 control	10	14.9 ± 2.1
Scoop	9	19.2 ± 2.7
LA4 + scoop	9	17.1 ± 2.4
LA4[t=0] + scoop	9	19.0 ± 2.3
LA8 + scoop	8	15.3 ± 2.8

Table 3.1: Plasma cortisol concentrations (mean ± SEM) in calves 0.33 hours before treatment.

concentration returned to control levels by 7.33 hours after dehorning.

LA4 + scoop

In calves which were scoop dehorned 20 minutes after local anaesthetic administration, a significant and protracted rise in mean cortisol concentration was observed 4.33 hours after treatment, followed by a return to Control and LA4 control levels by 9.33 and 8.33 hours, respectively (Figure 3.5). At 6.33 and 7.33 hours the mean concentrations were significantly higher than those of the Scoop calves.

During the first four hours after dehorning, the mean cortisol concentration did not differ significantly from that of Control and LA4 control calves, except at 0.66 and 2.83 hours when they were higher than for Control group calves.

LA4[t=0] + scoop

In calves which were scoop dehorned immediately after local anaesthetic administration, a protracted rise similar to that observed in the LA4 + scoop group occurred, except that the mean cortisol concentration did not rise significantly above that of LA4 control calves until 4.83 hours after treatment and returned to Control and LA4 control levels by 8.33 hours (Figure 3.5). At 7.33 hours the mean concentration was significantly higher than that of the Scoop calves.

The mean concentration was not significantly different from LA4 control calves during the first 4 hours after dehorning. When compared to Control calves during the same 4 hour post-dehorning period, a significantly greater mean cortisol elevation was noted only at 0.66 hour.

There was no significant difference between the mean cortisol concentrations of calves dehorned immediately after and 20 minutes after local anaesthetic administration.

LA8 + scoop

The mean cortisol concentration of calves given local anaesthetic 20 minutes before and again 4 hours after scoop dehorning differed significantly from that of Control and LA8 control calves only at 3.83, 8.33 and 9.33 hours (Figure 3.6). A significant elevation in mean cortisol concentration was also noted at 8.33 and 9.33 hours

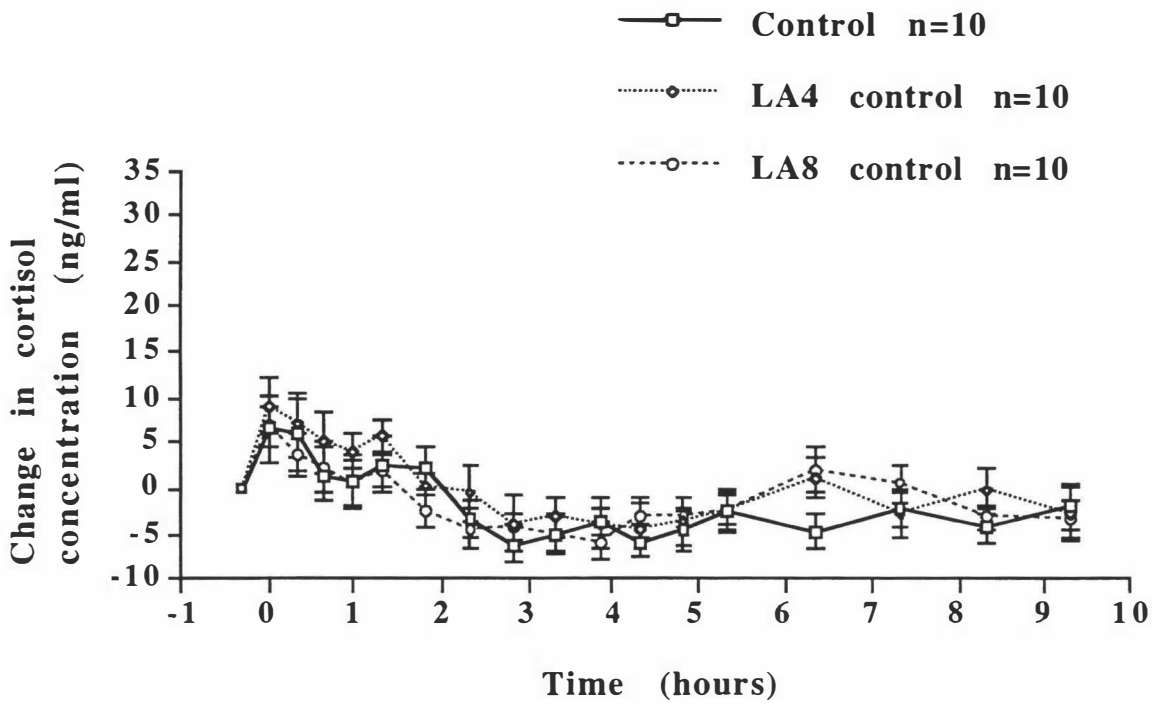


Fig 3.4: Changes in plasma cortisol concentration in calves in response to handling and local anaesthetic administration.

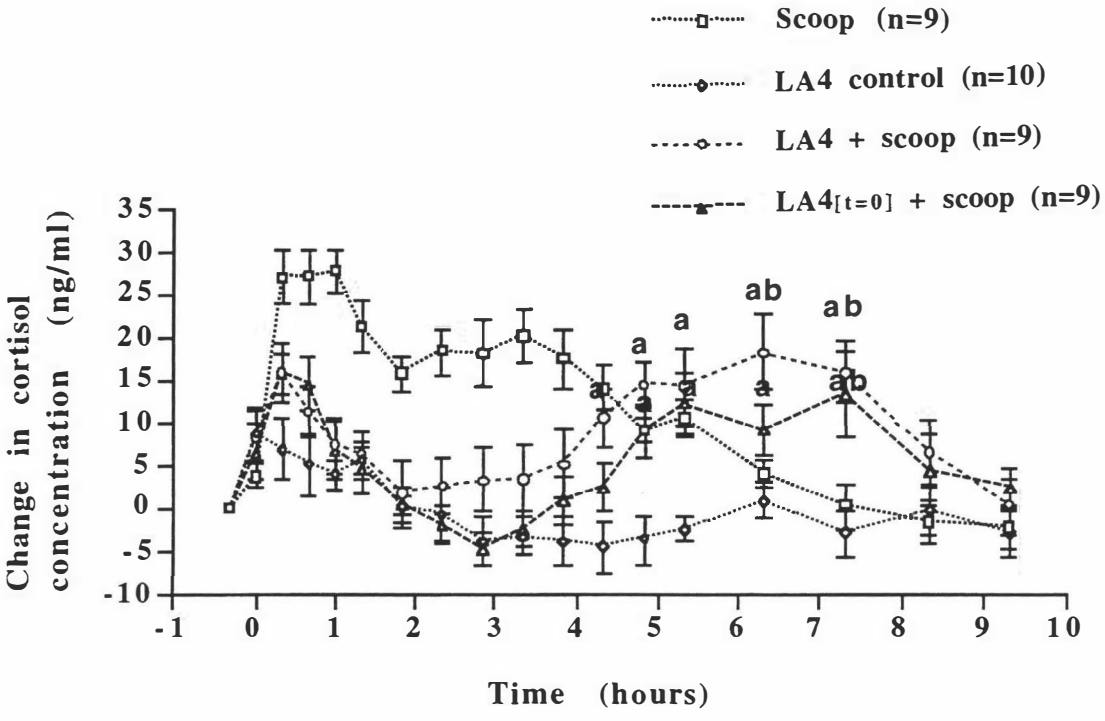


Fig 3.5: Changes in plasma cortisol concentration in calves in response to scoop dehorning with and without LA4. Time 0represents time of dehorning. [a = significantly different from LA4 control; b = significantly different from Scoop].

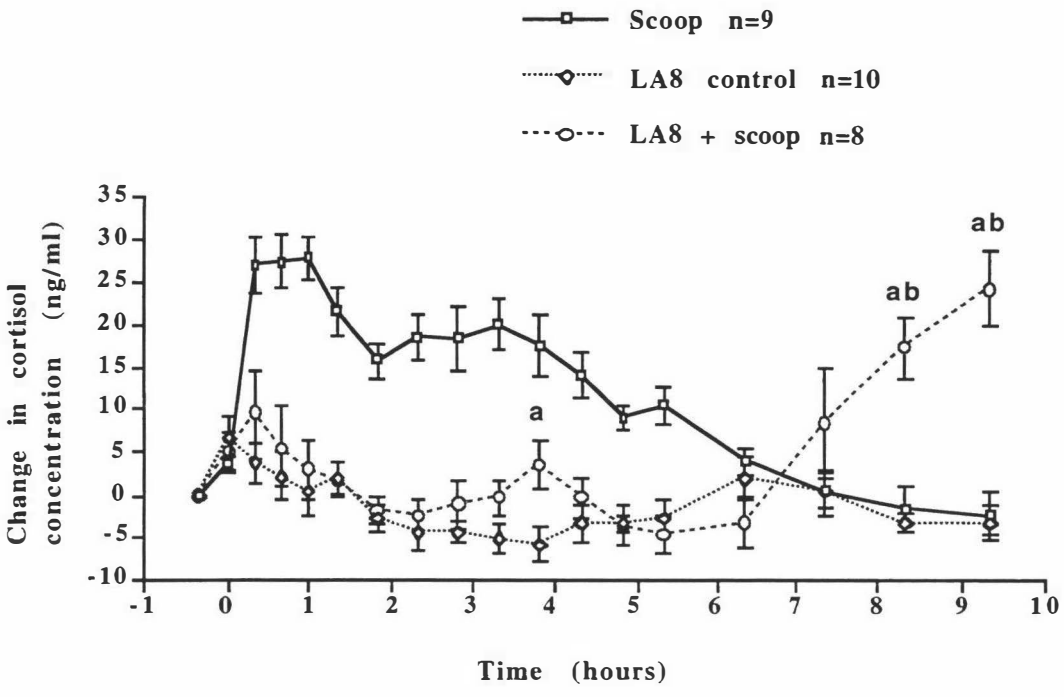


Fig 3.6: Changes in plasma cortisol concentration in calves in response to scoop dehorning with and without LA8. Time 0 represents time of dehorning. [a = significantly different from LA8 control; b = significantly different from Scoop].

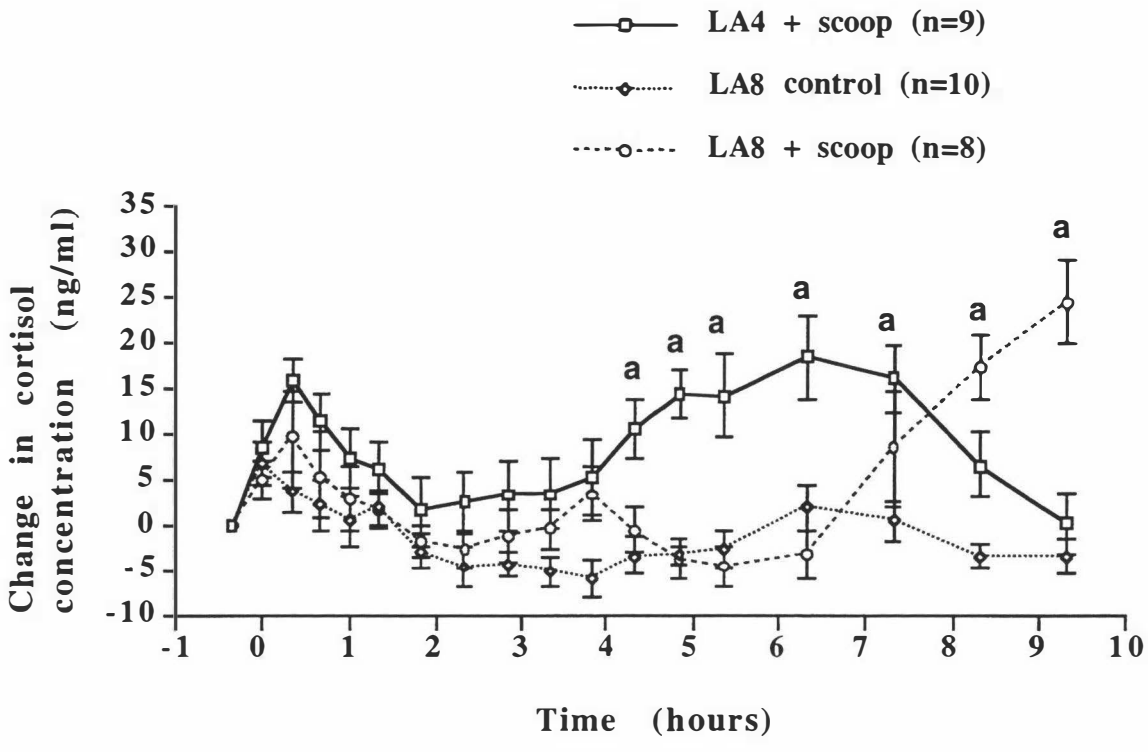


Fig 3.7: Changes in plasma cortisol concentration in calves in response to scoop dehorning with LA4 or LA8. Time 0 represents time of dehorning. [a = significantly different from LA8 control].

Table 3.2: The mean (± SEM) integrated cortisol responses for each group over the complete recording period of 9.33 hours, the first 3.83 hours and final 5.50 hours after treatment. Also noted are the overall durations of the responses. Different superscripts in each column indicate significant differences ($p < 0.05$).

		Integrated cortisol response (ng/ml.min)		
Treatment	Duration* (hours)	Whole 9.33 (hours)	First 3.83 (hours)	Final 5.50 (hours)
Control	0.00	1080 ± 373[a]	813 ± 316[a]	267 ±120[a]
LA4 control	0.33	1642 ± 509[a]	949 ± 305[a]	693 ± 257[a]
LA8 control	0.33	940 ± 184[a]	503 ± 139[a]	437 ± 111[a]
Scoop	7.33	6708 ± 822[b]	4713 ± 509[b]	1995 ± 404[b]
LA4 + scoop	8.33	6021 ± 713[b]	1838 ± 412[a]	4184 ± 610[b]
LA4[t=0] + scoop	8.33	4221 ± 841[b]	1281 ± 309[a]	2940 ± 670[b]
LA8 + scoop	Incomplete	3543 ± 620[#]	910 ± 418[a]	2633 ± 455[#]

*The time after treatment when the cortisol concentration returned to pretreatment values.
[#] The cortisol response was not complete at 9.33 hours.

comparison with the mean response of Scoop calves. Three calves in this group were noted to have exhibited a decline in plasma cortisol concentration before the trial's end.

3.41 Integrated cortisol responses

The integrated cortisol responses were calculated for three main periods: from 0.33 hour before until 9.33 hours after treatment, from 0.33 hour before until 3.83 hours after treatment, and from 3.83 hours to 9.33 hours after treatment (Table 3.2).

The total integrated cortisol response was significantly greater in the Scoop calves than in the Control, LA4 control, LA8 control and LA8 + scoop calves, but was similar to the responses of LA4 + scoop and LA4[t=0] + scoop calves. During the first 3.83 hours after treatment the responses of the LA4 + scoop, LA4[t=0] + scoop and LA8 + scoop calves were not significantly different from those of LA4 and LA8 control calves. After 3.83 hours to the trial's end, the responses of LA4[t=0] + scoop and LA8 + scoop calves did not differ significantly from that of Scoop group for the same period, whereas the response of LA4 + scoop calves was significantly greater. The total response of LA8 + scoop calves was significantly smaller than that of the LA4 + scoop group but not significantly different from that of the LA4[t=0] + scoop group. From 3.83 hours to the trial's end there was no significant difference between the responses of LA4 + scoop, LA4[t=0] + scoop and LA8 + scoop calves, but it must be noted that the cortisol response of the latter group was not complete so that the figure underestimates this response. There was no significant difference between the integrated responses of LA4 + scoop and LA4 [t=0] + scoop calves during all three time periods.

3.5 Discussion

Three main findings were derived from this study using comparative changes in plasma cortisol as an index of post-dehorning pain-induced distress. First, administration of long-acting local anaesthetic alleviated the pain-induced distress caused by scoop dehorning during its 4-hour period of action. However, it failed to reduce the overall cortisol distress response for the 9.5 hour period following dehorning in calves, due to a delayed plasma cortisol rise when the effect of local anaesthesia wore off. Hence, while administration of local anaesthetic prior to dehorning is to be

recommended in preference to giving no pain-relieving drugs as it reduces the initial pain of horn amputation, it appears to be inadequate in alleviating calf pain in the longer term. Other analgesic regimes need to be examined if scoop dehorning pain is to be relieved more effectively. Secondly, dehorning immediately after local anaesthetic administration was observed to cause no greater distress to calves compared to a 20 minute delay before dehorning. However, the plasma cortisol response may not be rapid enough to truly reflect the immediate pain-induced distress experienced by calves, so allowing time for local anaesthetic to act before dehorning is still recommended. Thirdly, the present study indicates that there was no significant hypothalamic-pituitary-adrenal axis activation independent of noxious sensory input from the scoop wounds. This is assuming that local anaesthetic does not directly inhibit the inflammatory response at the wound sites, independently of its nerve-block action.

As previously observed (Petrie *et al.*, 1996a,b; Sylvester., *et al* 1993; McMeekan *et al.*, 1997 [Chapter 2]) the mean cortisol concentrations of all Control and LA control groups showed only small rises above their pretreatment values during the first 20 minutes of the trial (Figure 3.4). This suggests that the handling, blood sampling and/or other novel features of the research environment caused minor distress to the calves initially, but that the calves quickly habituated to this within the first hour. Handling and injection of bupivicaine did not cause significantly greater calf distress than handling alone.

The cortisol response to scoop dehorning showed a biphasic response. Initially there was a peak followed by lower plateau concentrations and then a decline to pretreatment values (Figure 3.5). This has been observed in other scoop dehorning studies involving 6 to 8 week-old (Petrie *et al.*, 1996a), 14 week-old (McMeekan *et al.*, 1997 [Chapter 2]) and 6 month-old calves (Sylvester., *et al* 1993), thereby confirming that the distress response to scoop amputation is similar over a range of ages.

Administration of local anaesthetic to scoop dehorned calves resulted in a substantially reduced cortisol response during the period of nerve-block analgesic action (Figure 3.5 & 3.6). One exception to this was at 3.83 hours after dehorning in the calves given two bupivicaine injections (Figure 3.6). However, this corresponds to the time at which the nerve-block effects of the first injection would be expected to begin wearing off. This study has

thus established that administering long-acting local anaesthetic either once prior to dehorning or again 4 hours later, abolishes the distress response for 4 hours and 7-8 hours respectively. The effectiveness of local anaesthetic in alleviating the pain-induced distress of dehorning has previously only been shown with the short-acting anaesthetic lignocaine (Morisse *et al.*, 1995; Petrie *et al.*, 1996a; SP Sylvester, DJ Mellor, KJ Stafford, RA Bruce, RN Ward, unpublished data).

It was anticipated that the pattern and duration of tissue reactions from the scoop wounds would be similar whether or not local anaesthetic had been administered (Petrie *et al.*, 1996a). Cornual nerve blockade for 4 hours (LA4 + scoop) was expected to abolish the cortisol response for at least 4 hours and have no other subsequent effects. Likewise, an 8-hour blockade (LA8 + scoop) was expected to abolish the whole response, because its duration would exceed the usual 7-8 hours of the distress response. However, the results from LA4 + scoop and LA8 + scoop groups did not support these assumptions. The mean cortisol rise after the local anaesthetic wore off in LA4 + scoop calves exceeded that of the Scoop calves at the equivalent time, with concentrations significantly greater at 6.33 and 7.33 hours (Figure 3.5). Also the mean cortisol concentration for this group did not resolve to LA4 control levels until 8.33 hours after dehorning, a time two hours after that of the Scoop calves (Figure 3.5). There was no significant difference in the total integrated cortisol response of those calves dehorned with (LA4 + scoop) and without (Scoop) local anaesthetic (Table 3.2). Thus it is apparent that while single administration of bupivicaine served to alleviate the pain-induced distress which follows dehorning during its 4-hour period of nerve-block action, the overall distress response was not significantly reduced, but simply delayed. Prior injection with lignocaine local anaesthetic, which has half the duration of action of bupivicaine (Link and Smith, 1956), was similarly ineffective in reducing the overall cortisol response to scoop dehorning of 6-week-old calves (Petrie *et al.*, 1996a).

The rise in mean cortisol concentration once the local anaesthesia effect passed in the LA4 + scoop calves was probably due to increased noxious sensory input, because in those calves which received a second local anaesthetic injection this rise was absent (Figure 3.7). However, extending the nerve-block analgesic period to 7-8 hours did not abolish the distress response completely, for the mean cortisol concentrations became significantly elevated once

that nerve blockade ended (Figure 3.6). Since this cortisol rise remained unresolved by the trial's end, it is not known whether the total acute cortisol distress response would have been significantly reduced in calves given two injections of bupivicaine. However, it is clear that as with those calves given one bupivicaine injection, the cortisol distress response to scoop dehorning had been delayed, with the response of calves dehorned in the absence of local anaesthesia having resolved to control levels at least 2 hours earlier.

These results suggest that providing nerve-block analgesia to a scoop dehorned calf could cause it greater distress in the later stages of its response when the analgesic effects has worn off. This may occur for a number of reasons: it could be a predominantly psychological response to the novel experience of pain. By 4 hours, those calves not given bupivicaine may have become habituated to the noxious sensory input originating from the wound site. That is, they may still experience pain but after 4 hours it is not perceived to be as noxious and so does not elicit such a large distress response compared to those animals which are likely experiencing pain for the first time as the local anaesthetic wears off. The importance of novelty in hypothalamic-pituitary-adrenal axis activation has been noted in numerous studies (Mason, 1968; Bassett *et al.*, 1973; Stephens, 1980; Manteca and Deag, 1994).

Alternatively, it is possible that provision of local anaesthesia in dehorned calves suppresses important central and/or peripheral endogenous pain-relieving mechanisms. For instance, it has been suggested that the immediate and marked pain caused by horn amputation may induce stress-induced analgesia in calves (Petrie *et al.*, 1996a), such that in the subsequent hours less pain is actually experienced by these animals due to endogenous endorphin release. It could follow that those calves given bupivicaine prior to scoop dehorning either fail to activate or have delayed stress-induced analgesia and so perceive greater pain when the nerve-block effect initially ends, causing a concomitant greater cortisol release. There is however evidence to discount this possibility. No significant increase in plasma endorphin was observed in 6 month old calves within the first hour after scoop dehorning (Cooper *et al.*, 1995). Also the opioid antagonist naloxone did not significantly alter the cortisol response and effected only minor behavioural changes in 1-week old lambs after castration and docking (Wood *et al.*, 1991).

At present there appears to be more evidence to support the possibility that local anaesthesia indirectly enhances inflammatory pain in dehorned calves. The mechanism may depend on markedly reduced noxious input during the period of nerve-block analgesia resulting in lower plasma levels of cortisol, which is itself a potent anti-inflammatory substance in mammals (Buckwalter, 1995). Adrenalectomised rats (Flower *et al.*, 1986) and those given a glucocorticoid antagonist (Laue *et al.*, 1988) have their inflammatory response to carrageenin greatly enhanced. The susceptibility of Lewis rats to developing of chronic severe inflammatory disease can be due to their inability to activate their hypothalamic-pituitary-adrenal axis appropriately, with replacement doses of dexamethasone decreasing this susceptibility (Sternberg *et al.*, 1989; Karalis *et al.*, 1995). It is also recognised that the anti-inflammatory properties of glucocorticoids are exhibited when plasma concentrations are similar to those attained after trauma (Buckingham, 1985) and stress (Munck *et al.*, 1984). Furthermore, post-operative pain is significantly lower in human patients given corticosteroids (Skjelbred and Lokken, 1982). Since inflammatory pain has been shown to contribute markedly to the acute distress response following scoop amputation in calves (Chapter 4), prevention of the usual large cortisol response during the nerve-block analgesic period could cause an unimpeded progression of the inflammatory reaction at the amputation wound sites. This in turn could cause greater inflammatory pain and thus enhancement of the distress response once the local anaesthetic effects passed.

There were no significant differences between the mean cortisol concentration of calves given bupivicaine immediately before versus 20 minutes before scoop dehorning (Figure 3.5). Nor did these two groups differ significantly in their integrated cortisol response during all three measured time periods (Table 3.2). Hence with regard to the initial pain of horn amputation, no greater distress was detected in those calves dehorned immediately after bupivicaine injection. This could be due to rapid onset of bupivicaine's nerve-block action such that waiting a further 20 minutes offered no great analgesic advantage. However, it is equally likely that a difference in the immediate pain perceived did exist but the plasma cortisol response failed to detect this difference because of the 20 minute delay between horn amputation and taking of the first post-treatment blood sample, and cortisol's own inherent secretory delay in response to stressors (Guyton, 1991). Further assessment involving indices of greater sensitivity to

immediate pain-induced distress is necessary, such as changes in behaviour (Morisse *et al.*, 1995; Taschke and Folsch, 1995; Lester *et al.*, 1996), heart rate, plasma catecholamine (Friend, 1980; Mason and Mendl, 1993) and ß-endorphin (Jephcott *et al.*, 1986; Shutt *et al.*, 1988) levels, before dehorning immediately after injection of local anaesthetic could be recommended as a means of avoiding double handling of calves.

The time at which the distress response was noted to be significantly higher than LA4 control levels following wearing off of local anaesthesia was 30 minutes later in calves given bupivicaine immediately before compared to 20 minutes before horn amputation (Figure 3.5), as expected. However, the reason for the discrepancy between the two groups with regard to their significant difference from dehorned calves given no local anaesthetic at 6.33 hours after dehorning is unknown (Figure 3.5). It could be a chance effect which would disappear upon increasing calf numbers, hence having little biological relevance. This is supported by the fact that for the rest of the trial, no similar discrepancy was noted. In both calf groups the time at which cortisol concentrations were observed to returned to LA4 control levels was 8.33 hours after dehorning.

There are a growing number of studies showing that inflammatory cytokines can directly stimulate the HPA axis. Interleukin-1(IL-1), IL-6 and tumor necrosis factor (TNF) have been found to directly stimulate the HPA axis *in vivo*, alone, or in synergy with each other, particularly at the hypothalamic level with the first cytokine the most potent (Sapolsky *et al.*, 1987; Tsagarakis *et al.*, 1989; Imura and Fukata, 1994; Chrousos, 1995; Kapcala *et al.*, 1995; Mastorakos *et al.*, 1995). Also plasma levels of IL-6 are reported to increase following surgery (Ayala *et al.*, 1991; Fukata *et al.*, 1993). Nevertheless the results of present study suggest that very little post-injury activation of the hypothalamic-pituitary-adrenal axis occurs independently of noxious sensory input, since plasma cortisol levels did not rise significantly during the 8 hour period of nerve-block analgesia in LA8 + scoop calves (Figure 3.6).

However, this conclusion is not without qualification. It is based on the assumption that local anaesthetic does not directly inhibit the acute inflammatory reaction following tissue injury, particularly cytokine synthesis and/or release. Ropivacaine and lidocaine have been reported to significantly inhibit proliferation of fibroblasts, vascular endothelial cells and epithelial cells within therapeutic concentrations, all of which are involved in inflammation

(Martinsson *et al.*, 1993). However, general anaesthesia in conjunction with an epidural local anaesthetic had no effect on elevated circulating IL-6 concentrations, despite markedly inhibiting plasma cortisol levels during surgery (Moore *et al.*, 1992). Also two calves in the present study elicited a substantial cortisol response despite receiving local anaesthesia (presumably because little or no nerve-block was achieved in these animals) thereby suggesting that local anaesthetic has no indirect inhibitory action on cortisol secretion independent of its pain relieving property. Clarification of this matter would be aided in future calf dehorning trials by injection of local anaesthetic at the horn base deliberately away from the major innervating cornual nerve, and comparing the resultant cortisol response to that following standard local anaesthetic administrative practice.

The present results support previous reports which have exposed limitations to short-acting local anaesthetic in alleviating the overall pain-induced distress following tissue amputation in cattle (Petrie *et al.*, 1996a; Fisher *et al.*, 1996), in showing that long-acting local anaesthetic also appears to be similarly ineffectual. [Note however, that both short- and long-acting local anaesthetic apparently abolish noxious sensory input throughout their durations of nerve-block action]. Hence, other analgesic strategies require investigation. Indeed, non-steroidal anti-inflammatory drugs show promise (Chapter 4) in improving pain alleviation in stock following the very common and necessary husbandry practice of horn removal.

CHAPTER FOUR: Effects of local anaesthetic and/or non-steroidal anti-inflammatory analgesic on the acute cortisol responses to scoop dehorning in calves.

4.1 Chapter Summary

Changes in cortisol concentrations were used to monitor the pain-induced distress in 3-4 month-old Friesian calves during the 9.5 hours following scoop dehorning. Calves were given either no pain relieving drugs, or local anaesthetic and/or a non-steroidal anti-inflammatory drug (NSAID). The NSAID abolished the cortisol response, and by inference pain-induced distress, from around 2 hours after dehorning until the study's end. Local anaesthetic plus NSAID abolished the total cortisol response including the initial peak which followed scoop amputation and the plateau cortisol response which develops 2 hours later. This suggests that the distress experienced by calves in response to scoop dehorning is biphasic, involving pain at horn amputation and a later inflammatory pain. It is thus apparent that administration of an anti-inflammatory drug in addition to the usual injection of local anaesthetic prior to scoop dehorning is likely to improve pain relief in calves undergoing this amputation procedure.

4.2 Introduction

Administering local anaesthetic to calves prior to horn removal has been investigated as a means of alleviating dehorning pain in calves. Using plasma cortisol as an index, lignocaine (Morisse *et al.*, 1995; Petrie *et al.*, 1996a; SP Sylvester, DJ Mellor, KJ Stafford, RA Bruce, RN Ward, unpublished data) and bupivicaine (Chapter 3) were shown to reduce pain-induced distress in calves during the nerve-block period. However in the majority of these studies, local anaesthetic was not effective in reducing the overall distress response, indicated by a rise in plasma cortisol as the nerve-block effects wore off (Morisse *et al.*, 1995; Petrie *et al.*, 1996a; Chapter 3). Similar limitations have been observed for local anaesthetic used for alleviating the overall pain-induced distress in cattle following castration (Fisher *et al.*, 1996). The apparent inability of local anaesthetic to reduce the overall pain-induced distress in calves following the above amputation procedures may be due to

the fact that surgery generally leads to nociceptive input not only during the surgery itself, but also postoperatively as a result of the inflammatory response in the damaged tissue (Woolf and Chong, 1993). This second wave of nociceptive input could last considerably longer than the duration of nerve-blockade provided by the local anaesthetic. Inflammatory pain is considered to be a major contributor to post-operative pain following human surgery, with non-steroidal anti-inflammatory drugs (NSAIDs) reliably reducing this pain (Dahl and Kehlet, 1991; Hommeril *et al.*, 1994). The primary mode of NSAID analgesic action is via inhibition of prostaglandin production from arachidonic acid (Higgins and Lees, 1984). Prostaglandins are normally released in response to trauma or disturbance of the cell membrane (Dahl and Kehlet, 1991), and subsequently sensitise pain receptors to mechanical or chemical stimulation at the injury site, causing hyperalgesia (Raja *et al.*, 1988).

Thus it may be possible to alleviate the pain normally experienced by calves following dehorning by administering non-steriodal anti-inflammatory drugs (NSAIDs). This possibility was evaluated in the present study by administering the NSAID ketoprofen intravenously to calves prior to scoop dehorning. Ketoprofen has been shown to provide analgesia in horses with colic (Owens *et al.*, 1995), and in calves it prevents the increase in plasma concentrations of prostaglandins normally associated with *Escherichia coli* infection, a major cause of calf diarrhea (Semrad, 1993).

The distress response following scoop dehorning of calves has a duration of 6-7 hours, as measured by changes in plasma cortisol concentrations (Petrie *et al.*, 1996a; Sylvester *et al.*, 1993; Chapter's 2 and 3). Extending the duration of nerve-block analgesia to cover this period would require two injections of long-acting anaesthetic. This would be impractical under normal farming conditions. However, ketoprofen is thought to have a longer duration of action of approximately 12 hours (KJ Stafford; personal communication). Hence, a single injection of local anaesthetic plus one of ketoprofen prior to dehorning may alleviate both the initial pain of horn amputation (an already proven action of local anaesthetic) as well as extend the analgesic period following dehorning. To clarify these issues plasma cortisol responses were studied in dehorned calves given ketoprofen in conjunction with either lignocaine or bupivicaine.

4.3 Materials and Methods

4.31 Animals

This study was performed simultaneously with that described in Chapter Three. One hundred female Friesian calves, 3-4 months of age, weighing between 63 and 110 kg (mean 86 kg) were divided evenly between ten experimental groups. Four groups (Control, Scoop, LA4 control, LA4 + scoop) were the same as those reported previously.

Calves were housed in an open-fronted shed with sawdust bedding at 1700 hours on the evening before each trial day. They were immediately randomly allocated to a group and spray painted with an identification number. They were then held overnight with free access to water in two pens each containing 16 or 17 calves in total. At 0500 hours on the morning of each trial day, the mobs were moved quietly into two smaller pens located in the same open-sided shed (10.4 m^2). Blood sampling commenced at 0700 hours.

4.32 Blood sampling

Blood samples (10 ml) were taken by venepuncture from either jugular vein at 0.33 hour before treatment (-0.33 hour) and at 0.0, 0.33, 0.66, 1.0, 1.33, 1.83, 2.33, 2.83, 3.33, 3.83, 4.33, 4.83, 5.33, 6.33, 7.33, 8.33 and 9.33 hours after treatment. On each occasion the calf was restrained firmly but gently against a wall of the pen by two people while a third took the blood sample. The whole procedure from restraint to completion of a sample usually took less than 15 seconds. The order of bleeding the calves in each pen was the same on each occasion.

4.33 Treatments

There were ten treatments, each conducted while the calf was manually restrained against the pen wall by two people. Treatments took no longer than 30 seconds to perform.

Control (='Control')
The horn buds and adjacent skin were massaged firmly but not amputated.

Bupivacaine control (='LA4 control')
Local anaesthetic (6 ml of 0.25% bupivacaine hydrochloride; Marcain; Astra Pharmaceutical Pty Ltd, Australia) was injected around each cornual nerve, midway along the lateral edge of the frontal bone crest (Weaver, 1986), 20 minutes prior to calves having their horn buds massaged but not amputated. The duration of local anaesthetic action was approximately 4 hours (Link and Smith, 1956).

Ketoprofen Control (='K control')
The nonsteroidal anti-inflammatory drug ketoprofen (3 ml of 10% Ketofen, Rhône Mérieux, France) was injected intravenously into the jugular vein of calves 20 minutes prior to having their horn buds massaged but not amputated.

Lignocaine + Ketoprofen Control ('LA2:K control')
The local anaesthetic lignocaine (6 ml of 2% lignocaine hydrochloride; Lopain, Ethical Agents Ltd, Auckland), with a duration of action of approximately 2 hours (Link and Smith, 1956), was injected around each cornual nerve, as described for bupivicaine. At the same time ketoprofen was administered intravenously. Calves had their horn buds massaged but not amputated 20 minutes later.

Bupivicaine + Ketoprofen Control ('LA4:K control')
As described for LA2 + K control calves, except bupivicaine was injected instead of lignocaine.

Scoop ('Scoop')
The horns were removed with a standard dehorning scoop (Barnes Dehorners, Stones, USA) consisting of two interlocking semicircular blades each of diameter 56 mm × 38 mm attached to handles. The act of closing the blades, by separating the handles and pushing down towards the calf's head, amputated the horn, adjacent skin and some underlying bone (Petrie *et al.*, 1996a).

Bupivicaine + scoop ('LA4 + scoop')
Local anaesthetic was administered 20 minutes before the horns were removed with the scoop as described in the LA4 control group. Duration of nerve-block analgesia was approximately 4 hours.

Ketoprofen + Scoop (='K + scoop')
Ketoprofen was administered to calves as described for the K control group. Horns were amputated 20 minutes later with a standard dehorning scoop.

Lignocaine + Ketoprofen + Scoop ('LA2:K + scoop')
Lignocaine and ketoprofen were administered as described for the LA2 + K control group. Horns were amputated 20 minutes later with a standard dehorning scoop.

Bupivicaine plus Ketoprofen plus Scoop ('LA4:K + scoop')
Bupivicaine and ketoprofen were administered as described for the LA4 + K control group. Horns were amputated 20 minutes later with a standard dehorning scoop.

4.34 Plasma cortisol assay

Blood samples were collected in heparinised vacutainers, chilled immediately, then centrifuged and the plasma was removed and stored at -20 °C until required. Plasma cortisol concentrations were determined by using a non-extraction tritium radio-immuno-assay method (Endocrine Sciences, 4301 Lost Hills Rd, CA 91301); the lowest detectable concentration was 0.1 ng/ml and the intra-assay and inter-assay coefficients of variation were 7.6% and 9.5%, respectively.

4.35 Integrated cortisol responses

The integrated cortisol responses were calculated to give a single measure of the magnitude and the duration of any increase in plasma cortisol concentration after treatment. The integrated cortisol response is defined as the area between a horizontal line drawn through the pretreatment concentration (at -0.33 hour) and the cortisol response curve during defined periods after treatment when the concentrations were greater than the pretreatment value (Mellor and Murray 1989).

4.36 Statistical analysis

Where applicable the cortisol results have been expressed as the mean ± standard error of the mean (SEM). Except where otherwise stated all cortisol results refer to plasma cortisol concentrations adjusted by subtracting the pretreatment value. Hence the cortisol curves are expressed as the change from the -0.33 hour plasma

cortisol concentration. Significant differences between mean concentrations and between integrated cortisol responses were determined using Student's t-test assuming unequal variation (Microsoft Excel V 5.0, Microsoft Corporation, U.S.A). Students t-test: paired two sample for mean (Microsoft Excel V 5.0) was used to determine whether there were significant changes with time after each treatment.

4.4 Results

Twenty-three animals from control groups on trial days one and two were reused on the fourth day. The mean pretreatment plasma cortisol concentration of these calves was not significantly different from the mean pretreatment value of all other calves involved in this study. However, the mean pretreatment cortisol concentrations of calves used on the first trial day (23 ng/ml) were significantly higher ($p<0.01$) than that of calves studied on the fourth day (16 ng/ml). These differences were small in comparison with the responses observed in calves dehorned in the absence of a local anaesthetic or an anti-inflammatory drug. Blood sampling order was found to have a small but significant effect on cortisol pretreatment values ($r = 0.43$; $p<0.05$), and hence was of probable little biological significance (Figure 4.1).

No significant between-group differences were found in the mean pretreatment cortisol concentrations, due to calves of different treatments being evenly distributed throughout the blood sampling order and between all four trial days (Table 4.1).

Control

Restraint and massage of horn buds elicited a small significant rise of 6 ng/ml in the mean plasma cortisol concentrations which returned to pretreatment values 40 minutes after treatment. There was no other significant change in plasma cortisol concentration throughout the 9.5 hour sampling period, except for a fall of 4 to 6 ng/ml below pretreatment values at 2.83, 3.33, 3.83, 4.33, 6.33 and 8.33 hours ($p<0.05$).

LA4 control

A significant elevation in the mean cortisol concentration to 9 ng/ml above pretreatment level occurred 20 minutes after local anaesthetic administration (0 hour), and to 5 ng/ml 1.33 hours later. At all other sample times the mean cortisol concentration did not deviate significantly from pretreatment levels.

Treatment	n	Plasma cortisol concentration (ng/ml)
Control	10	18.5 ± 2.6
LA4 control	10	15.3 ± 3.0
K control	9	18.1 ± 2.5
LA2:K control	9	21.6 ± 2.6
LA4:K control	10	18.0 ± 2.7
Scoop	9	19.2 ± 2.7
LA4 + scoop	9	17.1 ± 2.4
K + scoop	9	19.3 ± 2.7
LA2:K + scoop	8	20.6 ± 3.3
LA4:K + scoop	7	16.6 ± 3.3

Table 4.1: Plasma cortisol concentrations (mean ± SEM) in calves 0.33 hours before treatment.

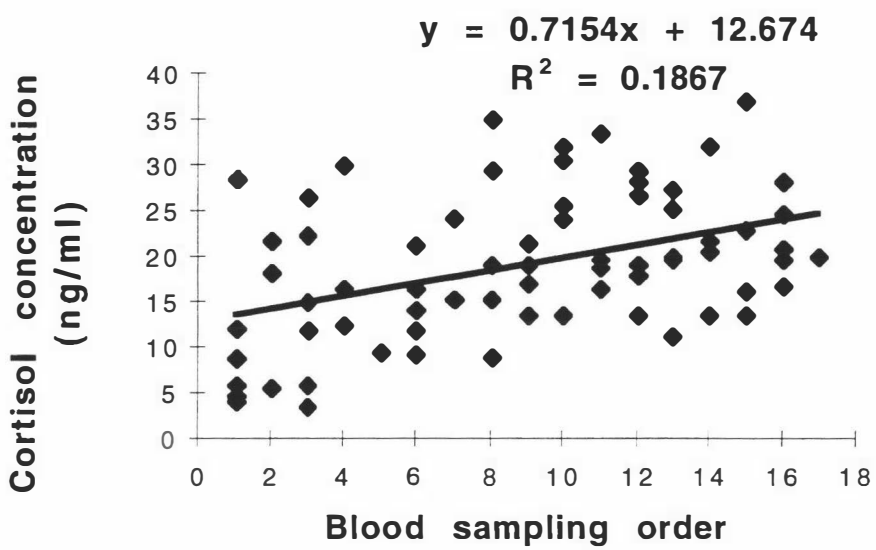


Fig 4.1: Relationship between plasma cortisol concentration and the sample order for the pretreatment blood sample.

There were no significant differences between the mean cortisol concentrations of Control and LA4 control calves (Figure 4.2).

K control

A significant elevation of 8 ng/ml above the mean pretreatment concentration ($p<0.05$) occurred 20 minutes after ketoprofen administration (0 hour). Thereafter, the mean cortisol concentration did not deviate significantly from the pretreatment level except at 2.33, 2.83, 3.33, 3.83, 4.33, 5.33 and 7.33 hours when it fell 5 to 8 ng/ml below this level ($p<0.05$).

There were no significant differences between the mean cortisol concentrations of Control and K control calves (Figure 4.2).

LA2:K control

The mean cortisol concentration increased significantly to 5 ng/ml above the pretreatment value 20 minutes after lignocaine and ketoprofen administration ($p<0.05$). At all other times there was no significant deviation, except at 2.33, 2.83, 3.33 and 3.83 hours when it fell 4 to 9 ng/ml below the pretreatment concentration ($p<0.05$).

There were no significant differences between the mean cortisol concentrations of Control and LA2:K control calves (Figure 4.2).

LA4:K control

The mean cortisol concentration increased significantly to 10 ng/ml above the pretreatment value 20 minutes after bupivicaine and ketoprofen administration ($p<0.01$). At all other times there was no significant deviation, except at 2.33, 2.83 and 9.33 hours when it fell 6 to 7 ng/ml below the pretreatment concentration ($p<0.05$).

There were no significant differences between the mean cortisol concentrations of Control and LA4:K control calves (Figure 4.2).

Scoop

Scoop dehorning caused a marked significant rise in mean cortisol concentration within the first 20 minutes after horn amputation ($p<0.01$), which peaked at 28 ng/ml above the pretreatment level. This was followed by a fall to plateau values which were maintained between 1.83 and 4.33 hours (Figure 4.3). The mean concentration returned to pretreatment levels by 7.33 hours after dehorning, and to LA2:K + scoop and LA4:K + scoop mean levels by 4.83 and 6.33 hours respectively (Figure 4.4 and 4.5).

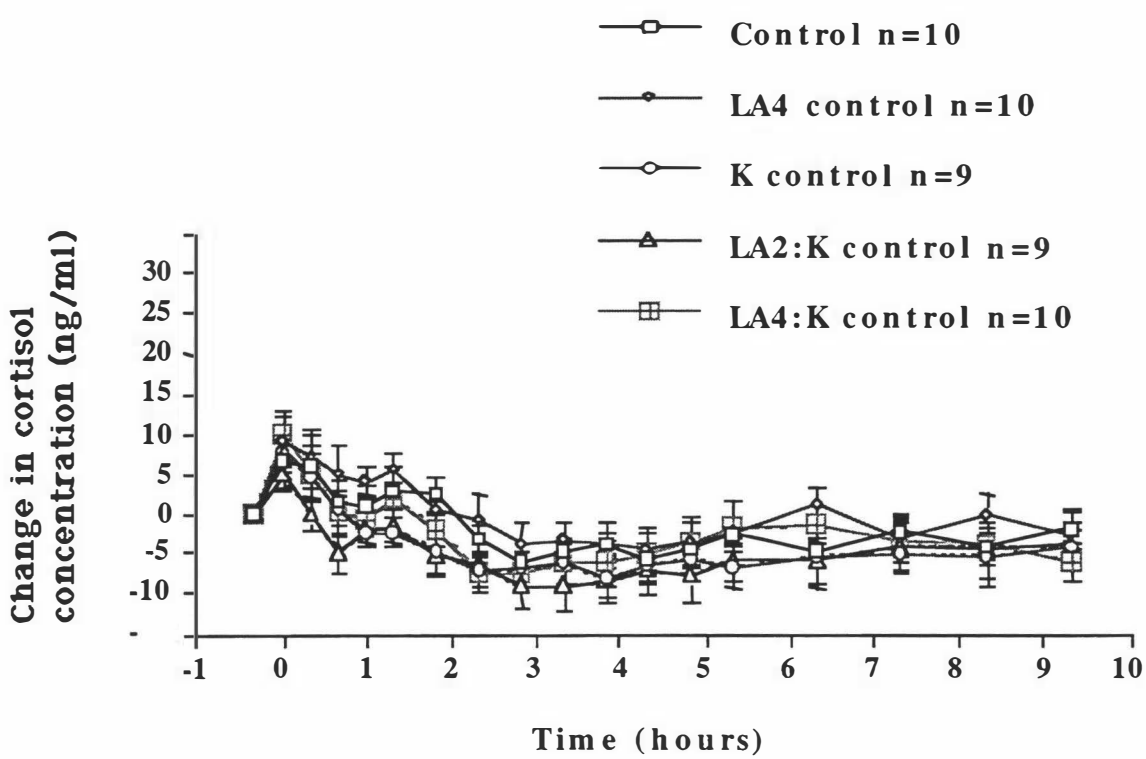


Fig 4.2: Changes in plasma cortisol concentrations (Mean ± SEM) in calves in response to handling, and local anaesthetic and/or NSAID administration.

LA4 + scoop

In calves which were scoop-dehorned 20 minutes after local anaesthetic administration, a significant and protracted rise in mean cortisol concentration was observed 4.33 hours after treatment, followed by a return to Control levels by 9.33 hours. Between 4.33 and 7.33 hours the mean concentrations were significantly higher than those of the LA4:K + scoop calves (Figure 4.6).

During the first four hours after dehorning, the mean cortisol concentration did not differ significantly from those of Control and LA4 control calves, except at 0.66 and 2.83 hours when they were higher than for the Control group

K + Scoop

Like Scoop calves, those animals injected with ketoprofen and dehorned exhibited a marked rise in mean cortisol concentration within the first 20 minutes following horn amputation ($p<0.01$), peaking at 22 ng/ml (Figure 4.3). Mean cortisol concentrations returned to the pretreatment and K control values by 1.83 hours. The only other significant deviation was at 9.33 hours, when the mean cortisol concentration fell 5 ng/ml below the pretreatment level ($p<0.05$).

During the first 1.33 hours after dehorning the mean cortisol concentrations did not differ significantly from those of Scoop calves except at 1.0 hour ($p<0.05$) (Figure 4.3).

LA2:K + Scoop

The mean cortisol concentration increased significantly to 5 ng/ml above the pretreatment value 20 minutes after dehorning in those calves administered both lignocaine and ketoprofen ($p<0.05$). There was no other significant deviation, except at 1.83, 2.33 and 2.83 hours when it fell 4 to 9 ng/ml below the pretreatment concentration ($p<0.05$).

Throughout the trial's duration there was no significant difference between the mean cortisol concentration of this group compared to that of LA2 + K control calves (Figure 4.4). When compared to Control calves, small but significant deviations of -6 and 4 ng/ml were noted at 1.83 and 8.33 hours respectively.

LA4:K + Scoop

There was a significant mean cortisol elevation above the pretreatment concentration between 0 and 0.83 hours after

dehorning in those calves given bupivicaine and ketoprofen, peaking at 14 ng/ml ($p<0.05$). The only other significant deviation from the pretreatment level was a rise of 9 ng/ml at 9.33 hours.

No significant difference was noted between the mean cortisol concentration of this group compared to Control and LA4:K control calves, except at 9.33 hours ($p<0.05$) (Figure 4.5).

4.41 Integrated cortisol responses

The integrated cortisol responses were calculated for three main periods: from 0.33 hour before until 9.33 hours after treatment, from 0.33 hour before until 3.83 hours after treatment, and from 3.83 hours to 9.33 hours after treatment (Table 4.2).

The total integrated cortisol response was significantly greater in the Scoop calves compared to all other groups, except for the LA4 + scoop group. There was no significant difference between LA2:K + scoop and LA4:K + scoop calves, and neither of values for these groups differed from those of any control groups.

Comparing K + scoop calves to LA2:K + scoop and LA4:K + scoop calves, there was no significant difference in their integrated responses during all three measured time periods, except between the former two groups during the first 3.83 hours, when lignocaine reduced the cortisol response.

The total and final 5.5 hour integrated cortisol responses of LA4 + scoop calves were significantly greater than in LA4:K + scoop calves. There was no significance difference when the same two groups were compared over the first 3.83 hours.

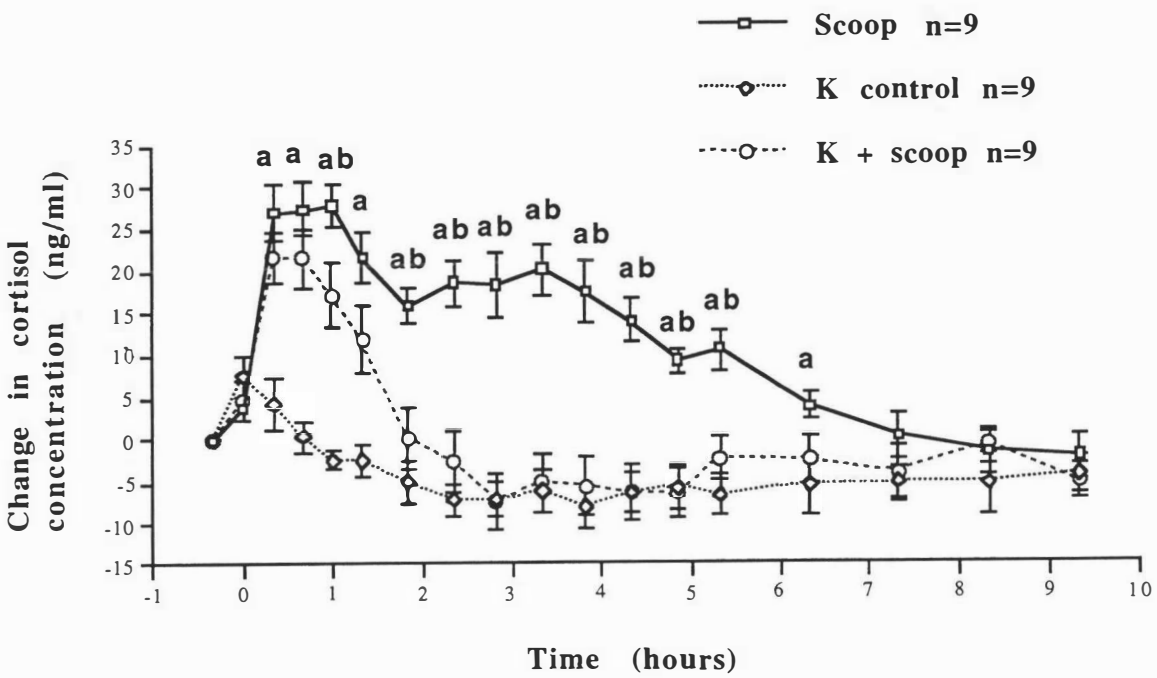


Fig 4.3: Changes in plasma cortisol concentration in calves in response to scoop dehorning with or without NSAID (ketoprofen). Time 0 represents time of dehorning. [a = significantly different from K control; b = significantly different from K + scoop].

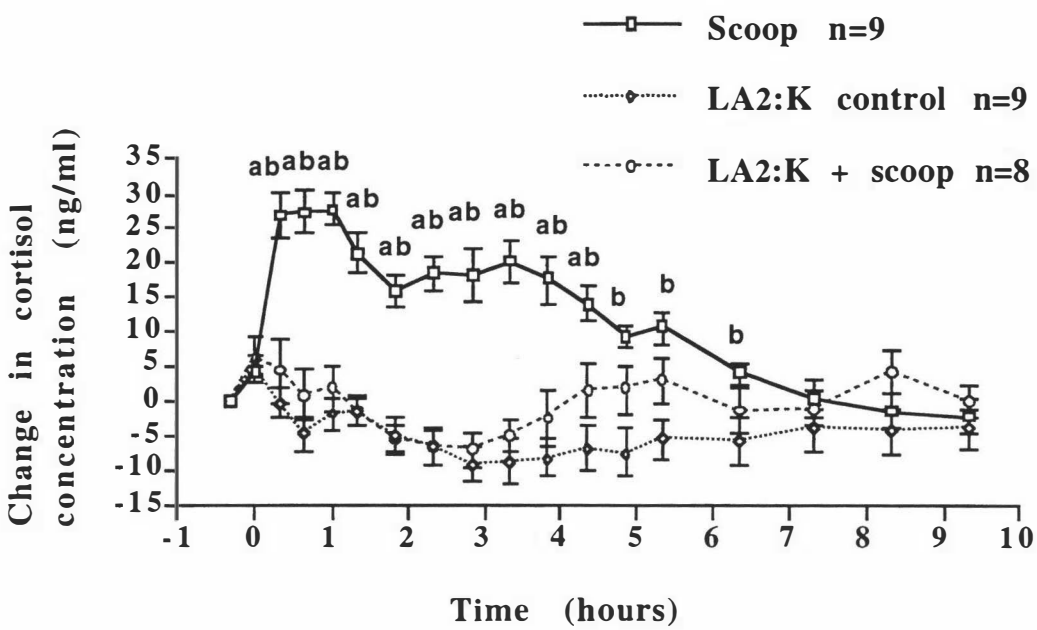


Fig 4.4: Changes in plasma cortisol concentration in calves in response to scoop dehorning with or without NSAID plus LA2. Time 0 represents time of dehorning. [a = significantly different from LA2:K + scoop; b = significantly different from LA2:K control].

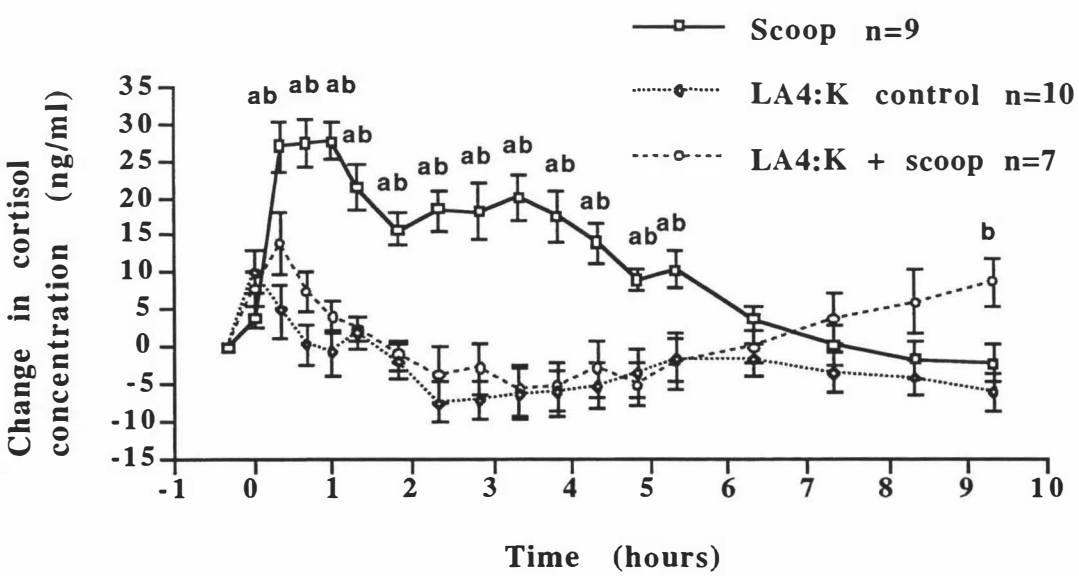


Fig 4.5: Changes in plasma cortisol concentration in calves in response to scoop dehorning with or without NSAID plus LA4. Time 0 represents time of dehorning. [a = significantly different from LA4:K + scoop; b = significantly different from LA4:K control].

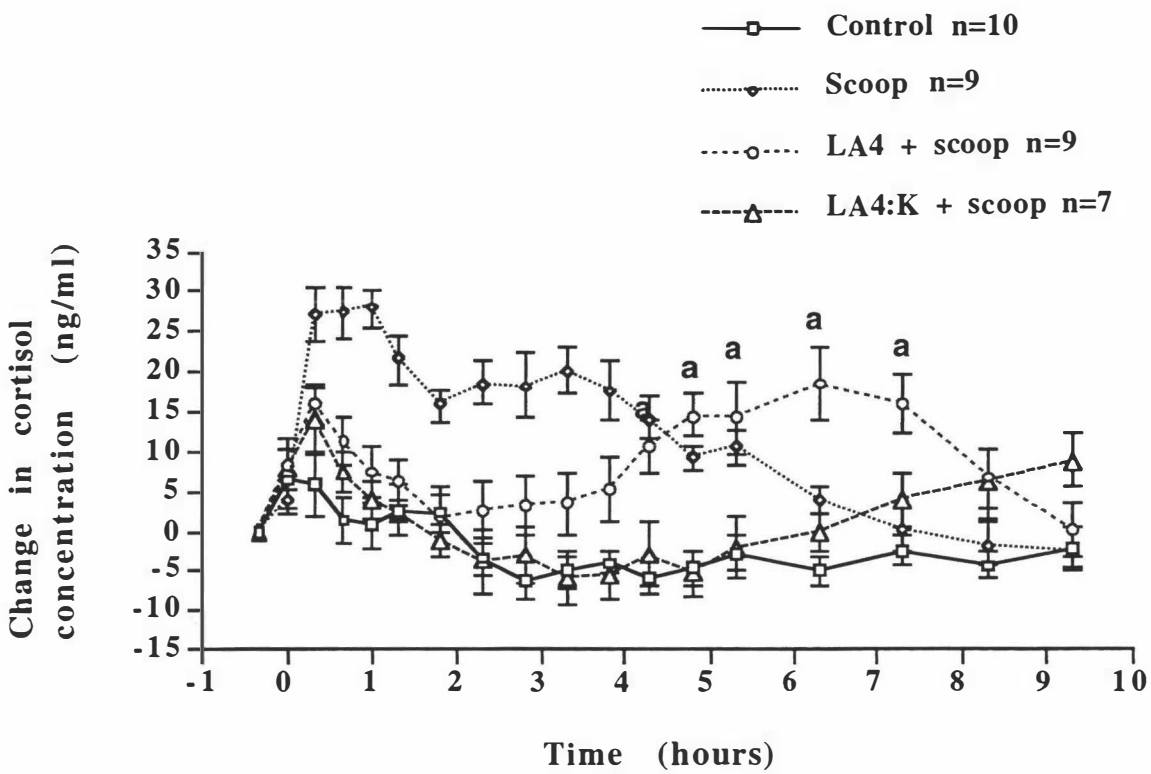


Fig 4.6: Changes in plasma cortisol concentration in calves in response to scoop dehorning with LA4 or NSAID plus LA4. Time 0 represents time of dehorning. [a = significantly different from LA4:K + scoop].

Table 4.2: The integrated cortisol responses (mean ± SEM) for each treatment group, over the complete recording period of 9.33 hours, the first 3.83 hours and final 5.50 hours after treatment. Also noted are the overall durations of the responses. Significant differences are described in the text.

Treatment	n	Duration[a] (hours)	Integrated cortisol response (ng/ml.min) over total 9.33 hours	over first 3.83 hours	over final 5.50 hours
Control	10	0.00	1080 ± 373	830 ± 316	267 ± 120
LA4 control	10	0.33	1642 ± 509	949 ± 305	693 ± 257
K control	9	0.33	664 ± 240	415 ± 137	249 ± 157
LA2:K control	9	0.33	617 ± 267	276 ± 89	341 ± 194
LA4:K control	10	0.33	1231 ± 400	704 ± 215	527 ± 243
Scoop	9	7.33	6708 ± 822	4713 ± 509	1994 ± 404
LA4 + scoop	9	8.33	6021 ± 713	1838 ± 412	4184 ± 610
K + scoop	9	1.83	2434 ± 654	1934 ± 499	499 ± 275
LA2:K + scoop	8	0.33	1821 ± 634	581 ± 203	1240 ± 482
LA4:K + scoop	7	1.00	2507 ± 661	989 ± 202	1518 ± 519

[a] the time after treatment when the cortisol concentration returned to pretreatment values.

4.5 Discussion

The present study has provided new insight into the qualitative nature of dehorning pain in calves, with the use of ketoprofen allowing two phases of the cortisol response and by inference two phases of pain-induced distress to be identified. First, a predominantly amputation response consisting of an initial distress peak, followed two hours later by a largely inflammatory phase consisting of a plateau and subsequent decline to baseline levels by 8 hours. Administration of an anti-inflammatory drug in addition to the usual injection of a local anaesthetic prior to scoop dehorning is thus recommended as a means of providing better pain relief for calves undergoing this amputation procedure.

The magnitude and duration of the distress response which follows scoop dehorning was similar to that demonstrated by previous studies (Sylvester *et al.*, 1993; Petrie *et al.*, 1996a; McMeekan *et al.*, 1997 [Chapter 2]). It consisted of an immediate plasma cortisol peak within 20 minutes of horn amputation and a subsequent decline at 1.83 hours to a plateau level. This plateau response was sustained above control levels until 7.33 hours after dehorning. When ketoprofen alone was given to calves before scoop dehorning the initial mean peak distress response was only slightly reduced. However, ketoprofen completely abolished the following plateau response, such that from 1.83 hours after dehorning to the end of the trial's duration (9.5 hours) their mean cortisol levels were equivalent to those of control calves (Figure 4.3). Calves which received ketoprofen plus local anaesthetic (either lignocaine or bupivicaine) prior to scoop dehorning had both the initial distress peak and subsequent plateau phase virtually abolished (Figure 4.4 and 4.5).

Since ketoprofen's analgesic action is primarily anti-inflammatory (Owens *et al.*, 1995), its effective alleviation of the distress response from 1.83 hours after dehorning suggests that significant inflammatory pain is not experienced by scoop dehorned calves until about this time. This delay in pain alleviation is unlikely to be due to a slow onset of ketoprofen action. It has been shown to have a fast distribution and inhibits the synthesis of the inflammatory mediator thromboxane by 89% in serum within one hour when administered intravenously to similarly aged calves at the same dose to that used in the present study (Landoni *et al.*, 1995). In humans an intravenous 100 mg bolus dose of ketoprofen, one third

the quantity used in the present study, produced analgesia by 10 minutes after administration (Debruyne *et al.*, 1987).

However, prior to 1.83 hours, it was apparent that dehorned calves given ketoprofen still experienced significant pain-induced distress, as indicated by the presence of a cortisol peak. This was effectively alleviated by the nerve-block actions of local anaesthetic. Since ketoprofen was largely ineffective in alleviating the distress response during this time, the initial pain is not likely to be inflammation-induced. Rather it is likely caused by the activation of damaged nerves at and shortly after horn bud severance.

The separation of dehorning distress into nociceptive and inflammatory pain phases is consistent with studies using noxious stimuli. Mice given injections of dilute formalin had two distinct periods of hind paw licking activity, with the NSAIDs indomethacin and naproxen inhibiting licking only during the second period, whereas centrally acting analgesics inhibited licking during both periods (Hunskaar and Hole, 1987). A similar finding was made when comparing the inhibition of thalamic nerve activity in arthritic rats by two NSAIDs of high and low prostaglandin inhibiting potencies (Braga *et al.*, 1987).

In a previous study (Petrie *et al.*, 1996a) calves disbudded by cautery without anaesthesia and/or analgesia showed cortisol responses consisting of a marked initial peak and a substantially reduced plateau distress phase. That observation was mirrored here after scoop dehorning calves given ketoprofen. Based on the above argument that the plateau distress phase represents inflammatory pain, this implies that when no pain relieving drugs are provided, calves dehorned by cautery experience less inflammatory pain than those dehorned by scoop. This may be attributed to the type of injury caused by each method. Scoop dehorning involves direct severance of the horn removing both skin and bone, whereas the cautery iron burns a ring of skin, killing the germinal tissue which prevents further bud growth. The fact that the horn is not removed in the cautery method implies an absence of, or certainly a reduced number of severed nerves and blood vessels, such that the inflammatory reaction may be both quantitatively and qualitatively different. It is also likely, as argued by Petrie *et al.*, 1996a, that cautery disbudding causes such extensive burns to the epidermal, dermal and subcutaneous tissues surrounding the horn bud, that nociceptors located in the dermis are destroyed. Hence, even if an appreciable inflammatory reaction occurs following horn cautery,

this may still not be registered as inflammatory pain due to a reduced nociceptive afferent input. The observed significant reduction in the overall plasma cortisol response of lambs following hot iron tailing compared to knife tailing has also been attributed to this phenomenon (Lester *et al.*, 1991a).

It is important to note that although NSAIDs in general produce analgesia primarily through their peripheral anti-inflammatory actions, they also have direct central analgesic actions which vary according to the drug (McCormack and Brune, 1991; Dart, 1992). The mechanisms involved in the latter are less certain, but NSAIDs have been noted to inhibit the synthesis of neurotransmitters involved in central pain pathways and to enhance nerve membrane potentials thereby reducing synaptic output (Liles and Flecknell, 1992). While ketoprofen has been described as having moderate (McCormack and Brune, 1991) to potent (Landoni *et al.*, 1995) anti-inflammatory action through its prostaglandin inhibiting capacities, there is a chance that it reduces dehorning pain via a central analgesic action as well. This may explain why the initial amputation peak was slightly reduced in those dehorned calves given ketoprofen (Compare K + scoop with Scoop: Figure 4.3). Administration of an NSAID with proportionately higher anti-inflammatory activity to central analgesic action such as phenybutazone (Dart, 1992), would help clarify this point.

Ketoprofen also has central effects independent of any analgesic action, having been found to significantly reduce melatonin secretion within 30 minutes of administration in sheep (Lapwood *et al.*, 1997). Such a rapid depressive action would be unlikely in the case of corticotrophic-releasing hormone (CRF) release from the hypothalamus or ACTH secretion from the anterior pituitary as in the present study the mean cortisol levels were not significantly lower during the first 1.33 hours, except for the 1 hour sampling point (Figure 4.3).

NSAIDs can cause undesirable side effects related to their inhibition of prostaglandin synthesis. These include gastrointestinal bleeding, renal function impairment and prolongation of clotting time (Liles and Flecknell, 1992; Woolf and Chong, 1993). In the present study no greater blood loss was observed in scoop dehorned calves administered ketoprofen compared to any other dehorned group.

In the present work (Chapter 3) it has been found that injection of local anaesthetic alone before scoop dehorning was ineffective in

reducing the total distress response in calves, due to a marked increase in cortisol once the nerve-block effects wore off. It was reasoned that local anaesthesia may indirectly enhance the development of inflammatory pain, since the usual rise in plasma cortisol, a potent anti-inflammatory substance (Buckwalter, 1995), is absent during the 4 hour nerve-block analgesic period. The fact that ketoprofen in the present study eliminated approximately 60% of the total dehorning cortisol response and by inference the pain-induced distress response (Table 4.2), demonstrates that inflammatory pain is indeed prevalent following horn amputation and therefore supports the previous argument. However, it would be helpful in future dehorning studies to extend the trial period beyond 9.5 hours in order to examine the longer term effects of ketoprofen administration in scoop dehorned calves. If the marked cortisol rise which follows wearing off of local anaesthetic action is indeed due to enhanced inflammatory pain, one would expect this rise to be absent or at least reduced when ketoprofen's anti-inflammatory analgesic action wore off. This has yet to be established.

It is apparent from the results of the present study however, that if local anaesthetic is to be administered to calves in order to help alleviate dehorning distress, a simultaneous injection of an anti-inflammatory drug is to be recommended. Otherwise the benefits of reducing the initial amputation pain are likely to be offset by a later enhancement of inflammatory pain, as shown in Figure 4.6. Although the benefits of implementing a dual anaesthetic-analgesic strategy to improve post-surgical pain alleviation in humans has been recognised for some time (Dahl and Kehlet, 1991; Woolf and Chong, 1993), this is the first study to demonstrate the need for a similar approach for calves under going scoop horn amputation.

CHAPTER FIVE: Effects of local anaesthetic and/or nonsteroidal anti-inflammatory analgesic on the behavioural responses to scoop dehorning in calves.

5.1 Chapter Summary

Eight different behaviours were examined in order to assess the pain experienced by 3-4 month-old calves scoop dehorned with and without local anaesthetic and/or a non-steroidal anti-inflammatory drug. Of these, lying, grazing/ruminating, tail shaking and ear flicking were distinctly different for at least the first 4-6 hours after treatment in control compared to scoop dehorned calves given no pain relieving drugs. This suggests that these behaviours are useful indices of acute dehorning pain. Behavioural differences between control and dehorned groups were markedly reduced, particularly during the first 4 hours after treatment, when lignocaine and ketoprofen were administered together prior to horn amputation. This was not so evident when either lignocaine or ketoprofen was administered alone prior to dehorning. Effectiveness of the different analgesic regimes in relieving dehorning pain in calves may have been more clearly defined had a greater number of animals been used. However, the results of this small study do support giving calves both local anaesthetic and an anti-inflammatory drug, rather than none or either drug alone, with regard to improving pain relief in these animals following scoop dehorning.

5.2 Introduction

In order to recognise when injury is giving rise to the subjective experience of pain, two sources of evidence may be used, physiological and behavioural (Dawkins, 1980). However, interpreting changes in either an animal's physiology or behaviour in isolation to assess its subjective state can be difficult (Rushen, 1986; Barnett and Hemsworth, 1990; Bateson, 1991). It is thus recommended that animal distress and pain be measured by more than one parameter to broaden the assessment base (Broom, 1986; Stafford and Mellor, 1993). This approach has been used in a number of farm husbandry studies to assess the pain experienced by stock following castration and tailing (Shutt *et al.*, 1988; Mellor

and Murrey, 1989; Mellor *et al.*, 1991; Wood *et al.*, 1991; Petrie, 1994; Robertson *et al.*, 1994; Kent *et al.*, 1995; Lester *et al.*, 1996), dehorning (Morisse *et al.*, 1995; Taschke and Folsch, 1995) and velvet antler removal (Pollard *et al.*, 1992; Weilburg, 1996).

In the previous two chapters the effects of various analgesic and anaesthetic regimes on alleviating dehorning pain were assessed by monitoring changes in calf plasma cortisol concentrations. The aim of the present study is to further assess dehorning pain in calves from a behavioural perspective. Behavioural observations are a valuable measure of pain for they involve minimal interference to the animal (Duncan and Poole, 1990). Also animals are innately motivated to avoid stimuli which give rise to negative emotional states such as pain, and may increase or decrease their behavioural activities in accordance with this objective (Kitchen *et al.*, 1987; Lawrence, 1991). However, pain perception and hence behavioural responses to pain can vary according to the site, duration and intensity of the stimulus, previous experience, emotional states and innate individual differences (Kitchen *et al.*, 1987). Guidelines for the recognition of pain and assessment of its intensity in animals have been well summarised in previous reviews (Morton and Griffiths, 1985; Sanford *et al.*, 1986).

Although cattle, like most ruminants, are considered to be less expressive in their behavioural responses to pain in comparison to other mammals (BVA Animal Welfare Foundation, 1985; Rollin, 1985), changes in activity have been observed following painful amputation procedures. Branding (Lay *et al.*, 1992), castration (Robertson *et al.*, 1994), docking (Petrie *et al.*, 1996b) and dehorning (Morisse *et al.*, 1995; Taschke and Folsch, 1995) have been associated with significantly increased foot stamping, head and tail shaking, ear flicking, vocalisation and/or general restlessness. That calves struggle less violently during dehorning when local anaesthetic is given, is a common observation among persons carrying out the procedure. However, controlled assessment of the apparent benefits of local anaesthetic and/or analgesic administration in terms of calf behavioural responses to dehorning, particularly in the hours following the procedure, has been minimal. One study showed a significant reduction in the intensity of immediate reactions during cautery disbudding of calves given lignocaine compared to those not given lignocaine, and a later non-significant trend towards more grooming and less head shaking after the local anaesthetic had worn off (Morisse *et al.*, 1995).

In the present study calves were given either lignocaine (a local anaesthetic) and/or ketoprofen (a non-steriodal anti-inflammatory drug) prior to scoop dehorning. Behaviour was compared to appropriate control and scoop dehorned calves given no pain-relieving drugs. There were three primary objectives:

- to identify specific behavioural responses which may be associated with dehorning pain.
- to assess the sensitivity of these behaviours such that they may differentiate between pain experienced by dehorned calves given different anaesthetic and analgesic regimes.
- to compare the behavioural responses following scoop dehorning with the plasma cortisol responses reported in Chapters 3 and 4, in order to identify possible associations between the two parameters which may further strengthen (or challenge) the conclusions made in these previous chapters.

5.3 Materials and Methods

5.31 Animals

Fifty-five female Friesian dairy calves, three to four months of age were used in this study comprised of three observation days. They were brought into the yards at 11 am on the first morning. Each calf was randomly allocated to one of seven treatments and sprayed on both sides of its body with an identification number. Treatment was then commenced. Immediately after this the calves were walked as a group along a race to the observation paddock approximately 50 meters away. The observation paddock (40m x 70m) allowed *ad libitum* access to grass and water. Throughout the above procedure the behavioural observer was absent.

5.32 Treatments

There were seven treatments. The Scoop group included seven calves. All other groups consisted of eight calves each.

There were three control groups:

Control (='Control')
The horn buds and adjacent skin were handled firmly but not amputated.

Local anaesthetic (2 hours) control ('LA2 control')
Local anaesthetic (6 ml of 2% lignocaine hydrochloride; Lopain; Ethical Agents Ltd, Auckland) was injected around each cornual nerve, midway along the lateral edge of the frontal bone crest (Weaver, 1986), 20 minutes prior to calves having their horn buds handled firmly but not amputated.

Ketoprofen Control ('K control')
The nonsteroidal anti-inflammatory drug ketoprofen (3 ml of 10% Ketofen, Rhône Mérieux, France) was injected intravenously into the jugular vein of calves 20 minutes prior to having their horn buds massaged but not amputated.

Four groups of calves were dehorned:

Scoop ('Scoop')
The horns were removed with a standard dehorning scoop (Barnes Dehorners, Stones, USA) in an identical manner to that described in Chapter Three.

Lignocaine plus scoop ('LA2 + scoop')
Local anaesthetic was administered 20 minutes before the horns were removed with the scoop, as described in the LA2 control group.

Ketoprofen plus Scoop ('K + scoop')
Ketoprofen was administered to calves as described for the K control group. Horns were amputated 20 minutes later with a standard dehorning scoop.

Lignocaine plus Ketoprofen plus Scoop ('LA2:K + scoop')
Lignocaine and ketoprofen were administered as described above. Horns were amputated 20 minutes later with a standard dehorning scoop.

5.33 Behaviour Measurements

Behavioural recording began after all calves had entered the observation paddock, allowing time for initial settling, approximately 2 hours after treatment. Each calf's treatment was unknown to the observer. Individual calves were observed consecutively for one minute in the same order as their treatment to minimise differences in time from treatment between animals. Eight behaviours, divided into five activities and three states, were recorded on pre-prepared data sheets. Behavioural states were recorded as being present or not present for each calf at the start of the one minute observation period. Behavioural activities were recorded as the number of times each activity occurred during each of the one minute periods.

Behavioural states:

1. **Lying:** the calf is sitting on the ground.
2. **Grazing**: the calf is grazing either while standing or sitting.
3. **Ruminating:** the calf is ruminating either while standing or sitting.

Behavioural activities:

1. **Tail shaking:** the animal is rapidly shaking its tail to and fro. The number of times the tail passed a fixed point were measured. This included both tail flicking behaviour used to dislodge flies and that of the more repetitive and vigorous tail flicking commonly observed after dehoning (KJ Stafford & DJ Mellor, personal communication).
2. **Ear flicking:** the animal is rapidly moving one or two ears to the front and back.
3. **Head shaking:** the calf is rapidly shaking its head from one side to the other.
4. **Foot stamping:** the animal is lifting either one of its legs and replacing it on the ground.
5. **Grooming:** the animal nibbles parts of its body it can reach with the teeth.

There were a total of seven one minute observation periods over the three trial days. These were at **2**, **4** and **6** hours after treatment on day one; **22** and **26** hours after treatment on day two; **46** and **50** hours after treatment on day three. Behaviours recorded during the two observation periods on day two were very similar to each other. These were pooled and a mean derived.

Likewise for the two observation periods on day three. Hence, figures for five observation periods have been presented.

5.34 Presentation and Statistical Analysis of Results

The behavioural activities of calves at each of the five observation periods following their particular treatment have been presented as the total number of activities observed for each group, and as the percentage of calves receiving each treatment which displayed the particular activity. The total number of behavioural activities observed in the Scoop group, which contained only 7 calves, was multiplied by 8/7, to avoid misleading comparisons with the six other groups containing 8 calves each. Behavioural states have been presented as the percentage of calves within each group displaying the particular position. Comparisons between total number of activities observed after different treatments were made using the Mann-Whitney U test (Prism, V 2.0, GraphPad Software Incorporated, 1995). Comparisons between the proportion of calves displaying a particular behavioural activity or state after different treatments were made using Fisher's exact test (Prism, V 2.0).

Also presented for each behavioural activity and state in each group is a mean percentage calculated from the three observation periods on day one (2, 4 and 6 hours) to assess any differences between groups across the first 6 hours as a whole. A regression analysis (Microsoft Excel V 5.0, Microsoft Corporation, U.S.A) was performed between this mean and the integrated cortisol responses calculated over the first 9.5 hours after treatment of different calves in Chapter's Three and Four. This comparison involved identical groups, with the exception that the integrated cortisol response of LA4 + scoop was substituted for LA2 + scoop, since the latter treatment was not included in the previous trial. In an another scoop dehorning study (Petrie *et al.,* 1996a) the mean integrated cortisol response of LA2 + scoop calves was very similar to that calculated for LA4 + scoop calves in Chapter 3 (6115 and 6021 ng/ml.min respectively).

The Fisher's exact test was also used to compare percentage changes in each behavioural action and position across time for all treatments.

Due to the very low incidence of rumination measured throughout the three observation days, this behaviour has been presented in

combination with grazing behaviour. Both behaviours are assumed to represent non-stressed activity.

5.4 Results

5.41 Comparison Between Treatments

Behavioural States:

1. Lying. All (100%) Scoop calves were lying 2 hours after dehorning (Figure 5.1), which was significantly higher than the 0% observed in Control, K control and LA2:K + scoop calves, the 12% observed in LA2 control calves, and the 25% observed in K + scoop calves ($p<0.01$). 50% of LA2 + scoop calves were lying 2 hours after dehorning, which was not significantly different from any other group.

Four hours after dehorning the percentage of LA2 + scoop calves lying increased to 100%, significantly greater ($p<0.05$) than the 0-38% observed in the three control groups. Lying in K + scoop calves increased to 75% at 4 hours which, like the 58% figure for Scoop calves, was significantly higher ($p<0.05$) than that for Control calves which remained at 0%. Fifty percent of LA2:K + scoop calves were lying 4 hours after dehorning, which was not significantly different from any the incidences in other groups.

At 6 hours after dehorning lying behaviour in Scoop calves (58%) was numerically but not significantly larger than all other dehorned (38%) and control groups (12-25%).

The mean percentage of lying over the first 6 hours (pooled data) was greatest in Scoop calves (72%), and that was significantly higher ($p<0.05$) than the values for Control and LA2 control calves (8-16%; Figure 5.2). LA2 + scoop, K + scoop and LA2:K + scoop calves had mean lying incidences of 62%, 45% and 29%, respectively. No significant differences were noted between these three percentages, nor between them and the values for any other group.

On day 2 and day 3 there was a general increase in lying behaviour in all three control groups, such that no significant difference between any group was noted (Figure 5.2).

2. Grazing and Ruminating. All (100%) of LA2:K + scoop calves and 88-100% of calves in all three control groups were either grazing or ruminating 2 hours after treatment (Figure 5.3). A significantly lower incidence was observed in Scoop calves (15%; $p<0.01$). K + scoop calves were also ruminating/grazing significantly less (38%) compared to Control, LA2 control and LA2:K + scoop calves ($p<0.05$). 50% of LA2 + scoop calves were grazing/ruminating 2 hours after dehorning.

Four hours after treatment 75-100% of calves in the three control group calves were grazing/ruminating. The percentage of LA2:K + scoop and LA2 + scoop calves grazing/ruminating were 38% and 25% respectively, values which were significantly less than in K control calves ($p<0.05$). No Scoop and 12% of the K + scoop calves were grazing/ruminating 4 hours after dehorning, incidences which were both significantly lower than in all three control groups ($p<0.05$).

The incidence of grazing/ruminating had increased in all four dehorned groups by 6 hours, such that no significant differences were observed at this time.

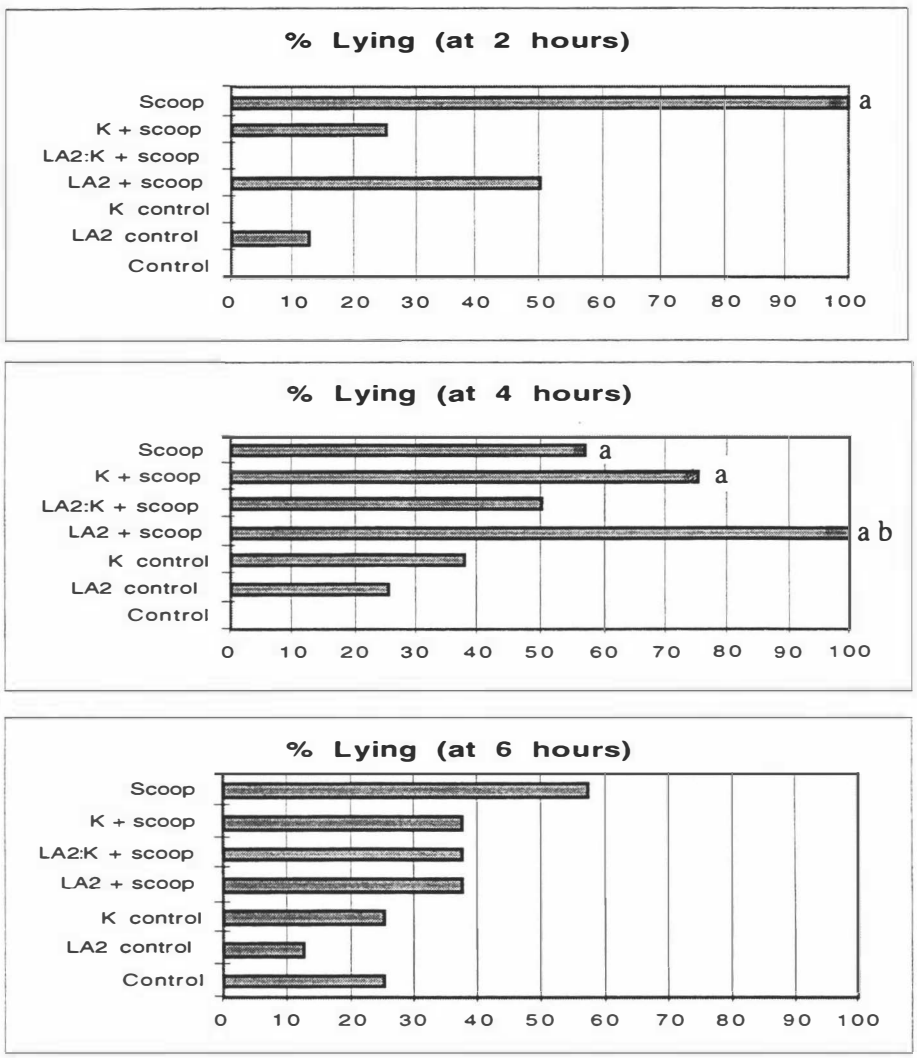
The mean percentage of grazing/ruminating across the first 6 hours (pooled data) was lowest in the Scoop calves (22%), being significantly less ($p<0.05$) compared to that in the three control groups (88-92%; Figure 5.4). The mean percentage was 38% in LA2 + scoop and K + scoop calves, and 62% in LA2:K + scoop calves.

There was a general increase in grazing and ruminating behaviour in all dehorned groups during day 2 and day 3, such that no significant between group differences were observed (Figure 5.4).

Behavioural Activities:

1. Tail Shaking. Two hours after dehorning 100% of the Scoop calves were exhibiting tail shaking behaviour (Figure 5.5), a proportion significantly greater ($p<0.05$) than in all three control groups (25-38%) and in the LA2:K + scoop calves (25%). Tail shaking was also significantly greater in K + scoop calves (88%) compared to incidences in LA2 control, K control and LA2:K + scoop calves ($p<0.05$). There was a 62% incidence in LA2 + scoop calves.

Fig 5.1: Percentage of calves lying at 2, 4 and 6 hours after each treatment.

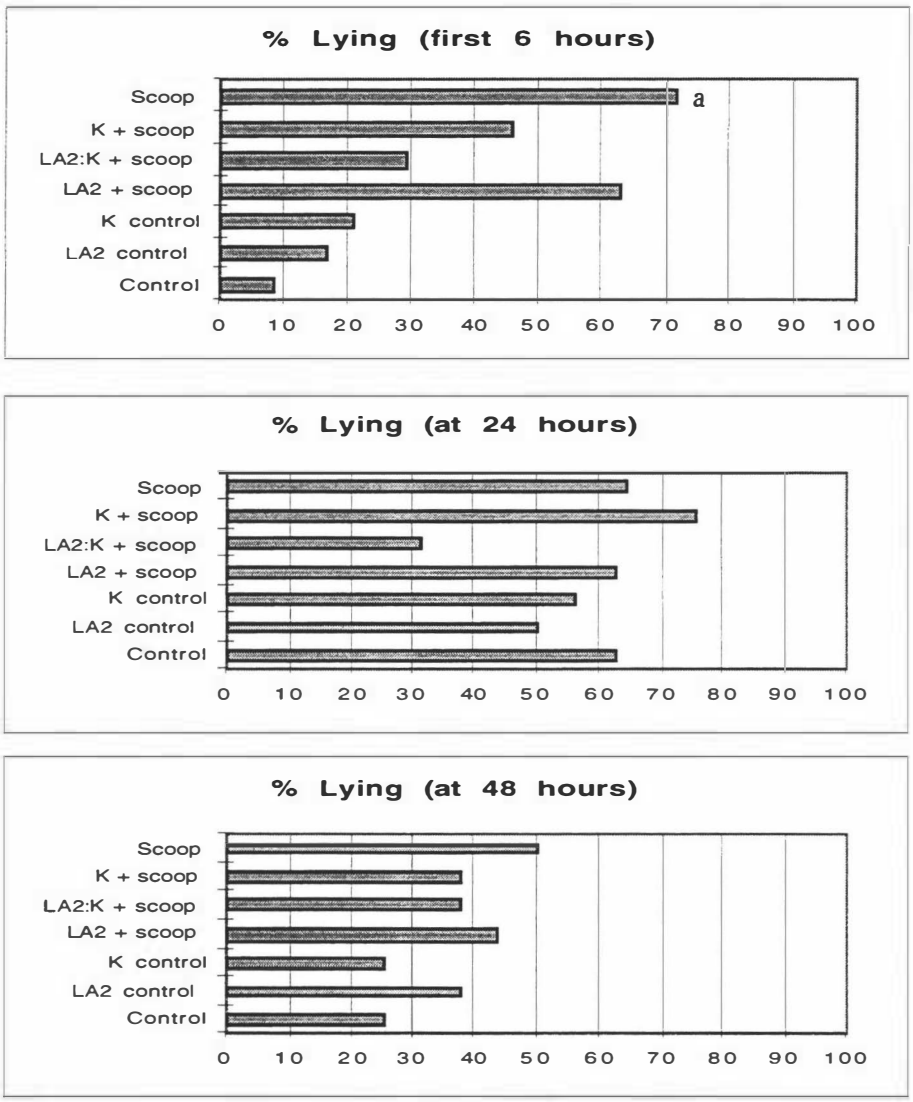


Significant Differences:

At 2 hours: a = From Control; LA2 + control; K + control; K + scoop; LA2:K + scoop ($p<0.05$).

At 4 hours: a = From Control ($p<0.05$).
b = From LA2 control; K control ($p<0.05$)

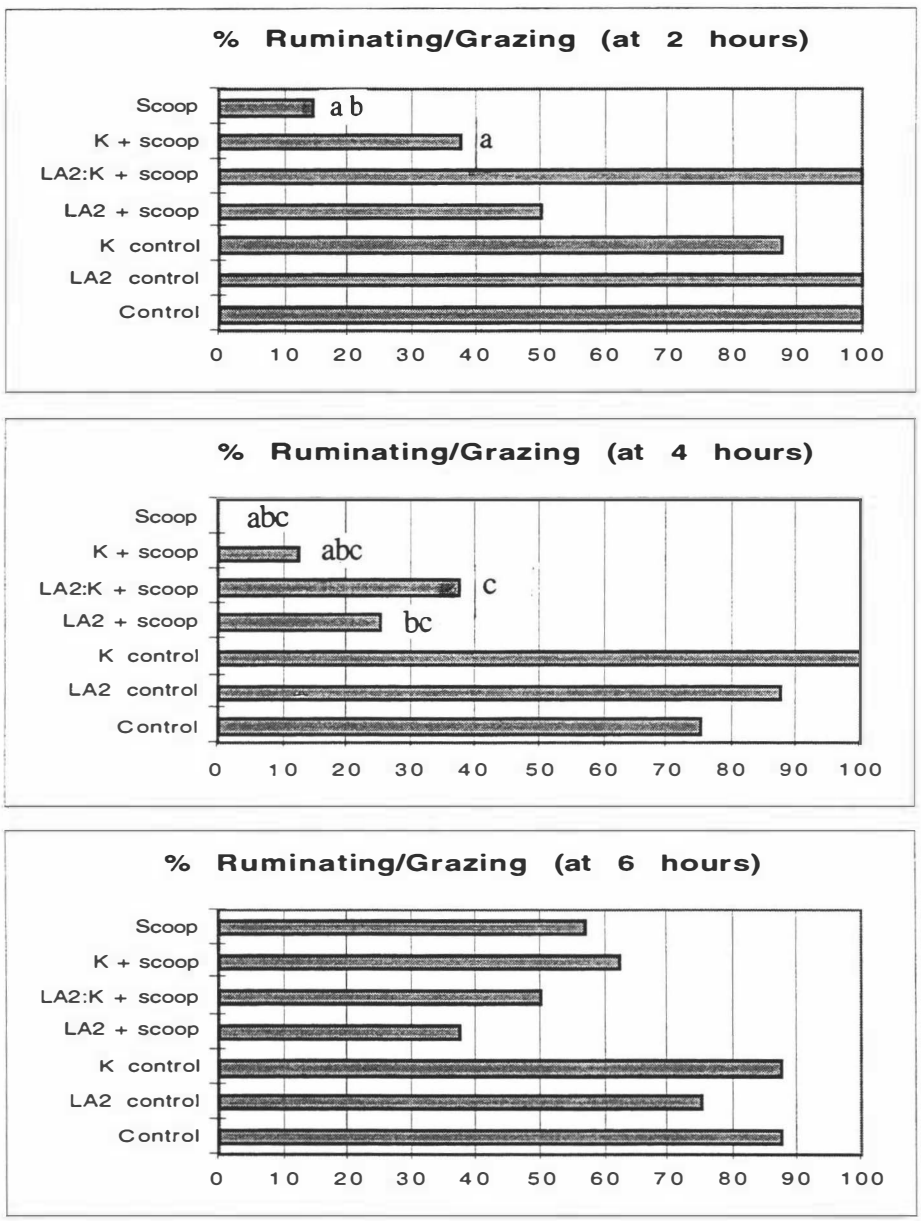
Fig 5.2: Mean percentage of calves lying during first 6 hours, and at 24 and 48 hours after each treatment



Significant Differences:

First 6 hours: a = From Control; LA2 control ($p<0.05$).

Fig 5.3: Percentage of calves ruminating/grazing at 2, 4 and 6 hours after each treatment.

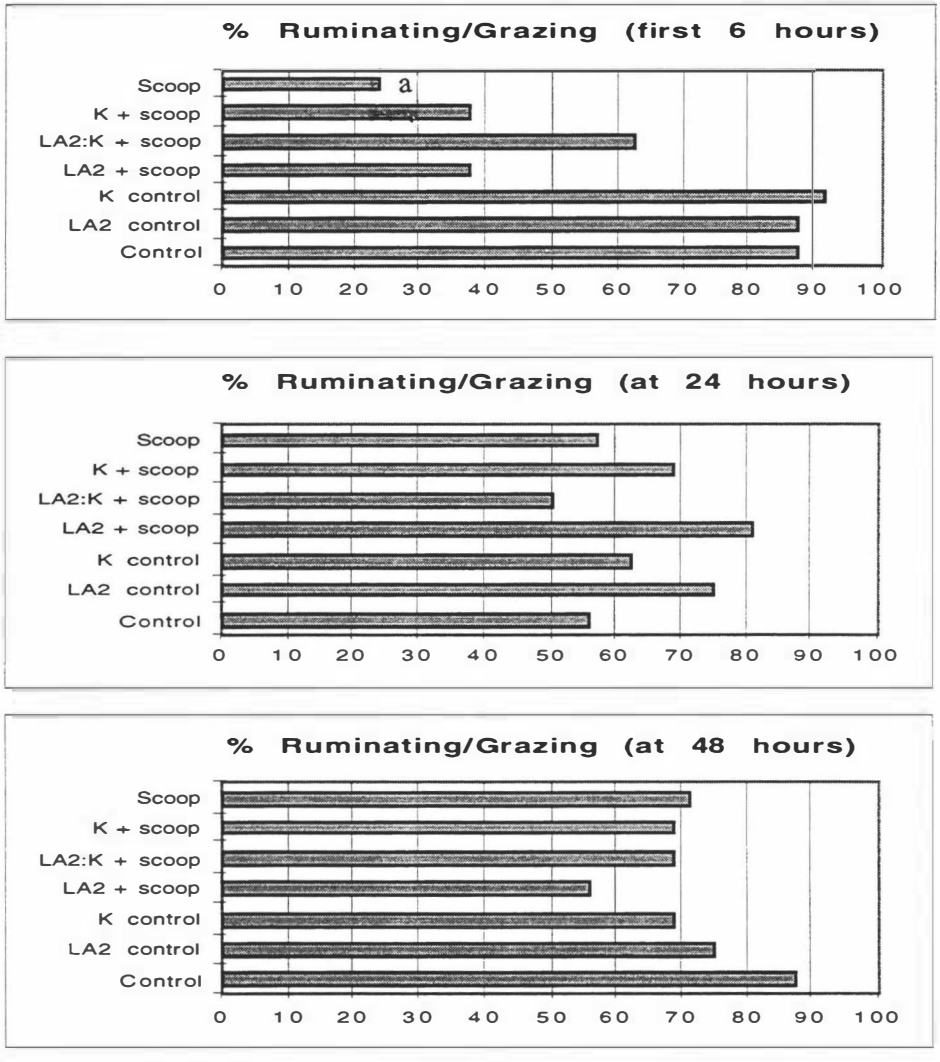


Significant Differences:

At 2 hours: a = From Control; LA2 control; LA2:K + scoop ($p<0.05$).

At 4 hours: a = From Control ($p<0.05$).
b = From LA2 control ($p<0.05$).
c = From K control ($p<0.05$).

Fig 5.4: Mean percentage of calves ruminating/grazing during first 6 hours, and at 24 and 48 hours after each treatment.



Significant Differences:

First 6 hours: a = From Control; LA2 control; K control ($p<0.05$).

The total number of tail shakes observed within each group 2 hours after dehorning was highest in the K + scoop calves with 55 in one minute (Figure 5.7), but averaged 8 tail shakes per calf (Table 5.1). The next highest total number of tail shakes was 47 observed in Scoop calves, averaging 6 per calf. The frequencies in both groups were significantly higher than those elicited by LA2 control, K control and LA2:K + scoop treatments (3-5 total tail shakes; $p<0.01$), while the frequency within the Scoop treatment was also significantly higher than that in LA2 + scoop calves (22; $p<0.05$). Control calves exhibited a total of 32 tail shakes (11 per calf), which was numerically but not significantly higher than in LA2 + scoop and LA2:K + scoop calves.

Four hours after treatment, 85% of Scoop calves were tail shaking, a percentage which was significantly higher than the 12-25% exhibited by LA2 control, K control, K + scoop and LA2:K + scoop calves ($p<0.05$) (Figure 5.5). 50% of LA2 + scoop and Control calves tail shook. Scoop calves also exhibited the highest total number of tail shakes at 4 hours (37), being significantly higher than in LA2 control and K control calves (3-5; $p<0.05$) (Figure 5.7). The frequencies for all other treatments ranged between 14-24 total tail shakes per minute.

Six hours after treatment, 71% of Scoop calves and 75% of K + scoop calves were tail shaking, figures which were both significantly higher than in all three control groups (0-12%; $p<0.05$). The figures for LA2:K + scoop calves remained the lowest for all the dehorned treatments with a 38% tail shaking incidence, which was not significantly different from any control group. The total number of tail shakes was greatest in K + scoop calves with 40 (7 per calf), and like Scoop calves with a total of 20 tail shakes (3 per calf), these values were significantly greater ($p<0.05$) than those for the K control and LA2 control calves (0-1 tail shakes in one minute). LA2 + scoop calves had a 24 tail shake total (5 per calf), but this was not significantly different from the figures in any other group. The total number in LA2:K + scoop and Control calves was very similar (9 and 11 respectively), although in the latter group all tail shaking occurred in one individual. The same calf had also been very active during the two previous observation periods.

The mean percentage of tail shaking across the first 6 hours after treatment (pooled data) was highest in Scoop calves (85%; Figure 5.6), a value which was significantly greater than those in LA2 control, K control and LA2:K + scoop calves (12-29%; $p<0.05$). LA2 +

scoop and K + scoop calves had similar means of 58% and 62% respectively. The Control calves reached a mean tail shaking percentage of 32%.

On day 2 and day 3 there was a general decline in the percentage of calves tail shaking and in the number of tail shakes occurring in all dehorned groups (Figure 5.6 and 5.8). Hence, no significant differences were observed during this time between all seven groups.

2. Ear Flicking. Two hours after treatment the highest percentage of ear flicking (88%) was observed in Control, K + scoop, LA2 + scoop calves, followed by a 72% incidence in Scoop calves (Figure 5.9). These incidences were all significantly greater ($p<0.05$) than that in K control calves which had 12% of calves ear flicking. Fifty percent of LA2 control and LA2:K + scoop calves were ear flicking, a percentage not significantly different from those for any other treatment. Unlike the K + scoop and LA2 + scoop treatments, the high percentage of ear flicking in the Control calves did not equate to a high total number of ear flicks (Figure 5.11). This was due to a low number of 3 ear flicks per calf in the Control calves (19 total ear flicks) compared to 7-8 ear flicks per calf in the K + scoop and LA2 + scoop calves (48-53 total ear flicks; Table 5.1). The total number of ear flicks in LA2:K + scoop calves 2 hours after dehorning was 19, equivalent to that of Control calves and significantly less than K + scoop calves ($p<0.05$).

Four hours after treatment there were no significant between-group differences in the percentage of ear flicking animals nor the total number of ear flicks. However, values for all dehorned groups were numerically larger than those for all controls.

At 6 hours the percentage range of ear flicks across the dehorned groups was narrow, being 58-62%. The values in these four groups were significantly greater than those in the three control groups, all of which exhibited zero ear flicks ($p<0.05$). The total number of ear flicks was highest at 44 in the K + scoop group, which again was significantly greater than for all three control groups ($p<0.05$).

The mean percentage of ear flicking across the first 6 hours was numerically but not significantly greater in the dehorned groups compared to all controls (Figure 5.10). Within the dehorned groups the highest percentage of ear flicking was in the LA2 + scoop calves (66%), and lowest in the LA2:K + scoop calves (50%).

On day 2, there was a general reduction in ear flicking elicited by dehorning treatments (Figure 5.10). Hence, no significant difference was noted between any groups on this day.

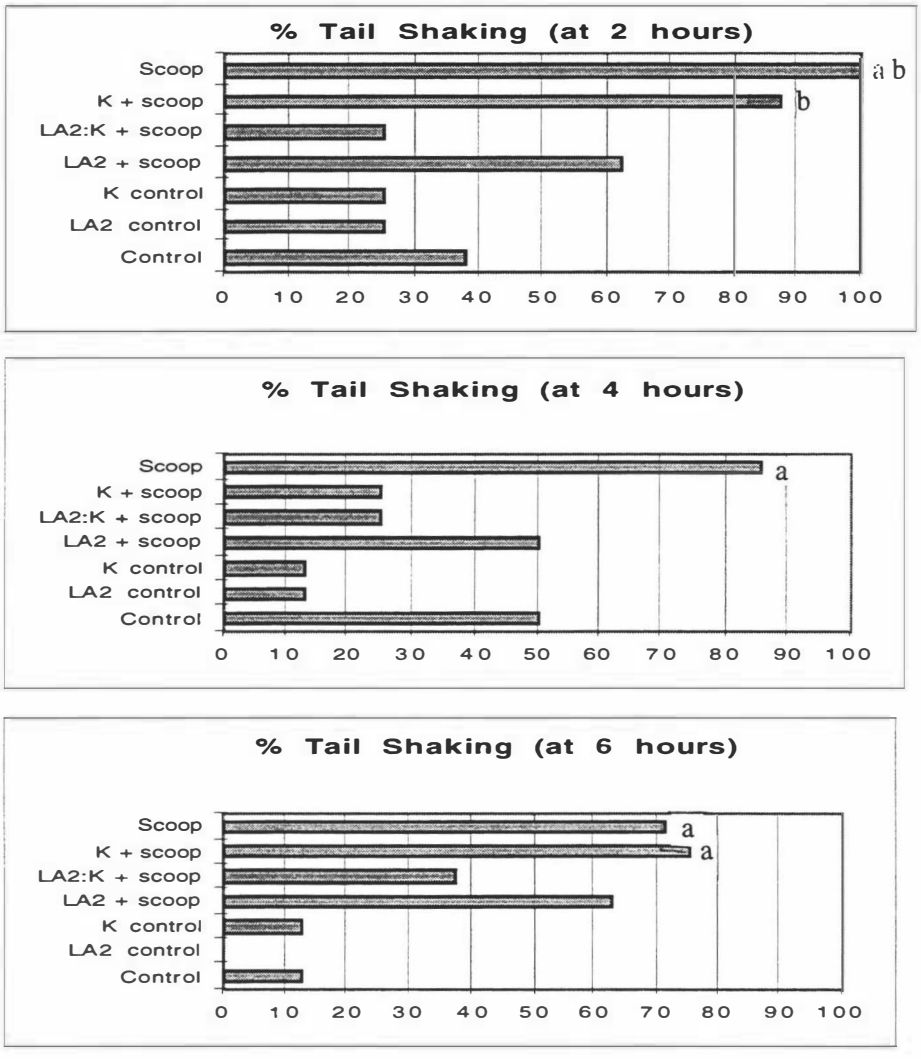
On day 3, the total number of ear flicks in K + scoop calves increased to be significantly greater than in all control and LA2:K + scoop treatments ($p<0.05$) (Figure 5.12). Ear flicking was the only recorded behaviour for which a significant between-group difference was observed on either day 2 or 3.

3. Head Shaking, Foot Stamping and Grooming. The recorded incidences of these behavioural actions were low throughout the three observation days (Figures 5.13-5.18). No significant between-group differences were detected for any of these actions. There were however numerical differences. All 17 calves which head shook and 10 of 13 calves which foot stamped were in the four dehorning groups, whereas 7 of 9 calves which groomed were in the three control treatments.

5.42 Regression Analysis With Integrated Cortisol Response

Significant correlations were found between the integrated cortisol response calculated for the first 9 hours after treatment (Chapter's 3 and 4) and the mean percentage of observed behaviours over the first 6 hours after treatment: sitting ($r=0.94$; $p<0.001$), ruminating/grazing ($r-0.86$; $p<0.01$) and tail shaking ($r=0.84$; $p<0.05$) behaviour (Figure 5.19; Table 5.2). A correlation was also noted between the integrated cortisol response and ear flicking behaviour ($r=0.73$), but this was not significant ($p<0.07$).

Fig 5.5: Percentage of calves tail shaking at 2, 4 and 6 hours after each treatment.



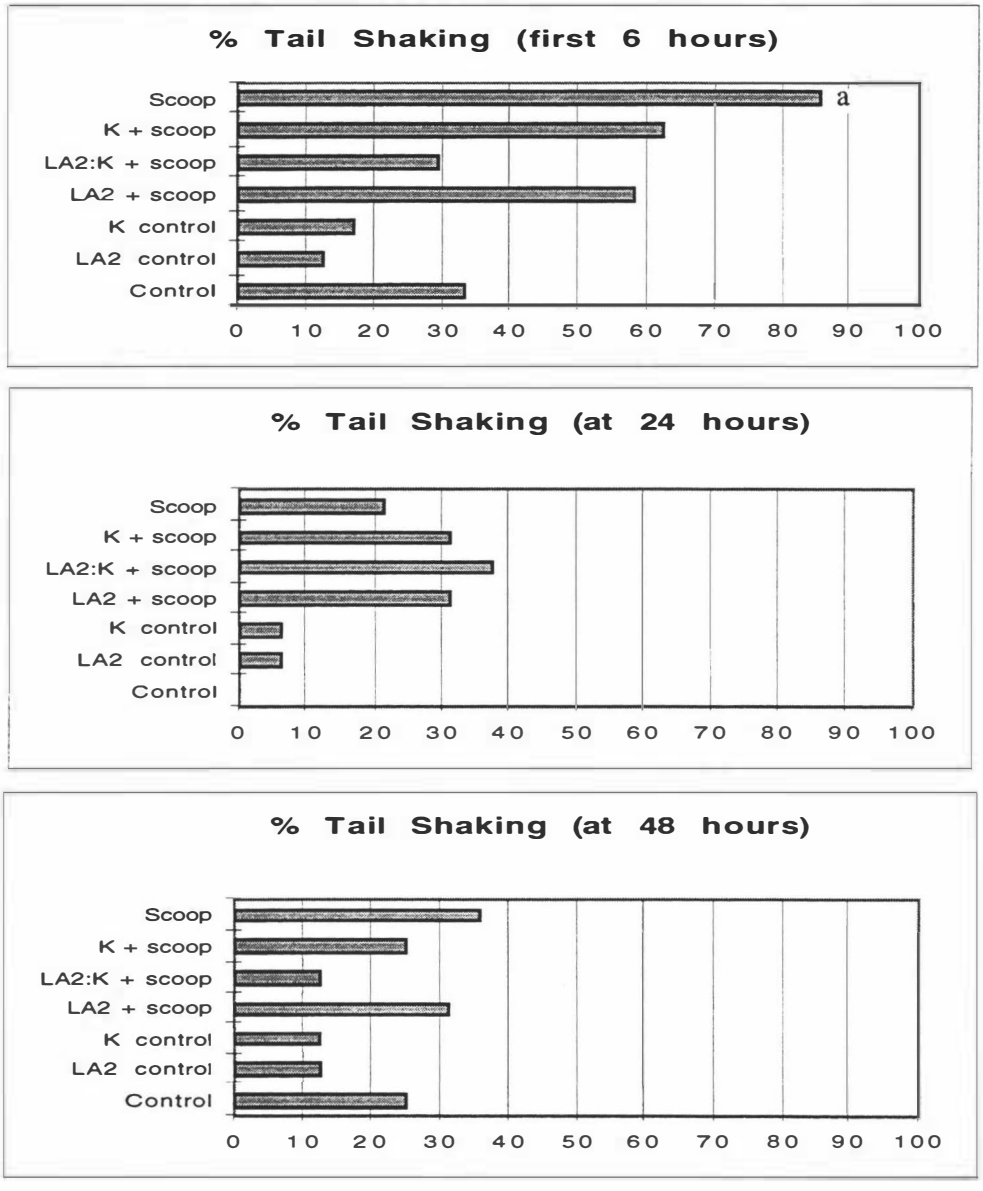
Significant Differences:

At 2 hours: a = From Control ($p<0.05$).
b = From LA2 control; K control, LA2:K + scoop ($p<0.05$).

At 4 hours: a = From LA2 control; K control, LA2:K + scoop; K + scoop ($p<0.05$).

At 6 hours: a = From Control; LA2 control; K control ($p<0.05$).

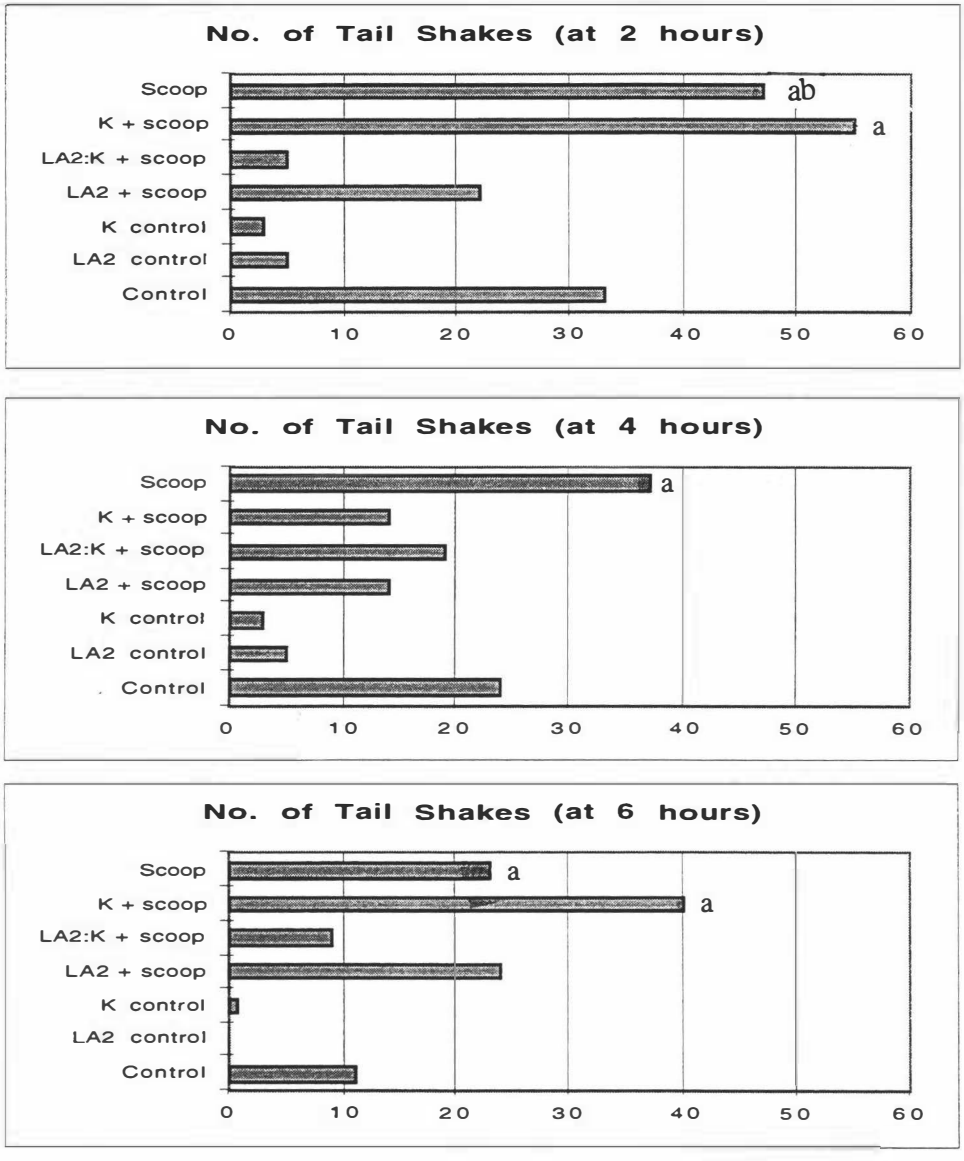
Fig 5.6: Mean percentage of calves tail shaking during first 6 hours, and at 24 and 48 hours after each treatment.



Significant Differences:

First 6 hours: a = From LA2 control; K control; LA2:K + scoop ($p<0.05$).

Fig 5.7: Total number of tail shakes observed at 2, 4 and 6 hours after each treatment.



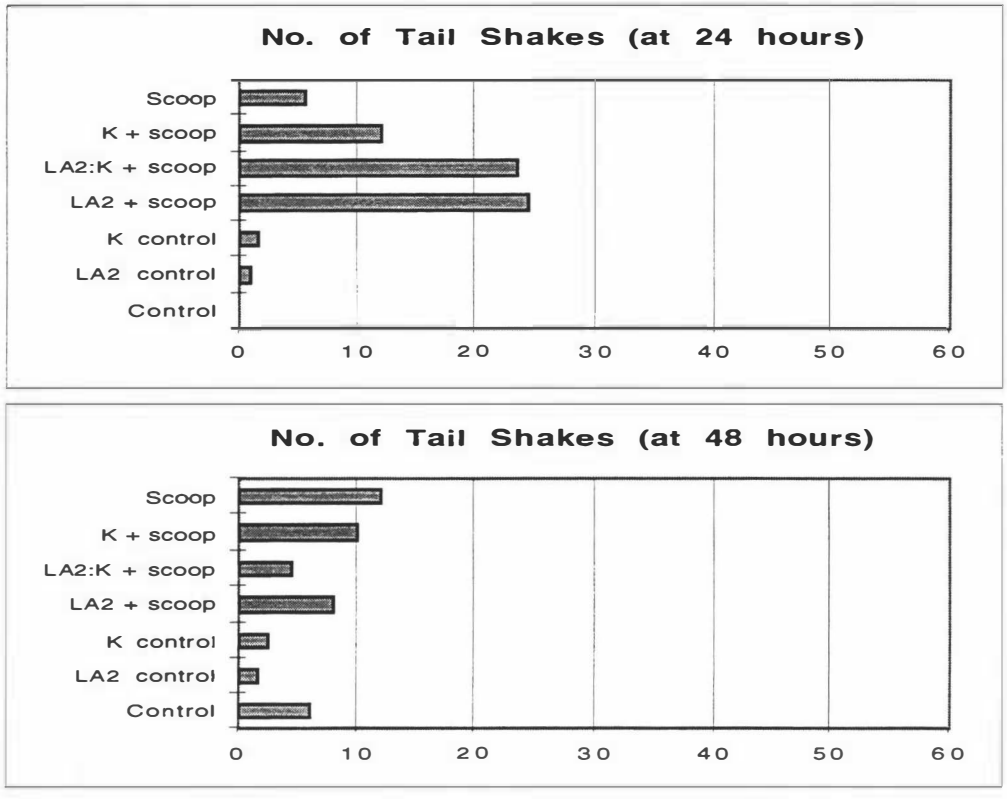
Significant Differences:

At 2 hours: a = From LA2 control; K control; LA2:K + scoop ($p<0.01$).
b = From LA2 + scoop ($p<0.05$).

At 4 hours: a = From LA2 control; K control ($p<0.05$).

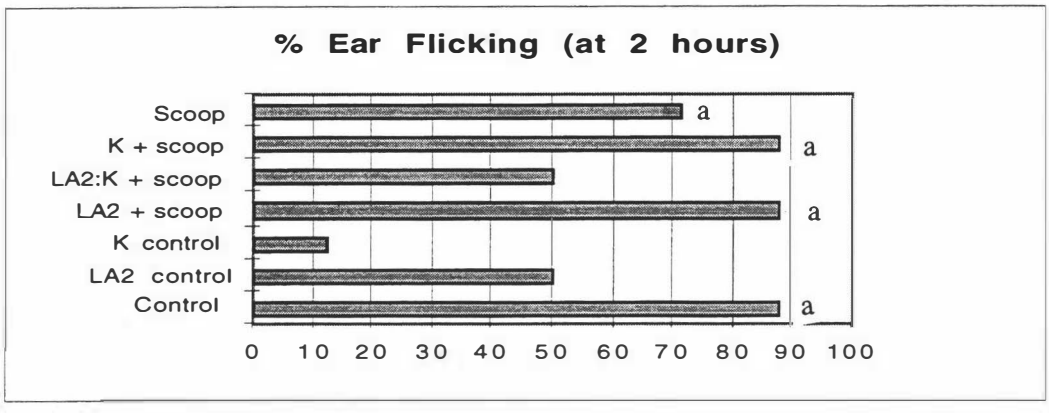
At 6 hours: a = From LA2 control; K control ($p<0.05$).

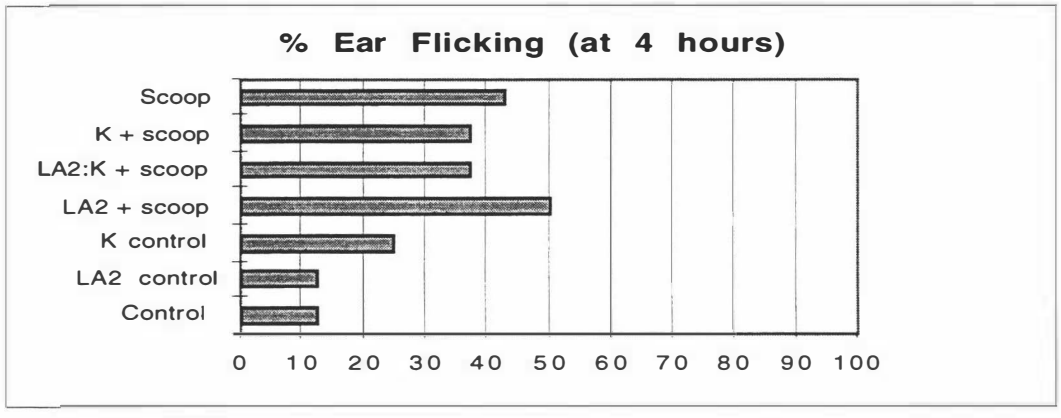
Fig 5.8: Total number of tail shakes observed at 24 and 48 hours after each treatment.

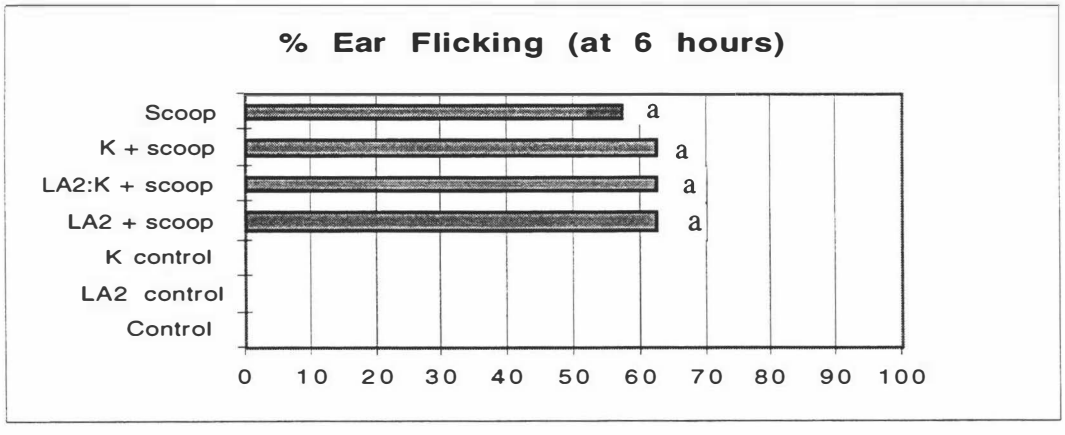


No significant differences.

FIG 5.9: Percentage of calves ear flicking at 2, 4 and 6 hours after each treatment.





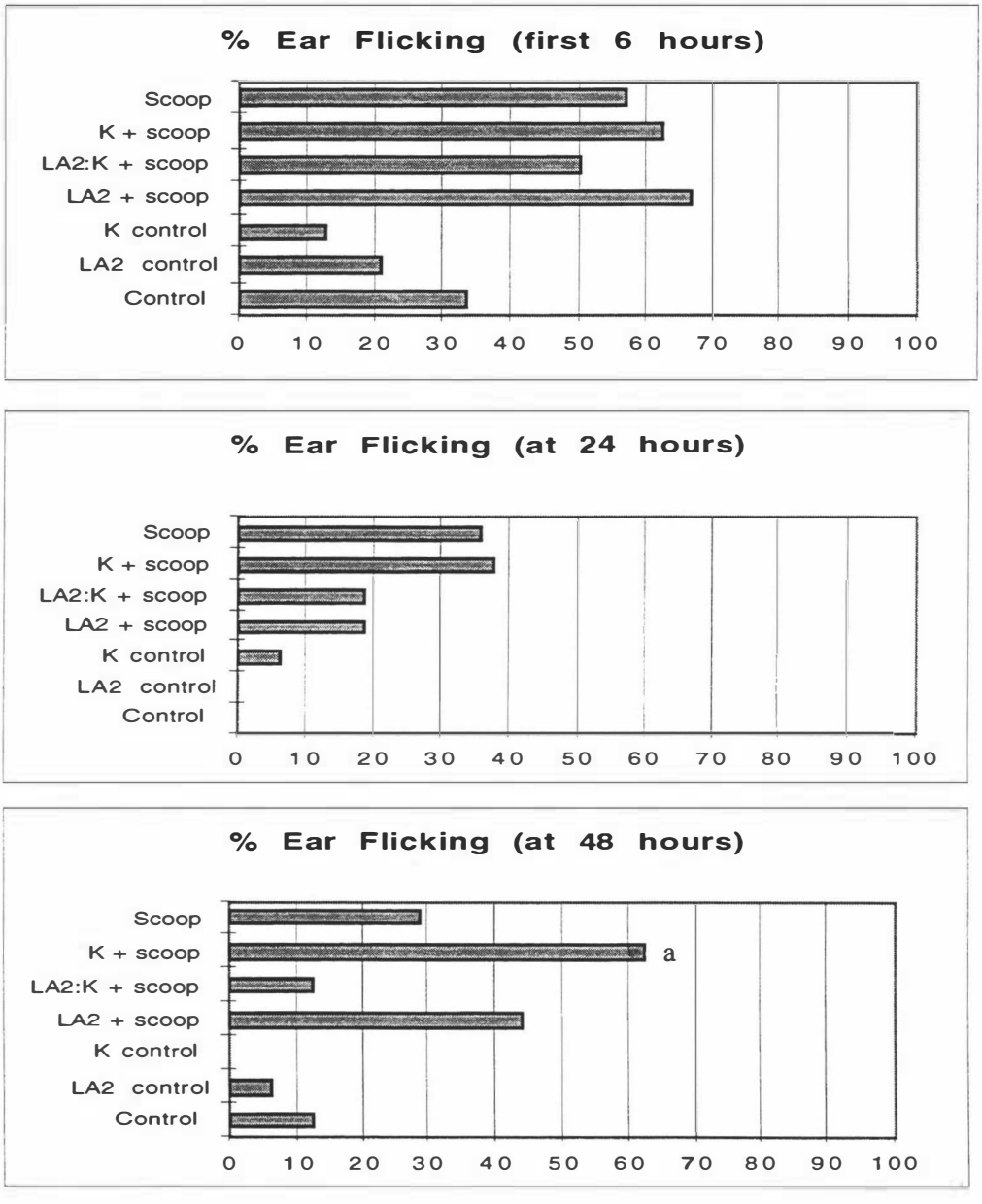


Significant Differences:

At 2 hours: a = From K control ($p<0.05$).

At 6 hours: a = From Control; LA2 control; K control ($p<0.05$).

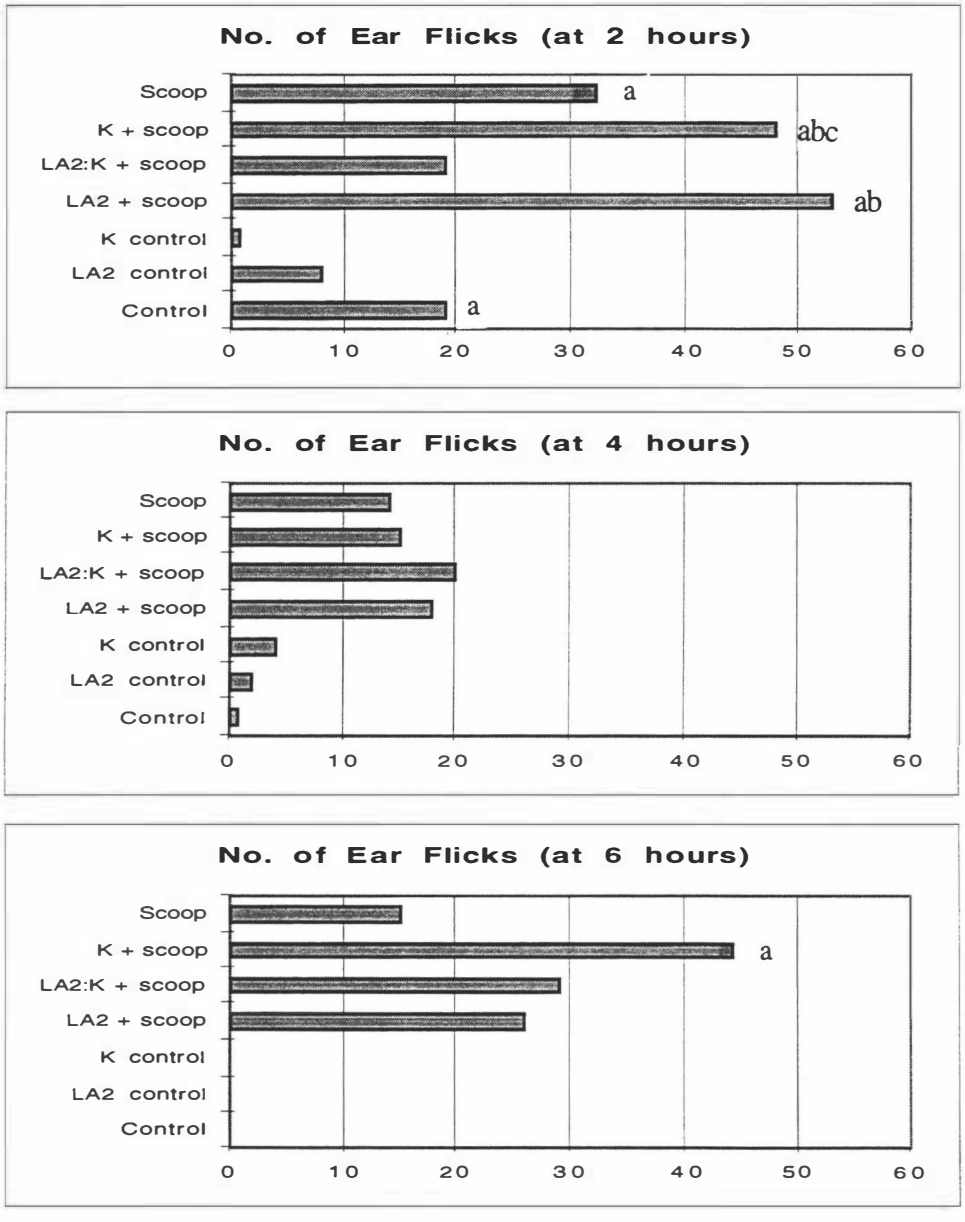
Fig 5.10: Mean percentage of calves ear flicking during the first 6 hours, and at 24 and 48 hours after each treatment.



Significant Differences:

At 48 hours: a = From K control ($p<0.05$).

Fig 5.11: Total number of ear flicks observed at 2, 4 and 6 hours after each treatment.

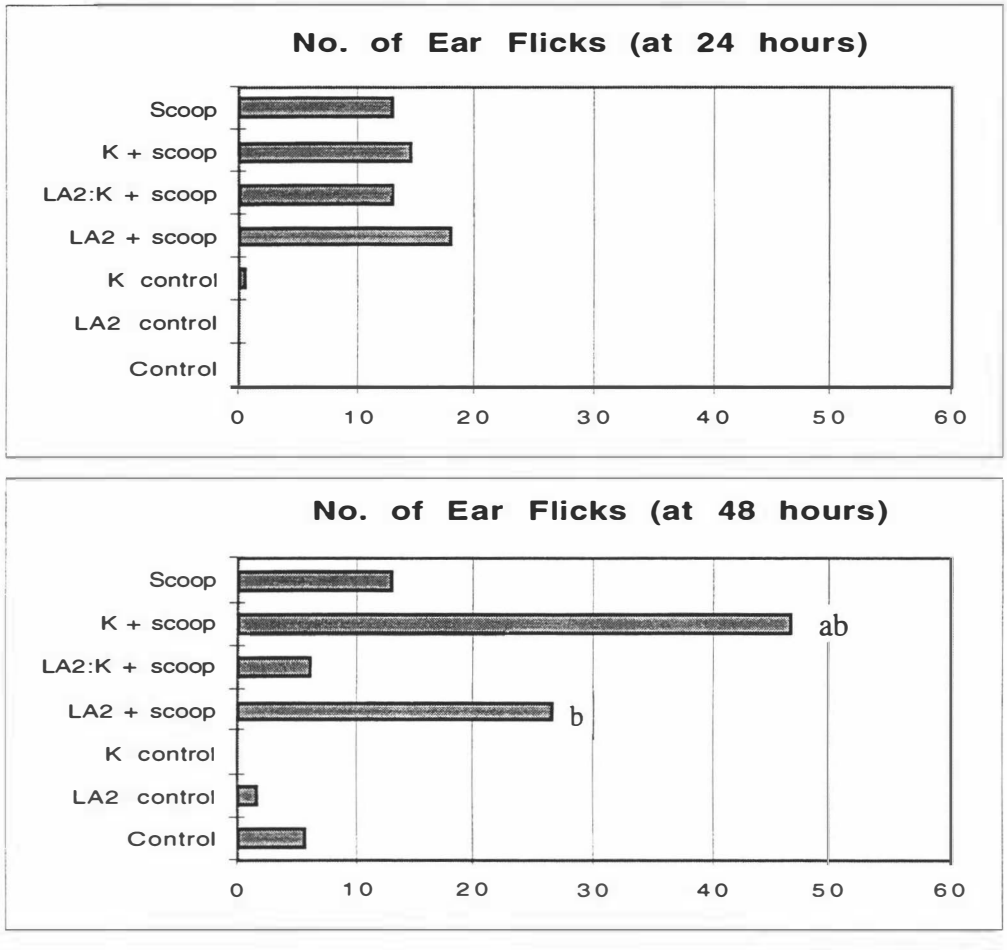


Significant Differences:

At 2 hours:
a = From K control ($p<0.05$).
b = From Control; LA2 control ($p<0.05$).
c = From LA2:K + scoop ($p<0.05$).

At 6 hours:
a = From Control; LA2:K + scoop ($p<0.05$).

Fig 5.12: Total number of ear flicks observed at 24 and 48 hours after each treatment.



Significant Differences:

At 48 hours:

a = From Control; LA2:K + scoop ($p<0.01$).

b = From LA2 control; K control ($p<0.05$).

EAR FLICKS

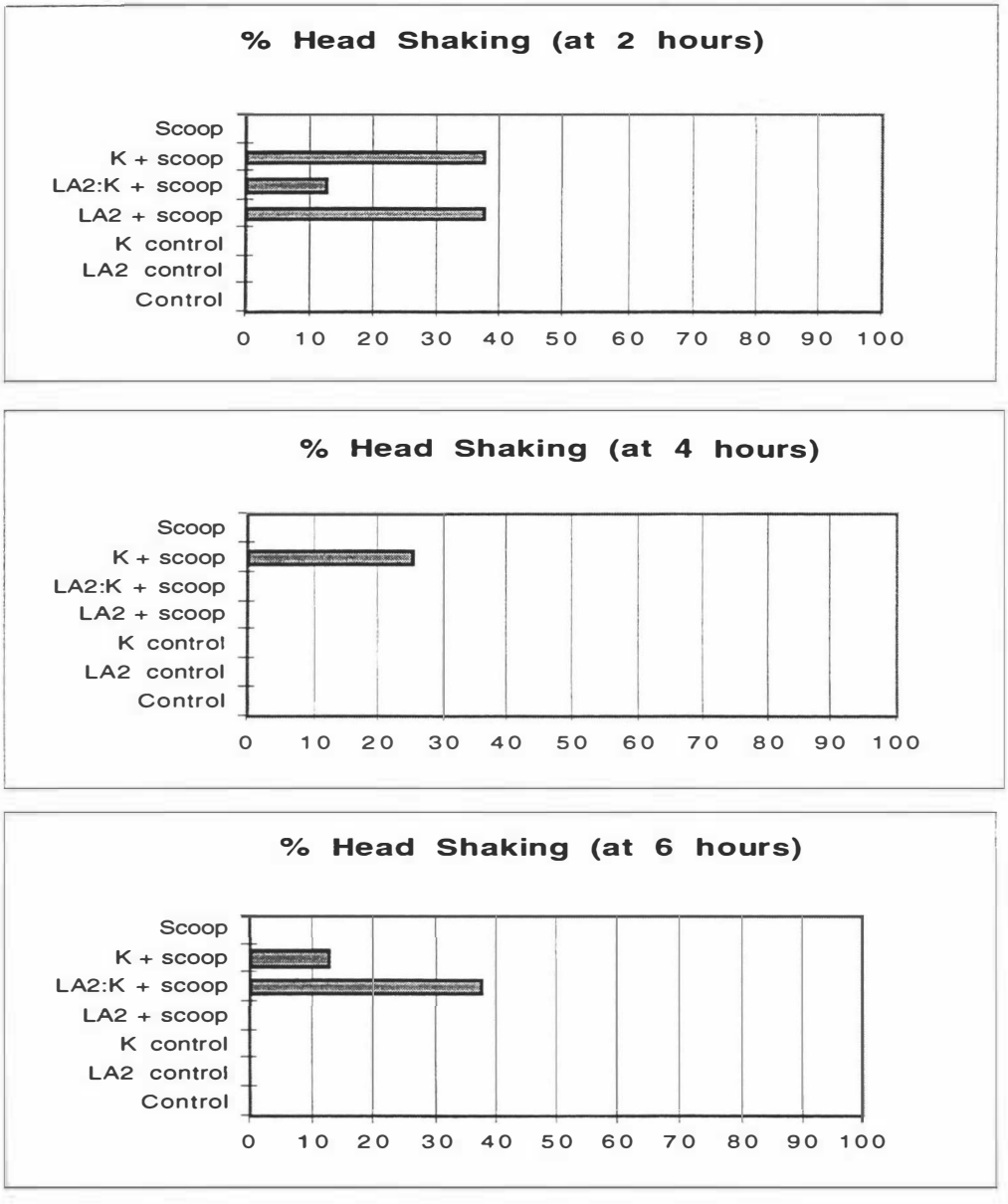
	Control	LA2 control	K control	LA2 + scoop	LA2:K + scoop	K+ scoop	Scoop
2 hrs	2.7	2.0	1.0	7.6	4.8	6.9	5.6
4 hrs	1.0	2.0	2.0	4.5	6.7	5.0	4.0
6 hrs	0.0	0.0	0.0	5.2	5.8	8.8	3.3
24 hrs	0.0	0.0	1.0	12.0	8.7	4.8	4.4
48 hrs	5.5	3.0	0.0	7.6	6.0	9.3	5.5

TAIL SHAKES

	Control	LA2 control	K control	LA2 + scoop	LA2:K + scoop	K+ scoop	Scoop
2 hrs	11.0	2.5	1.5	4.4	2.5	7.9	5.9
4 hrs	6.0	5.0	3.0	3.5	9.5	7.0	5.3
6 hrs	11.0	0.0	1.0	4.8	3.0	6.7	4.0
24 hrs	0.0	2.0	3.0	9.8	7.8	4.8	3.3
48 hrs	3.0	1.5	2.5	3.2	4.5	5.0	4.2

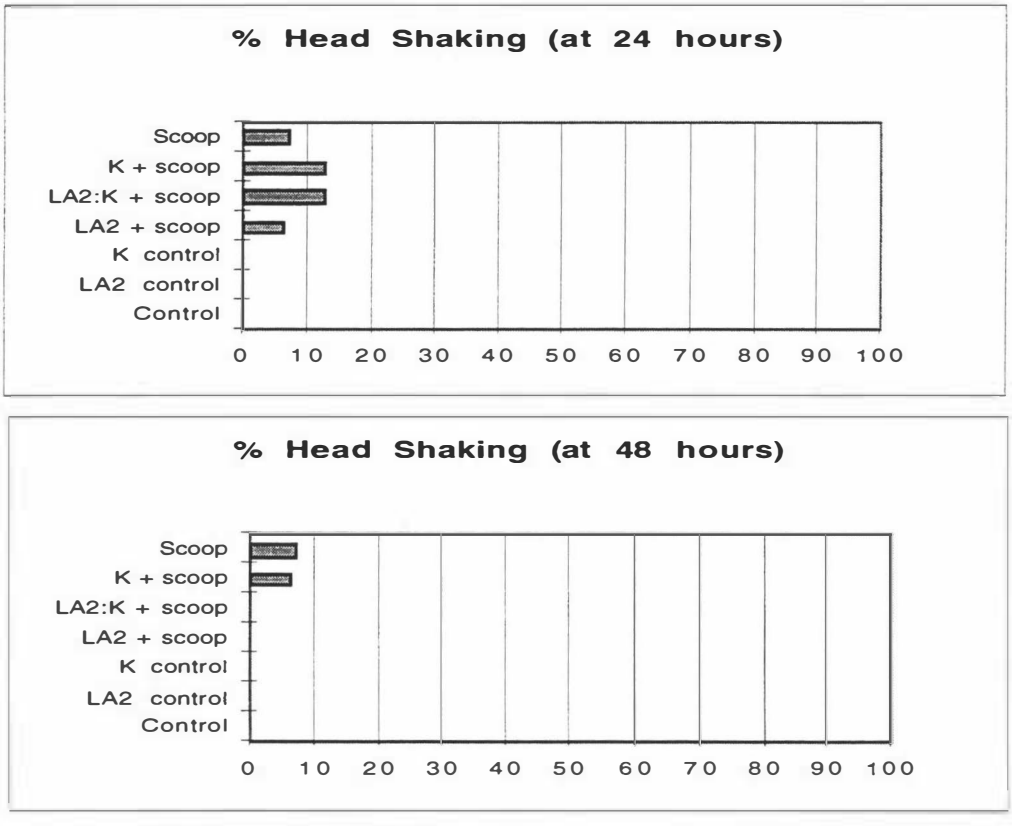
Table 5.1: Number of ear flicks and tail shakes observed per calf in the hours after each treatment.

Fig 5.13: Percentage of calves head shaking at 2, 4 and 6 hours after each treatment.



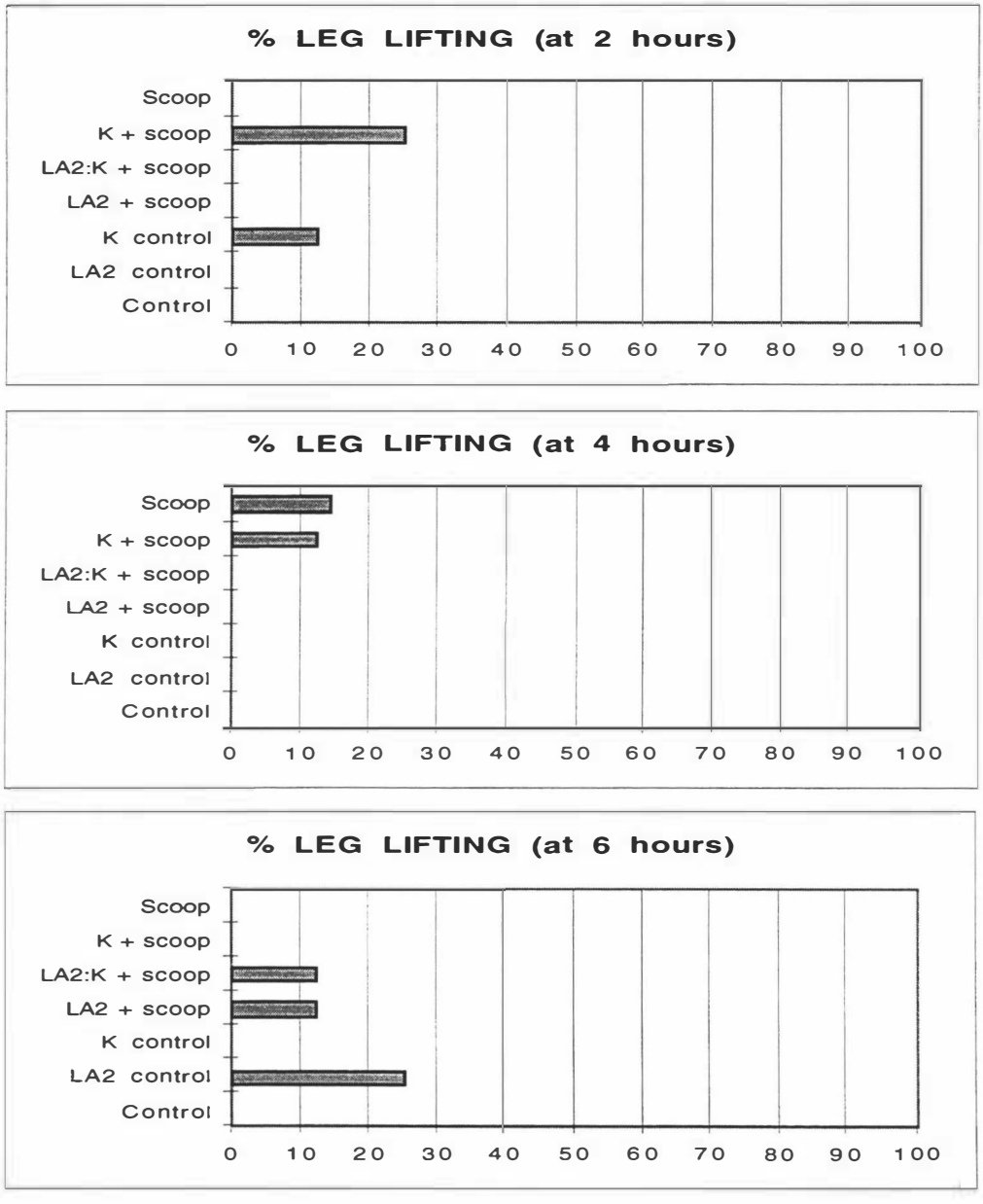
No significant differences.

Fig 5.14: Percentage of calves head shaking during the first 6 hours, and at 24 and 48 hours after each treatment.



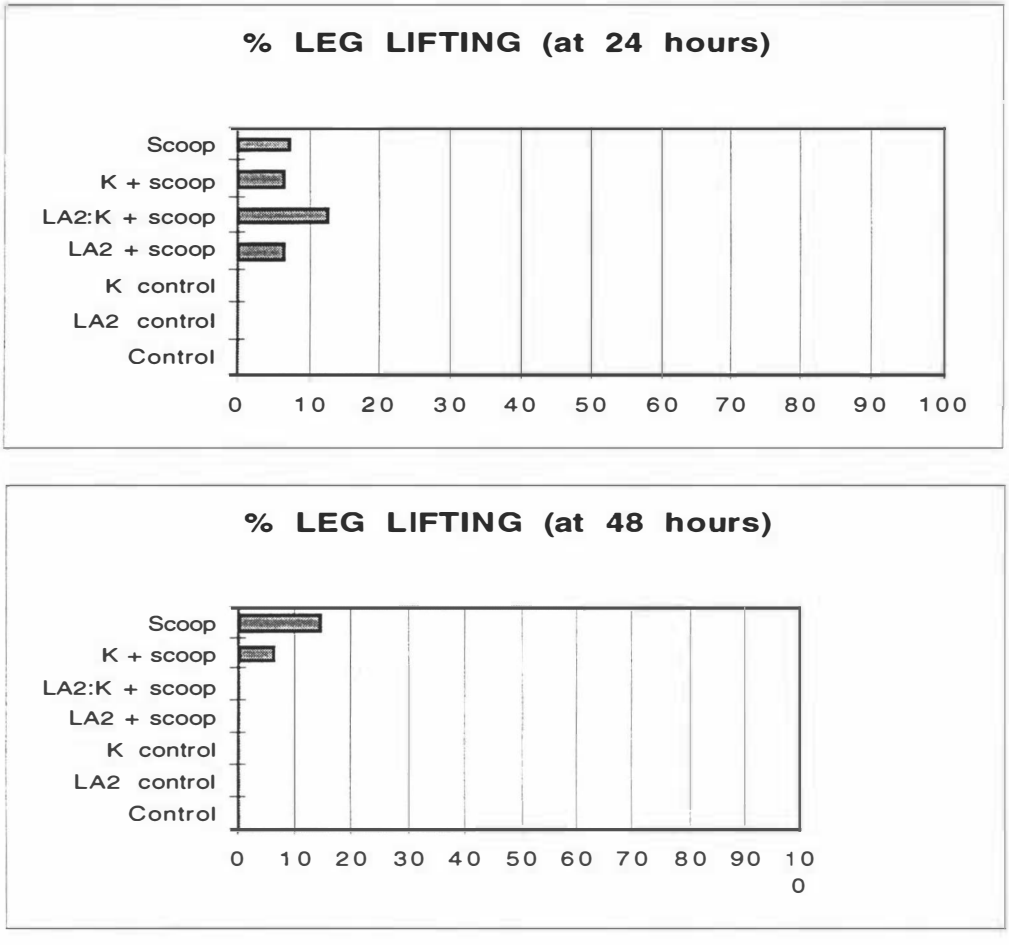
No significant differences.

Fig 5.15: Percentage of calves leg lifting at 2, 4 and 6 hours after each treatment.



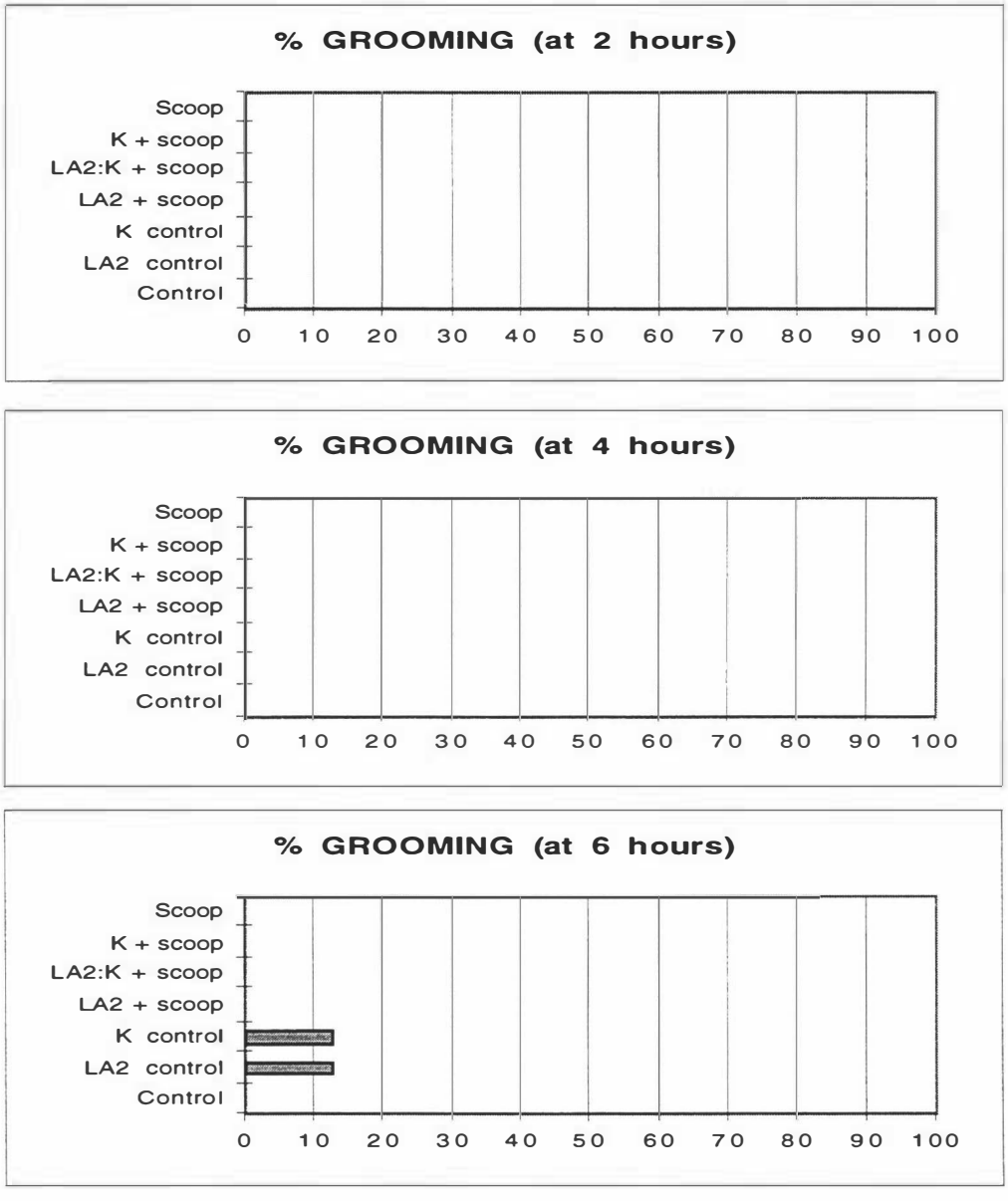
No significant differences.

Fig 5.16: Percentage of calves leg lifting at 24 and 48 hours after each treatment.



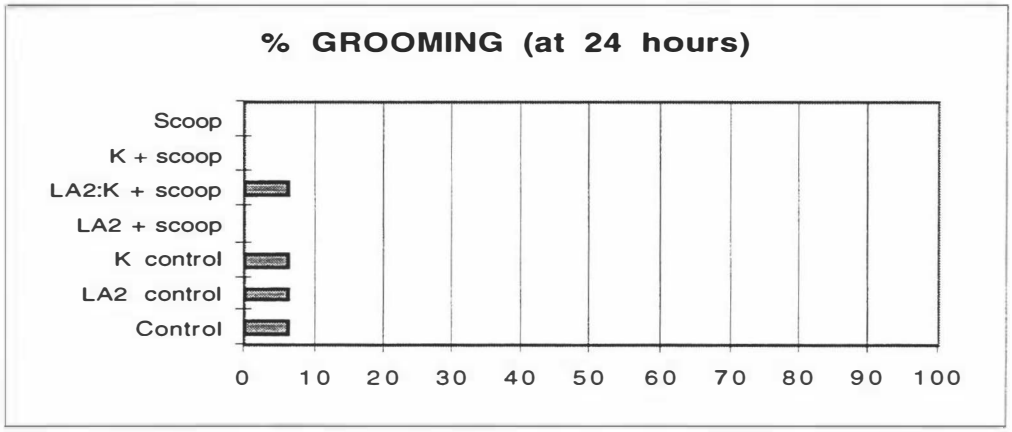
No significant differences.

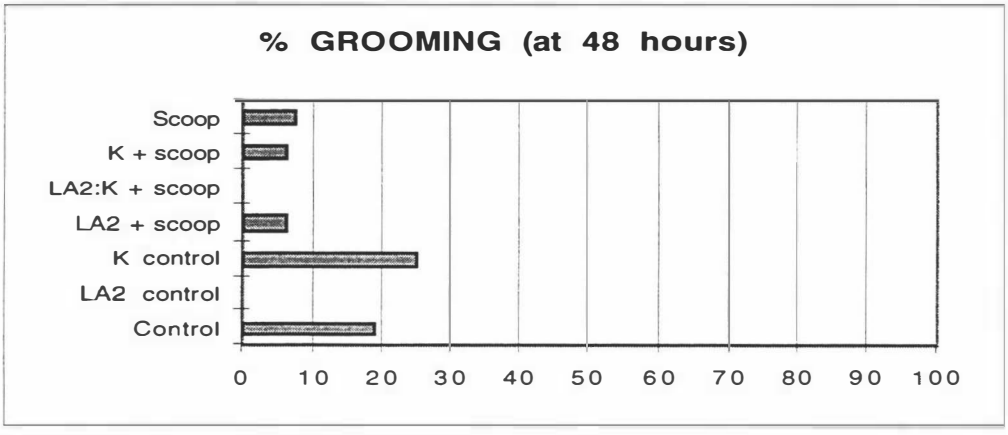
Fig 5.17: Percentage of calves grooming at 2, 4 and 6 hours after each treatment.



No significant differences.

Fig 5.18: Percentage of calves grooming at 24 and 48 hours after each treatment.





No significant differences.

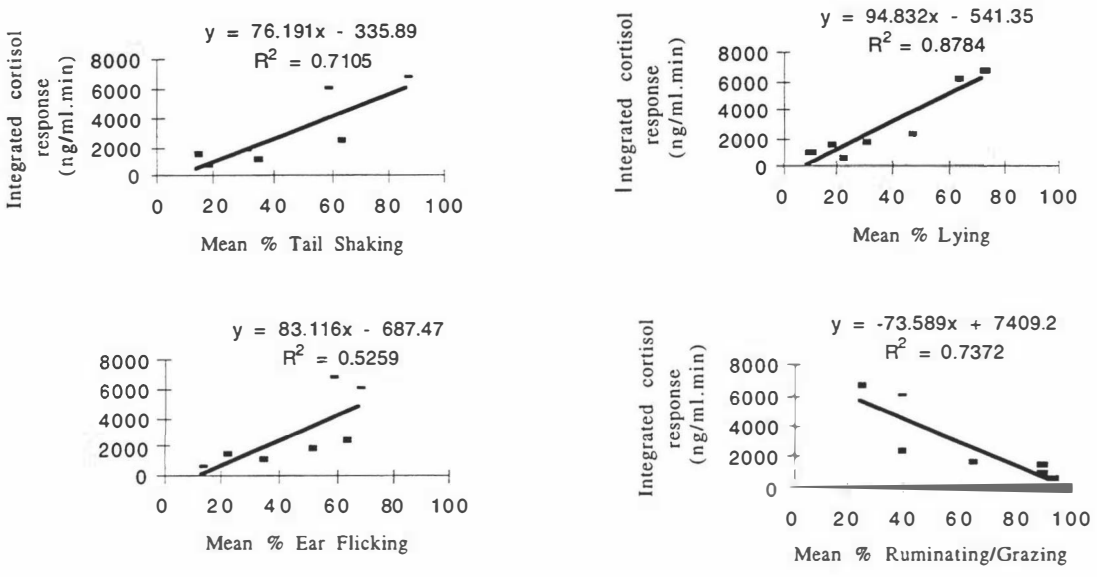


Fig 5.19: Linear regression of integrated cortisol response (over first 9 hours after treatment) versus mean percentage behaviour (over first 6 hours after treatment).

	r
Sitting	0.94 ($p<0.001$)
Ruminating/Grazing	-0.86 ($p<0.01$)
Tail Shaking	0.84 ($p<0.02$)
Ear Flicking	0.73 ($p<0.07$)

Table 5.2: R values for linear regression of integrated cortisol response versus mean percentage behaviour.

5.43 Comparisons Within Treatments Across Time

In K + scoop and Scoop calves tail shaking behaviour became significantly reduced at 4 hours and 24 hours after dehorning respectively. No other significant changes in the percentage expression of any behavioural activity or state across time were noted. Figures representing this data have thus not been presented in this thesis.

5.5 Discussion

Behaviour is an important parameter for recognising when the welfare of an animal may be at risk. However, determining which specific behaviours accurately measure welfare can be difficult (Blackshaw, 1986). In the present study, eight different behaviours were examined in order to assess the pain experienced by calves scoop dehorned with and without local anaesthetic (lignocaine) and/or a non-steroidal anti-inflammatory drug (ketoprofen). Of these, lying, grazing/ruminating, tail shaking and ear flicking were distinctly different for at least the first 4-6 hours after treatment in control compared to scoop dehorned calves given no pain relieving drugs. This suggests that these behaviours are useful indices of acute dehorning pain. Behavioural differences between control and dehorned groups were markedly reduced, particularly during the first 4 hours after treatment, when lignocaine and ketoprofen were administered together prior to horn amputation. This was not so evident when either lignocaine or ketoprofen was administered alone prior to dehorning. Thus the present behavioural study supports the recommendation that when local anaesthetic is used to reduce dehorning pain in calves, a simultaneous injection of an anti-inflammatory drug should also be given if improved pain relief is to be achieved.

The high incidence of lying and tail shaking (100%), and low incidence of grazing/ruminating (15%) behaviour in Scoop calves 2 hours after dehorning were the reverse of those elicited in Control calves, who exhibited these behaviours at 0-38% and 100%, respectively. This is consistent with previous studies in which a 90% tail shaking incidence immediately followed cautery disbudding (Taschke and Folsch, 1995), and reduced grazing and increased lying by deer occurred 3-4 hours after velvet antler removal (Pollard *et al.*, 1992). Since immobility and reduced food intake often characterise an animal in pain (Flecknell, 1986; Fraser

and Broom, 1990), the above behaviours indicate (unsurprisingly) that scoop dehorning is indeed a painful experience for calves. Behavioural responses of Control and Scoop calves were significantly different for at least 4-6 hours after treatment, which is comparable to the 5-7 hour plasma cortisol elevation above control levels reported in Chapter's 2 and 3.

The incidences of lying, grazing/ruminating, tail shaking and ear flicking behaviour 2 hours after treatment in dehorned calves given lignocaine were numerically between those of Control and Scoop calves, and significantly different from neither. This could be due to the nerve-block effects of lignocaine wearing off in some calves and not in others, since 2-3 hours represents its maximum duration of nerve-block action (Link and Smith, 1956), resulting in an overall intermediate anaesthetic effect for that group. Lying (100%) and ear flicking (62%) behaviour increased significantly above Control calves at 4 and 6 hours respectively, while grazing/ruminating behaviour (25%) declined at 4 hours, suggestive of increased pain in these animals after nerve-block anaesthesia ended. This is supported by the fact that Scoop calves, who one would intuitively expect to be still experiencing pain at this time, also exhibited significantly different incidences of these behaviours than Control calves at these same times. However, contrary to this was a lack of increased tail shaking in LA2 + scoop calves at 4 and 6 hours.

The incidence of lying (0%), grazing/ruminating (100%), tail shaking (25%) and ear flicking (50%) behaviour in those calves given both lignocaine and ketoprofen prior to scoop dehorning were very similar to those elicited in Control calves, 2 hours after treatment. This implies that pain experienced by these calves, if any, was minimal at this time. The disparity between this result and that reported above for LA2 + scoop calves at 2 hours suggests superior analgesia is achieved when both lignocaine and ketoprofen are given.

Whereas in those calves given ketoprofen alone prior to scoop dehorning, incidences of grazing/ruminating was significantly depressed and of tail shaking was significantly elevated compared with Control and LA2:K + scoop calves at 2 hours, the incidences of neither behaviour were significantly different from those in Scoop calves. Hence, the injection of an anti-inflammatory drug alone appeared to have only partially reduced the behaviours expressed in relative great abundance by Scoop calves 2 hours after dehorning, and hence to have only partially reduced probable pain-

associated behaviour. At 4 hours, lying, tail shaking and ear flicking behaviour in LA2:K + scoop calves was numerically less and grazing/ruminating numerically greater than in Scoop and LA2 + scoop calves. This was less obvious at 6 hours. The conclusion here, albeit a tentative one since these differences reached significance only for tail shaking, is that dual administration of ketoprofen and lignocaine reduced the pain experienced by calves for at least 4 hours after dehorning. Effectiveness of the above analgesic regimes in relieving post-dehorning pain in calves may have been more clearly defined in this study if it had not been constrained by small calf numbers.

The mean incidence of head shaking, foot stamping and grooming throughout the three observation days were low with no significant between-group differences observed. Nevertheless, these behaviours may have been more useful indices of an irritant or noxious effect of dehorning if they had been recorded more frequently than occurred here, because all 17 head shaking calves, 10 of 13 foot stamping calves and only 2 of the 9 grooming calves were scoop dehorned. Disbudding by caustic paste and cautery have been shown previously to be associated with less grooming and more head shaking in calves (Morisse *et al.*, 1995).

Some of the behaviours that were examined did appear to differentiate between pain being present and absent but did not indicate different pain intensities, due to an inherent high reactivity in response to noxious stimuli. Thus the expression of ear flicking and tail shaking behaviour may have been disproportionately higher in the presence of minimal pain, such that dehorned calves given lignocaine and/or ketoprofen could actually have perceived less pain than was indicated by the above results.

Since most behaviours were observed over a one minute period, the problem that a behaviour may be missed, noted as being a limitation with instantaneous recording (Mellor and Murray, 1989; Petrie, 1994), was largely avoided. However, this did mean that within each observation period there was a 55 minute delay between the behavioural recording of the first and the last calf. Thus while the study's primary aim was assessment of treatment effects on calf behaviour, there was potential for time to be an additional complicating variable during the behavioural observations. This confounding effect should have been minimal however, since recording from calves which received the seven different treatments was evenly distributed throughout each 55

minute observation period. This was unknown to the observer at the time who simply recorded the calf behaviour in numerical order. In future studies it would be advisable to have more than one behavioural observer so that recording delays are minimised.

It is also important to note that calf motivation priorities may change over time (Barnard and Hurst, 1996) causing their behaviour to deviate from that normally associated with pain, even if pain is still being perceived. For example, it is commonly reported that cattle tend to exhibit allomimetic (copy-cat) grazing behaviour (Kilgour and Dalton, 1984; Petrie, 1994), which may be expressed even in the presence of pain (Rollin, 1985). Although at 2 and 4 hours a difference in grazing/ruminating and lying behaviour was apparent between control and dehorned calves suggestive of greater pain in the latter, that this difference was absent by 6 hours does not necessarily reflect pain resolution by this time. Rather it could indicate simply that the motivation to alleviate hunger or to conform to behaviour expressed by the herd's majority had overridden that of remaining inactive. This is perhaps supported by the fact that at 6 hours ear flicking and tail shaking in Scoop calves remained significantly elevated above control calves. Hence, in addition to recording gross behaviours such as whether an animal is grazing/not grazing, measurement of the behavioural compromises it may make in order to meet motivational goals (such as area covered while grazing or number of chews before swallowing) could provide a more accurate assessment of the discomfort being experienced, particularly in the later hours following trauma.

Despite the above mentioned interpretative difficulties, there was good agreement between the behavioural assessment and the previously reported physiological assessments (Chapter's 3 and 4) of the overall acute distress experienced by calves following each treatment (Table 5.2). In both studies the responses of scoop dehorned calves given no pain-relieving drugs deviated most strongly from that of pain-free control calves, while the reverse was true for those administered both lignocaine and ketoprofen before dehorning. It is concluded here that from a pragmatic perspective, dual administration of a local anaesthetic and an anti-inflammatory drug is the most effective way of alleviating scoop dehorning pain in calves.

CHAPTER SIX: General Discussion

A number of husbandry procedures which cause pain are carried out on farm animals. Many of these procedures are considered necessary for important long-term hygiene, production, safety and/or welfare reasons. Calf dehorning is no exception, with its purpose being to reduce injury to herd-mates and stock handlers and to reduce carcass bruising (Armstrong, 1985; Stafford and Mellor, 1993; Rollin, 1995). However, this does not imply that animal pain should be an inevitable consequence of these practices, particularly if it can be alleviated in a practical manner.

6.1 Major Conclusions

In the present study the effects of local anaesthetic and/or a non-steriodal anti-inflammatory drug as a means of alleviating calf pain-induced distress,as indicated by plasma cortisol resposnes and/or behaviour, during and in the hours after scoop dehorning were examined. The major conclusions are as follows:

1. Horn amputation using the scoop method is very painful for calves if no pain-relieving drugs are given, causing them significant distress for at least 7 hours, but this distress probably lasts for longer.

2. The distress in calves caused by scoop dehorning is biphasic: an initial marked pain of horn amputation, followed about 2 hours later by inflammatory pain originating from the horn wound area which lasts for at least 5 hours, and probably for longer.

3. Local anaesthetic alleviated calf distress during its period of nerve-block action, including the distress caused by the initial pain of horn amputation. However this benefit was offset by an apparent delaying effect on inflammatory pain which manifested once nerve-block anaesthesia ended.

4. Administration of a non-steroidal anti-inflammatory drug alleviated calf distress during the inflammatory pain-phase, but it did not greatly reduce the initial distress of horn amputation. Whether or not inflammatory pain was experienced by calves after the drug's analgesic action ended is unknown.

5. The usual marked increase in cortisol secretion, a potent endogenous anti-inflammatory substance, which occurs immediately after horn amputation by scoop in calves given no pain-relieving drugs may play an important role in the resolution of inflammatory pain.

The above findings support the following recommendations:

6. Calves should be given an analgesic (eg, anti-inflammatory drug) in addition to the usual local anaesthetic injection prior to scoop dehorning if improved pain relief is to be achieved in these animals.

7. Based on the results of a previous dehorning study (Petrie *et al.*, 1996a), an alternative approach to reducing inflammatory pain in calves after dehorning, is to use a cautery instead of a scoop dehorner.

The benefits of a dual local anaesthetic/anti-inflammatory administrative regime in providing post-surgical pain relief in humans have been recognised for some time (Dahl and Kehlet, 1991; Woolf and Chong, 1993). Perhaps it is not suprising then that the same approach appeared to provide superior pain relief in scoop-dehorned calves compared to the sole injection of either drug, since similar principal biological changes occur in damaged tissue when mammals are subjected to trauma: i.e. an initial excitation of nociceptors immediately after the noxious insult, followed by the local release of numerous inflammatory mediators which sustain nociceptor sensitivity (Benson, 1994; Meller, 1994). It is logical to assume that the principles of pain relief in response to trauma would thus be similar across different mammalian species, noting of course that drug dosages will vary (Davis, 1983). Indeed it is this assumption of inter-species biological continuity which supports the extrapolation to humans of the outcomes of animal pain research.

However, this is not to say that an expected outcome which has hitherto not been examined specifically is an adequate substitute for scientific proof, particularly if it is to be a basis for future practical recommendations. This was emphasised by the present study which began with the intuitive prediction that giving calves local anaesthetic prior to scoop dehorning would lead them to experience less distress than giving them no local anaesthetic. Although this appeared to be true during the initial horn

amputation procedure and subsequent hours of the nerve-block period, it was not apparent beyond this time as evidenced by the initiation of a substantial cortisol response. This response was greater than that at the equivalent time in calves dehorned without local anaesthetic, casting doubt on the drug's ability to reduce the *overall* pain-induced distress following horn amputation. This may be due to the fact that cortisol is a potent anti-inflammatory mediator (Buckwalter, 1995) and its release in calves as part of the distress response to dehorning pain is prevented during nerve-block analgesia. This then could allow for unimpeded progression of the inflammatory reaction at the amputation wound site, thereby enhancing inflammatory pain. This apparent longer-term disadvantage of local anaesthetic administration was an unexpected finding, and may have relevance for other tissue amputation procedures in farm stock (eg, velvet antler removal) as well as for companion animal medicine.

If cortisol is such an important endogenous anti-inflammatory mediator involved in the early resolution of inflammatory pain in dehorned calves as suggested by the results of the present study, then one could be tempted to ask the following two questions: why bother giving calves an exogenous anti-inflammatory drug prior to dehorning, and how can increased plasma cortisol concentrations be considered both an index of pain-induced distress and a possible alleviator of inflammatory pain? Both can be answered by considering the likely initiator of increased cortisol secretion in scoop dehorned calves: i.e. perception of severe head pain. We assume this since pain-free control calves and those dehorned with prior injections of drugs known to provide pain relief in humans do not exhibit a marked cortisol response. Hence, if endogenous cortisol secretion alone were to be relied upon to help reduce inflammatory pain in scoop dehorned calves, practically this secretion must first be initiated by pain, an obviously self-defeating approach.

Likewise, if cortisol release following tissue injury serves a protective function by preventing an overshoot of the inflammatory response within damaged tissue (Munk and Náray-Fejes-Tóth, 1994; Kapula *et al.*, 1995), this does not negate the likelihood of pain perception originating from the injury site being its major cause for release. Indeed, this may be expected teleologically to be the adaptive rationale for the mechanism. The other possibility, that the presence of an injury *per se* evoked cortisol secretion rather than any associated pain was assessed by observing whether or not

cortisol secretion increased in dehorned calves given local anaesthetic during the nerve-block period. No increase was observed, which supports the conclusion that noxious sensory input was the major stimulus to the observed cortisol release in calves. However, the point should be made that this assumes local anaesthetic has no direct inhibitory action on the inflammatory reaction at the horn wound site, an action which in turn may inhibit stimulation of the hypothalamic-pituitary-adrenal axis by inflammatory mediators. If this did actually occur then the absence of pain during nerve-block analgesia would not be why cortisol concentrations were basal during this time. There is little evidence for local anaesthetic having such an action, but the possibility cannot be completely discounted. As the expression of pain-related behaviours in dehorned calves correlated well with the integrated cortisol responses, this supports the usefulness of cortisol as a physiological index of pain-induced distress.

6.2 Experimental Design and Limitations

All calves were dehorned by the same scoop amputation method. Hence, difficulty in assessing animal distress from behavioural responses to two or more types of amputation procedures, when those behaviours are elicited in a procedure-specific fashion, was avoided. This has been noted as an experimental limitation in assessment of lamb distress following ring (characterised by high restlessness) versus knife (immobility) castration (Lester *et al.*, 1996). No physical constraints such as pen confinement were imposed on the study calves, which may have otherwise artificially modified their behaviour. Rather they were free to express their full behavioural repertoire in a large open paddock. However, this did mean that time taken to identify individual animals was extended. Also only one observer was used in the dehorning behavioural study. Hence, differences in the behaviour expressed by individual calves at each observation period may not only be related to the different treatments to which they were subjected, but also to differences in time from treatment. This effect should have been minimised however, since behavioural recording of calves from each of the different groups was evenly distributed throughout each observation period.

The cortisol and behaviour studies were not carried out at the same time. Instead they involved separate animals in separate trials. It cannot be assumed therefore that calves from which behaviour was

recorded experienced similar levels of distress to those blood sampled in the pens, despite the treatments (except one) being the same. It is possible that in the latter animals, repetitive handling and venepuncture may have caused additional distress to that already caused by dehorning pain. Although in lambs following castrating and tailing such an effect could not be detected (Lester *et al.*, 1991b), it is not known whether this is also true for the present dehorning study. However, if indeed there was some exaggeration of the distress responses to dehorning by repeated handling, the responses here will at least represent the worst likely outcomes on the farm (Petrie, 1994).

The effect of blood sampling order on pretreatment cortisol concentrations was examined by regression analysis in the present study. Those calves sampled last did have significantly higher cortisol levels compared to those calves sampled early in the order. However, the actual regression gradient was minor suggesting that the increased distress in calves due to anticipation of human handling was also minor.

A final important consideration with regard to plasma cortisol levels is whether or not true basal pre-treatment levels were obtained at the start of the trial, particularly since these initial concentrations were subtracted from all subsequent cortisol concentrations within each group. If the pre-treatment cortisol concentrations were extremely high in study calves then the reported change in cortisol concentration in response to treatment would be under-estimated. To avoid this calves with abnormally high baseline pre-treatment cortisol values (2 times the standard deviation away from the mean) were excluded from the study. For the majority of calves (117 of a total of 130) pre-treatment values were within normal range (Herd, 1989). Habituating calves to the trial environment during the few days beforehand, such that pen confinement and close human contact were not such a novel experience may have reduced the number of calves with high initial cortisol concentrations. However, this would have then removed the dehorning study from the reality of usual farming practice. It is of interest to note that there was one calf in the study who expressed non-fearful behaviour in the presence of handlers, freely approaching them at will throughout the trial day. This calf had the lowest of all recorded pre-treatment cortisol concentrations (4 ng/ml), thereby supporting the argument that it is an animal's perception of the environment, rather than the environment *per se* which determines whether or not it experiences distress. A similar

example has also been reported in a previous dehorning study (Boandl *et al.*, 1989).

6.3 Practical Considerations

The question of whether or not administration of a local anaesthetic and an anti-inflammatory drug will offer significant relief of acute pain in scoop dehorned calves is not hard to answer. If administered properly all the indications here are that it will. There are however other issues that need addressing, such as the safety, efficacy, practicality, cost, and possible long term detrimental effects of this administrative regime. These issues were not investigated during the present study, except to say that successful injection of both drugs (local anaesthetic subcutaneously around each cornual nerve; ketoprofen intra-venously into the jugular vein) was a quick procedure, rarely taking more than one minute.

It was apparent that in a minority of calves only partial nerve-block analgesia was achieved, as shown by a substantial distress response immediately after scoop dehorning. The occasional ineffectiveness of local anaesthetic in relieving dehorning pain even when administered correctly has been attributed to random branching of the cornual nerve below the point of injection (Dyce *et al.*, 1987). Hence, in large herds alleviation of the initial pain of horn amputation is unlikely to achieve 100% efficacy. That ketoprofen appeared to slightly reduce the initial distress associated with horn amputation adds supports to its use in combination with local anaesthetic in such instances.

6.4 Future Directions

Possible long-term beneficial or detrimental effects of dual local anaesthetic/analgesic administration in dehorned calves may become apparent in future studies if they are extended beyond the period examined here. For instance, improved pain relief may prevent depressions in calf growth-rates through grazing disruption, a problem sometimes evident in the weeks following dehorning (S Wilson, personal communication). Conversely, provision of total analgesia may have long-term negative effects such as increased wound dehiscence in the days following horn

amputation. It would also be of interest to examine the cortisol distress response beyond 9.5 hours to assess whether or not inflammatory pain is evident at the end of ketoprofen's analgesic action, as it apparently was after nerve-block analgesia ended in the present study.

Future alternative approaches to alleviating dehorning pain may include administering inhibitors or enhancers of specific neurotransmitters involved in the pain pathway (Cook, 1995). However, the best solution to prevention of dehorning pain is obviously not having to carry out the procedure itself. There has been some effort put into breeding for polled cattle, but these are perceived to be inferior in desirable production traits to non-polled animals (Rollin, 1995). Advances in genetic engineering may resolve this problem in the future.

6.5 Personal Comments

As an end note one may be forgiven for wondering why in a world where exploitation and pain are common place we should even concern ourselves with whether or not a calf feels sore for a few days or so after having its horns removed. There are of course numerous practical and economic reasons for doing so (Cook *et al.*, 1989; Stafford, 1994), but in terms of considering the animal's well-being *per se* is it of any great relevance, particularly when many people in less fortunate countries are not subject to the same ethical considerations and find survival itself a daily struggle.

It was once written by Albert Schweitzer that *"the world offers us the disconcerting spectacle of a will to live in conflict with itself. One life is sustained at the expense of another. The world is truly nothing but horror in the midst of magnificence, absurdity in the midst of intelligence, suffering in the midst of joy."* (A Schweitzer, 1967). Although ours is an imperfect world, and to have it otherwise is perhaps even an undesirable concept, should we then accept the fact that in the process of living pain is inevitable? Should we accept this even when, as in the case of calf dehorning, we are the direct instigators of pain in other animals? Should we accept this still further in the knowledge that some of this pain may be easily prevented? If we resign ourselves to the belief that all pain is inevitable, we run the risk of becoming complacent in our views with regard to the quality of life of other animals. How then shall we regard ourselves when in doing so we are also losing a

grasp of the basic quality of compassion? If we as a species remain perceptive and sensitive enough during our interactions with other animals, then through our concern for their welfare, perhaps we may progress towards a deeper understanding of ourselves.

References

Animal Welfare Advisory Committee, The Animals Protection Act 1960 and its implications for those responsible for farm animals, Code of Animal Welfare, no. 12, Wellington, N.Z, 1994.

Armstrong JR. Surgical and other husbandry procedures. In: Grazing Animal Welfare. Eds. BL Moore and PJ Chenoweth. Australian Veterinary Association (Queensland Branch), 21-30, 1985.

Axelrod J, Reisine TD. Stress hormones: their interaction and regulation. Science 224, 452-459, 1984.

Ayala A, Wang P, Ba ZF, Perrin MM, Ertel W, Chaudry IH. Differential alterations in plasma IL-6 and TNF levels after trauma and hemorrhage. American Journal of Physiology 260, R167-R171, 1991.

Baddeley C. New Zealand's animal welfare image and its effect on produce and marketing. In: Animal Welfare in New Zealand, Veterinary Continuing Education, 144, 103, 1992.

Barnard CJ, Hurst JL. Welfare by design: the natural selection of welfare criteria. Animal Welfare 5, 405-433, 1996.

Barnett JL, Hemsworth PH. The validity of physiological and behavioral measures of animal welfare. Applied Animal Behaviour Science 25, 177-187, 1990.

Bassett JR, Cairncross KD, King MG. Parameters of novelty, shock, predictability and response contingency in corticosterone release in the rat. Physiology and Behaviour 10, 901-907, 1973.

Bateson P. Assessment of pain in animals. Animal Behaviour 42, 827-839, 1991.

Beaulieu S, Di Paolo T, Barden N. Control of ACTH secretion by the central nucleus of the amygdala. Neuroendocrinology 44, 247-254, 1986.

Benson GJ. Supportive measures during animal pain control. Animal Pain and its Control, Proceedings 226, Postgraduate Committee in Veterinary Science, University of Sydney, 137-139, 1994.

Blackshaw JK. Objective measures of welfare in farming environments. Australian Veterinary Journal 63:11, 361-364, 1986.

Boandl KE, Wohlt JE, Carsia RV. Effects of handling, administration of a local anesthetic and electrical dehorning on plasma cortisol in Holstein calves. Journal of Dairy Science 72, 2193-2197, 1989.

Braga PC, Biella G, Tiengo M. Effects of Tenoxicam on nociceptive thalamic neuronal firing in arthritic rats. Drugs Experimental and Clinical Research XIII:7, 389-398, 1987.

British Veterinary Association Animal Welfare Foundation. Detection and relief of pain. The Veterinary Record 116, 509-511, 1985.

Broom DM. Indicators of poor welfare. British Veterinary Journal 142, 524-526, 1986.

Broom DM. Assessing welfare and suffering. Behavioural Processes 25, 117-123, 1991.

Buckingham JC. Hypothalamic-pituitary responses to trauma. British Medical Bulletin 41:3, 203-211, 1985.

Buckwalter JA. Pharmacological treatment of soft-tissue injuries. Journal of Bone and Joint Surgery 77:A(12), 1902-1914, 1995.

Burchfield SR. The stress response: a new perspective. Psychosomatic Medicine 41:8, 661-673, 1979.

Carter PD, Johnston NE, Corner LA, Jarrett RG. Observations on the effect of electro-immobilisation on the dehorning of cattle. Australian Veterinary Journal 60, 17-19, 1983.

Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. The New England Journal of Medicine 332:20, 1351-1362, 1995.

Cohen RDH, King BD, Thomas LR, Janzen ED. Efficacy and stress of chemical versus surgical castration of cattle. Canadian Journal of Animal Science 70, 1063-1072, 1990.

Cook CJ, Devine CE, Gilbert KV, Maasland SA. Animal welfare research in the meat industry. In: Animal Welfare in New Zealand, Veterinary Continuing Education, 144, 59-68, 1992.

Cook CJ. Pain in farmed animals: awareness, recognition and treatment. In: Farm Animals in Biomedical and Agricultural Research. Australia New Zealand Council for the Care of Animals in Research and Teaching Proceedings, Wellington, August, 75-81, 1995.

Cooper C, Evans ACO, Cook S, Rawlings NC. Canadian Journal of Animal Science 95, 197-201, 1995.

Crookshank HR, Elissalde MH, White RG, Clanton DC, Smalley HE. Effects of transportation and handling of calves upon blood serum composition. Journal of Animal Science 48:3, 430-483, 1979.

Curtis SE, Stricklin WR. The importance of animal cognition in agricultural animal production systems: an overview. Journal of Animal Science 69, 5001-5007, 1991.

Dahl JB, Kehlet H. Non-steroidal anti-inflammatory drugs: rationale for use in severe postoperative pain. British Journal of Anaesthesia 66, 703-712, 1991.

Dantzer R, Mormede P. Stress in farm animals: a need for reevalulation. Journal of Animal Science 57:1, 6-18, 1983.

Dart CM. The basis for the use of steroids/nonsteroidal anti-inflammatory drugs (NSAID) for pain control in clinical practice. Animal Pain and its Control, Proceedings 226, Postgraduate Committee in Veterinary Science, University of Sydney, 351-366, 1992.

Davis LE. Species differences in drug disposition as factors in alleviation of pain. In: Animal Pain: Perception and Alleviation, Edited by Kitchell RL & Erickson HH, American Physiological Society, Maryland, 161-178, 1983.

Dawkins M. The subjective experience of animals. In: Animal Suffering: The Science of Animal Suffering. Chapman and Hall, London, Chapt. 2, 11-26, 1980.

Dawkins MS. Suffering, health and 'productivity'. In: Animal Suffering: The Science of Animal Suffering, Chapman and Hall, London, Chapt. 3, 27-37, 1980.

Dawkins M. From an animal's point of view: motivation, fitness and animal welfare. Behavioural and Brain Sciences 13, 1-61, 1990.

Debruyne D, Hurault de Ligny B, Ryckelynck JP, Albessard F, Moulin M. Clinical pharmacokinetics of ketoprofen after single intravenous administration as a bolus or infusion. Clinical Pharmacokinetics 12, 214-221, 1987.

Dinniss AS. Assessment and alleviation of castration distress in lambs. MSc Thesis, Massey University, 1996.

Dinniss AS, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. Acute cortisol resposnes of lambs to castration using a rubber ring and/or a castration clamp with or without local anaesthetic. New Zealand Veterinary Journal 1997 (submitted).

Duncan I, Dawkins M. The problem of assessing 'well-being' and 'suffering' in farm animals. In: Indicators Relevant to Farm Animal Welfare, Edited by D Smidt, Boston, 13-24, 1983.

Duncan IJH, Poole TB. Promoting the welfare of farm and captive animals. In: Managing the Behaviour of Animals. Edited by P Monaghan & P Wood-Gush, Chapman & Hall, Chapt. 7, 193-232, 1990.

Dyce KM, Sack WO, Wensing CJG. The head and neck of the ruminants. In: Textbook of Veterinary Anatomy, WB Sanders Company, Philadelphia, PA, Chapt. 25, 596-622, 1987.

Fisher AD, Crowe MA, Alonso de la Varga ME, Enright WJ. Effect of castration method and the provision of local anaesthesia on plasma cortisol, scrotal circumference, growth and feed intake of bull calves. Journal of Animal Science 74:10, 2336-2343, 1996.

Flecknell PA. Recognition and alleviation of pain in animals. In: Advances in Animal Welfare Science, Edited by MW Fox, LD Mickley, Martinus Nijhoff Publishers 61-77, 1986.

Flower RJ, Parente L, Parsico P, Salmon JA. A comparison of the acute inflammatory response in adrenalectomised and sham-operated rats. British Journal of Pharmacology 87, 57-62, 1986.

Fraser AF, Broom DM. Farm Animal Behaviour and Welfare, 3rd Edition, Bailliere Tindall, Chapter 9, 79-98, 1990.

Friend TH. Stress: what is it and how can it be quantified? International Journal for the Study of Animal Problems 1:6, 366-374, 1980.

Fukata J, Imura H, Nakao K. Cytokines as mediators in the regulation of the hypothalamic-pituitary-adrenocortical function. Journal of Endocrinological Investigation 16, 141-155, 1993.

Goldstein I. Inflammation: Basic Principles and Clinical Correlates, 2nd Edition, Edited by Gallin J, Goldstein I & Snyderman R, Raven Press, New York, Chapt. 58, 1127-1137, 1992.

Guyton AC. The adrenocortical hormones. In: Textbook of Medical Physiology (8th Ed.) WB Saunders Co. Philadelphia, 842-854, 1991.

Hall LW, Clarke KW. General principles of local analgesia. In: Veterinary Anaesthesia, 9th Edition, Bailliere Tindall, Chapt. 10, 173-188, 1991.

Harrison R. Animal Machines, Stuart and London, London, 1964.

Headley PM. Mechanisms of pain and its control. The Veterinary Record, August 14, 153-155, 1993.

Herd RM. Serum cortisol and "stress" in cattle. Australian Veterinary Journal 66:10, 341-342, 1989.

Higgins AJ, Lees P. The acute inflammatory process, arachidonic acid metabolism and the mode of action of anti-inflammatory drugs. Equine Veterinary Journal 16:3, 163-175, 1984.

Hommeril JL, Bernard JM, Gouin F, Pinaud M. Ketoprofen for pain after hip and knee arthroplasty. British Journal of Anaesthesia 72, 383-387, 1994.

Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 30, 103-114, 1987.

Hutson G. Animal welfare science: a discipline for the future or an ephemeral preoccupation? In: Animal Welfare in the Twenty-first Century: Ethical, Educational and Scientific Challenges, Australia New Zealand Council for the Care of Animals in Research and Teaching, April, Christchurch, New Zealand, 47-52, 1994.

Imura H, Fukata J. Endocrine-paracrine interaction in communication between the immune and endocrine systems. Activation of the hypothalamic-pituitary-adrenal axis in inflammation. European Journal of Endocrinology 130, 32-37, 1994.

Jänig W. Pathophysiological mechanisms operating in reflex sympathetic dystrophy. In: Advances in Pain Research and Therapy Edited by Sicuteri F, Raven Press, New York, 20, 111-127, 1992.

Jephcott EH, McMillen IC, Rushen J, Hargreaves A, Thorburn GD. Effect of electroimmobilisation on ovine plasma concentrations of ß-endorphin/ ß-lipotropin, cortisol and prolactin. Research in Veterinary Science 41, 371-377, 1986.

Kapcala LP, Chautard T, Eskay RL. The protective role of the hypothalamic-pituitary-adrenal axis against lethality produced by immune, infectious, and inflammatory stress. Annals of the New York Academy of Sciences 771, 419-437, 1995.

Karalis K, Crofford L, Wilder RL, Chrousos GP. Glucocorticoid and/or glucocorticoid antagonist effects in inflammatory disease-susceptible Lewis rats and inflammatory disease-resistant Fischer rats. Endocrinology 136:7, 3107-3112, 1995.

Kent JE and Ewbank R. The effect of road transportation on the blood constituents and behaviour of calves. I. Six months old. British Veterinary Journal 139, 228-235, 1983.

Kent JE and Ewbank R. The effect of road transportation on the blood constituents and behaviour of calves. II. One to three weeks old. British Veterinary Journal 142, 131-140, 1986a.

Kent JE and Ewbank R. The effect of road transportation on the blood constituents and behaviour of calves. III. Three months old. British Veterinary Journal 142, 326-335, 1986b.

Kent JE, Molony V, Robertson IS. Changes in plasma cortisol concentration in lambs of three ages after three methods of castration and tail docking. Research in Veterinary Science 55, 246-251, 1993.

Kent JE, Molony V, Robertson IS. Comparison of the Burdizzo and rubber ring methods for castrating and tail docking lambs. Veterinary Record 136, 192-196, 1995.

Kilgour R, Dalton C. Cattle. In: Livestock Behaviour - A Practical Guide. Granada Publishing, London, Chapter 2, 7-53, 1984.

Kitchen H, Aronson AL, Bittle JL, McPherson CW, Morton DB, Pakes SP, Rollin B, Rowan AN, Sechzer JA, Vanderlip JE, Will JA, Clark AS, Gloyd JS. Panal report on the colloquium on recognition and alleviation of animal pain and distress. Journal of American Veterinary Medical Association 191:10, 1186-1191, 1987.

Laden SA, Wohlt JE, Zajac PK, Carsia RV. Effects of stress from electrical dehorning on feed intake, growth and blood constituents of Holstein heifer calves. Journal of Dairy Science 68, 3062-3066, 1985.

Landoni MF, Cunningham FM, Lees P. Pharmacokinetics and pharmacodynamics of ketoprofen in calves applying PK/PD modelling. Journal of Veterinary Pharmacology and Therapeutics 18, 315-324, 1995.

Lapwood KR, Bhagat L, Simpson MP. Analgesic effects on endogenous melatonin secretion. Journal of Pineal Research 22, 20-25, 1997.

Laue L, Kawai S, Brandon DD, Brightwell D, Barnes K, Knazek RA, Loriaux DL, Chrousos GP. Receptor-mediated effects of glucocorticoids on inflammation: Enhancement of the inflammatory response with a glucocorticoid antagonist. Journal of Steroid Biochemistry 29:6, 591-598, 1988.

Lawrence AB. The biological basis of handling animals. In: Practical Animal Handling. Edited by RS Anderson & ATB Edney, 1-13, 1991.

Lay DC, Friend TH, Bowers CL, Grissom KK, Jenkins OC. A comparative physiological and behavioural study of freeze and hot-

iron branding using dairy cows. Journal of Animal Science 70, 1121-1125, 1992.

Lester SJ, Mellor DJ, Ward RN, Holmes RJ. Cortisol responses of young lambs to castration and tailing using different methods. New Zealand Veterinary Journal 39, 134-138, 1991a.

Lester SJ, Mellor DJ, Ward RN. Effects of repeated handling on the cortisol responses of young lambs castrated and tailed surgically. New Zealand Veterinary Journal 39, 147-149, 1991b.

Lester SJ, Mellor DJ, Holmes RJ, Ward RN, Stafford KJ. Behavioural and cortisol responses of lambs to castration and tailing using different methods. New Zealand Veterinary Journal 44, 45-54, 1996.

Liles JH, Flecknell PA. The use of non-steroidal anti-inflammatory drugs for the relief of pain in laboratory rodents and rabbits. Laboratory Animals 26, 241-255, 1992.

Link RP, Smith JC. Comparison of some local anaesthetics in cattle. Journal of the American Veterinary Medical Association 129, 306-309, 1956.

Livingston A, Ley S, Waterman A. Tactile and pain perception. In: Farm Animals and their Environment, Edited by Phillips C & Piggins D, Chapt. 11, 201-208, 1992.

Livingston A, Waterman AE, Chambers JP. Neurological evaluation of effective post-operative pain therapy. Animal Pain and its Control, Proceedings 226, Postgraduate Committee in Veterinary Science, 189-203, 1994.

Manteca X, Deag JM. Individual variation in response to stressors in farm animals: Implications for experimenters. Animals Welfare 3, 213-218, 1994.

Martinsson T, Haegerstrand A, Dalagaard C. Ropivacaine and lidocaine inhibit proliferation of non-transformed cultured adult human fibroblasts, endothelial cells and keratinocytes. Agents and Actions 40, 78-85, 1993.

Mason JW. A review of psychoendocrine research on the pituitary-adrenal cortical system. Psychosomatic Medicine 30:5, 576-607, 1968.

Mason G, Mendl M. Why isn't there one simple way of measuring animal welfare? Animal Welfare 2, 301-319, 1993.

Mastorakos G, Magiakou M, Chrousos GP. Effects of the immune/inflammatory reaction on the hypothalamic-pituitary-adrenal axis. Annals of the New York Academy of Sciences 771, 438-448, 1995.

Matthews LR. Behavioural measures of animal welfare. In: Animal Welfare in New Zealand, Veterinary Continuing Education, 144, 37-42, 1992.

McCormack K, Brune K. Dissociation between the antinociceptive and anti-inflammatory effects of the nonsteroidal anti-inflammatory drugs. Drugs 41:4, 533-547, 1991.

McMeekan CM, Mellor DJ, Stafford KJ, Bruce RA, Ward RN, Gregory NG. Effect of shallow and deep scoop dehorning on plasma cortisol concentrations in calves. New Zealand Veterinary Journal 1997 (In Press).

Meller ST. Mechanisms of hyperalgesia. Animal Pain and its Control, Proceedings 226, Postgraduate Committee in Veterinary Science, University of Sydney, 205-209, 1994.

Mellor DJ and Pearson RA. Some physiological changes in pregnant sheep and goats before, during and after surgical insertion of uterine catheters. Research in Veterinary Science 19, 102-104, 1975.

Mellor DJ and Murray L. Effects of tail docking and castration on behaviour and plasma cortisol concentrations in young lambs. Research in Veterinary Science 46, 387-391, 1989.

Mellor DJ, Murray L. Changes in the cortisol responses of lambs to tail docking, castration and ACTH injection during the first seven days after birth. Research in Veterinary Science 46, 392-395, 1989.

Mellor DJ, Molony V, Robertson IS. Effects of castration on behaviour and plasma cortisol concentrations in young lambs, kids and calves. Research in Veterinary Science 51, 149-154, 1991.

Mellor DJ, Reid CSW. Concepts of animal well-being and predicting the impact of procedures on experimental animals. In: Improving the Well-being of Animals in the Research Environment, Australia New Zealand Council for the Care of Animals in Research and Teaching Proceedings, Sydney, Australia, 3-18, 1993.

Mitchell G, Hattingh J, Ganhao M. Stress in cattle assessed after handling, after transport and after slaughter. Veterinary Record 123, 201-205, 1988.

Minton JE. Function of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system in models of acute stress in domestic farm animals. Journal of Animal Science 72, 1891-1898, 1994.

Moore CM, Desborough JP, Burrin JM, Hall GM. IL-6 and the pituitary hormone response to surgery. Journal of Endocrinology 132 (Suppl.), 207, 1992.

Morisse JP, Cotte JP, Huonnic D. Effect of dehorning on behaviour and plasma cortisol responses in young calves. Applied Animal Behaviour Science 43, 239-247, 1995.

Morton DB, Griffiths PHM. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Veterinary Record 116, 431-436, 1985.

Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine Reviews 5:1, 25-44, 1984.

Munck A, Náray-Fejes-Tóth A. Glucocorticoids and stress: Permissive and suppressive actions. Annals of the New York Academy of Sciences 746, 115-133, 1994.

Owens JG, Kamerling SG, Stanton SR, Keowen ML. Effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in the horse. Equine Veterinary Journal 27:4, 294-300, 1995.

Petrie NJ. Assessment of tail docking and disbudding distress and its alleviation in calves. MSc Thesis, Massey University, 1994.

Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. Cortisol responses to two different methods of disbudding used with or

without local anaesthetic. New Zealand Veterinary Journal 44, 9-14, 1996a.

Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. Cortisol responses to two different methods of tail docking used with or without local anaesthetic. New Zealand Veterinary Journal 44, 4-8, 1996b.

Pollard JC, Littlejohn RP, Johnstone P, Laas FJ, Suttie JM. Behavioural and heart rate responses to velvet antler removal in red deer. New Zealand Veterinary Journal 40, 56-61, 1992.

Raja SN, Meyer RA, Campbell JN. Peripheral mechanisms of somatic pain. Anesthesiology 68:4, 571-590, 1988.

Regan T. The Case for Animal Rights, University of California Press, Berkely, 1983.

Robertson IS, Kent JE, Molony V. Effect of different methods of castration on behaviour and plasma cortisol in calves of three ages. Research In Veterinary Science 56, 8-17, 1994.

Rollin B. Animal pain. Advances in Animal Welfare Science, 91-105, 1985.

Rollin BE. The beef industry. In: Farm Animal Welfare: Social, Bioethical and Research issues. Chapt. 3, 55-72, 1995.

Rushen J. Aversion of sheep to electro-immobilisation and physical restraint. Applied Animal Behavioural Science 15, 315-324, 1986.

Rushen J. Some problems with the physiological concept of 'stress'. Australian Veterinary Journal 63:1, 359-364, 1986.

Rushen J, de Passille AM. The scientific assessment of the impact of housing on animal welfare: a critical review. Canadian Journal of Animal Science 72, 721-743, 1992.

Sandoe P, Simonsen HP. Assessing animal welfare: where does science end and philosophy begin? Animal Welfare 1, 257-267, 1992.

Sanford J, Ewbank R, Molony V, Tavernor WD, Uvarov O. Guidelines for the recognition and assessment of pain in animals. Veterinary Record 118, 334-338, 1986.

Sapolsky R, Rivier C, Yamamoto G, Plotsky P, Vale W. Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. Science 238, 522-524, 1987.

Schweitzer A. Albert Schweitzer: An anthology. Edited by ER Joy, Boston, Beacon Press, 1967.

Semrad SD. Comparative efficacy of flunixin, ketoprofen, and ketorolac for treating endotoxemic neonatal calves. American Journal of Veterinary Research 54:9, 1511-1516, 1993.

Seyle H. A syndrome produced by diverse noxious agents. Nature, 138, 32, 1936.

Shutt DA, Fell LR, Connell R, Bell AK. Stress responses in lambs docked and castrated surgically or by the application of rubber rings. Australian Veterinary Journal 65:1, 5-7, 1988.

Singer P. Animal Liberation: Towards an End to Man's Inhumanity to Animals, Jonathan Cape, London, 1975.

Skjelbred P, Lokken P. Post-operative pain and inflammatory reaction reduced by injection of corticosteroid. European Journal of Clinical Pharmacology 21, 391-396, 1982.

Stafford KJ, Mellor DJ. Castration, tail docking and dehorning - What are the constraints? Proceedings of the New Zealand Society of Animal Production 53, 189-194, 1993.

Stafford KJ. The welfare of dairy cattle in New Zealand: implications for dairy farmers. Dairy Farmer Annual, 33-45, 1994.

Sternberg EM, Hill JM, Chrousos GP, Kamilaris T, Listwak SJ, Gold PW, Wilder RL. Inflammatory mediator-induced hypothalamic-pituitary-adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. Proceedings in the National Academy of Science USA 86, 2374-2378, 1989.

Stephens DB. Stress and its measurement in domestic animals: A review of behavioural and physiological studies under field and

laboratory situations. Advances in Veterinary Science and Comparative Medicine 24, 179-210, 1980.

Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. Cortisol responses to different methods of dehorning in six month old calves. Proceedings of the Physiological Society of New Zealand 13, 21, 1993.

Taschke AC, Folsch DW. Effects of electrical dehorning on behaviour and on salivary cortisol in calves. Proceedings of International Congress of Applied Ethology, 3rd Joint Meeting, Berlin. Edited by M. Nichelmann, HK Nierenga & S Braun, 1995.

Tsagarakis S, Gillies G, Rees LH, Besser M, Grossman A. Interleukin-1 directly stimulates the release of corticotrophin releasing factor from rat hypothalamus. Neuroendocrinology 49, 98-101, 1989.

van Adrichem PWM, Vogt JE. The effect of isolation and separation on the metabolism of sheep. Livestock Production Science 33, 151-159, 1993.

Weaver AD. Bovine Surgery and Lameness. Blackwell Scientific Publications, Oxford, 1986.

Webster J. Man's domain. In: Animal Welfare - A Cool Eye Towards Eden. Blackwell Science Ltd, Oxford, Chapt. 1, 3-16, 1994.

Weilburg V. Prevention of antler growth in deer. MSc Thesis, Massey University, 1996.

Wiepkema PR, Koolhaas JM. Stress and animal welfare. Animal Welfare 2, 195-218, 1993.

Wood GN, Molony V, Fleetwood-Walker SM, Hodgson JC, Mellor DJ. Effects of local anaesthetic and intravenous naloxone on the changes in behaviour and plasma cortisol produced by castration and tail docking with tight rubber rings in young lambs. Research in Veterinary Science 51, 193-199, 1991.

Woolf CJ, Chong M. Preemptive analgesia: Treating post-operative pain by preventing the establishment of central sensitisation. Analgesia and Anaesthesia 77, 362-379, 1993.

Zavy MT, Juniewicz PE, Phillips WA, Von Tungelin DL. Effect of initial restraint, weaning and transport on baseline and ACTH-stimulated responses of beef calves of different genotypes. American Journal of Veterinary Research 53:4, 551-557, 1992.