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The use of the alpha2- agonist xylazine (Rompun®) in a multimodal analgesic protocol for orthopaedic intervention in lateral recumbency on a surgical tipping table in dairy cows

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at University of Veterinary Medicine Hannover

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This work was dedicated to my parents, wife and lovely sons

(Ahmed & Mohammed)

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2. Rizk A., H. Meyer, A. Zaghloul and J. Rehage (2009):
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4. Rizk A., S. Herdtweck, H. Meyer, A. Zaghloul and J. Rehage (2010):
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List of abbreviations

ABP	Arterial blood pressure
ACTH	Adrenocorticotrophic hormone
ALA	After local anaesthesia
BE	Base excess
BL	Baseline
BLA	Before local anaesthesia
BP	Barometeric pressure
BW	Body weight
C_aO_2	Arterial oxygen content
C_cO_2	Pulmonary capillary oxygen content
Cl	Chloride
CNS	Central nervous system
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CRH	Corticotrophin releasing hormone
C_vO_2	Venous oxygen content
DAP	Diastolic arterial pressure
ECG	Electrocardiogram
e.g.	For example
ER	Oxygen extraction ratio
Fig.	Figure
gm	Gram
h	Hour
Hb	Haemoglobin
HBS	Beta hydroxybutrates
HCL	Hydrochloride
$HCO3_s$	Standard bicarbonate concentration
HPA	Hypothalamic-pituitary adrenal axis

HR	Heart rate
I.U.	International unit
IASP	International association for the study of pain
IM	Intramuscular
IV	Intravenous
IVRA	Intravenous regional analgesia
K	Potasium
Kg	Kilogram
l	Litre
LA	Local anaesthesia
LR	Lateral recumbency
LS	Lameness score
MAP	Mean arterial pressure
MEq L^{-1}	milli equivalent in litre
mg	Milligram
min.	Minute
ml	Millilitre
mmHg	Millimeter mercury
mmol	Millimol
n	Number
Na	Sodium
NaCl	Sodium chloride
NC	North Carolina
ND	Not determined
NEFA	Non esterified fatty acids
ng	Nanogram
n.s.	Not significant
OP	Operation
Plac	Placebo
P_aCO_2	Arterial partial pressure of carbon dioxide

P_aO_2	Arterial partial pressure of oxygen
P_vCO_2	Venous partial pressure of carbon dioxide
P_vO_2	Venous partial pressure of oxygen
pH	Logarithm of the reciprocal of hydrogen ion concentration
PCV	Packed cell volume
PLT	Platelets
PIO_2	Inspired partial pressure of oxygen
Q_s/Q_t	Pulmonary shunt volume
RBC_s	Red blood cells
RR	Respiratory rate
RT	Rectal temperature
S_aO_2	oxygen saturation of haemoglobin
SAP	Systolic arterial pressure
SAS	Statistical Analysing System
SD	Standard deviation
SEM	Standard error of the mean
St	standing
Tab	Table
UK	United Kingdom
USA	United State of America
WBC_s	White blood cells
Xyl	Xylazine
α_2	Alpha-2
x	Multiplication
%	Percent
µg	Microgram
C°	Grad celsius
®	Trademark

1 Introduction

The incidence of lameness in high producing dairy cows varies between 15 and 40%. Lameness is beside infertility and mastitis the most common cause for involuntary culling (CLARKSON et al. 1996; WHITAKER et al. 2000). Ninety percent of limb affections in cattle results from claw disorders (CLARKSON et al. 1996). The claw health disorders lead to traumatisation of tissue which is perceived by the affected animal as pain. The pain experience of lame dairy cows results in decreased productivity such as feed intake, fertility and milk yield (RAJALA-SCHULTZ and GROHN 1999; MELENDEZ et al. 2003) as well as it may change animal behaviour (GALINDO and BROOM 2002).

For treatment of lame cows commonly surgical interventions with proper analgesia and restraining are important in order to ensure adequate and safe surgical conditions for the surgeon and the animal as well as for animal welfare reasons. Although restraining is perceived by the treated animal as stress (TAGAWA et al. 1994; PESENHOFER et al. 2006), it is still uncommon to use sedatives for stress alleviation as pre-emptive treatment for cows which are laid down on surgical tipping tables.

Alpha2- agonists have strong sedative, mild analgesic and myo-relaxing effects in cattle. Also it has dose dependent adverse effects such as cardio-pulmonary depression, salivation, reduced rumen motility, ataxia, and increased uterine tone are described (GREENE and THURMON 1988). It has been reported that lateral and dorsal recumbency in adult cattle per se may lead to respiratory depression (WATNEY 1986a; WAGNER et al. 1990; TAGAWA et al. 1994).

Recently, there was a controversial discussion about the use of sedatives in lame cows treated on surgical tipping tables (STARKE et al. 2006; CLUTTON et al. 2007a, b; STARKE et al. 2007a, b). There were controversial arguments pro and con the use of xylazine in cattle layed down on a tipping table. It was finally concluded that, more research is necessary to provide conclusively evidence that α2-agonists like xylazine alleviate stress and that adverse effects are mild or even negligible.

Thus, the presented experimental studies, aimed to investigate the effects of pre-emptive xylazine treatment on cardiovascular, respiratory, hormonal, metabolic and behavioural stress responses in healthy dairy cows undergoing painless claw trimming in lateral recumbency on a surgical tipping table. A clinical study was performed to clarify the effects of xylazine in a multimodal analgesic protocol on stress and short-term analgesia during surgical treatment of claw disorders in lateral recumbency on a surgical tipping table of lame dairy cows.

2 Review of literature

2.1 Pain

2.1.1 Definition of pain

Pain has been defined by the International Association for the Study of Pain (IASP) as ‘’an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage’’ (MERSKEY 1983). However, this definition has not been accepted as being relevant to non-verbal animals. Therefore, (ZIMMERMANN 1986) modified this definition in animals as the pain is an aversive sensory experience that elicits protective motor actions, results in learned avoidance and may modify species-specific traits of behaviour, including social behaviour. While MOLONY and KENT (1997) defined pain as an aversive sensory and emotional experience representing awareness by the animal of damage or threat to the integrity of its tissues, it changes the animal’s physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery. Pain is a complex phenomenon involving patho-physiological and psychological components that are frequently difficult to recognize and interpret in animals (ACVA 1998).

There are many similarities between animals and humans regarding pain perception (MORTON and GRIFFITHS 1985). Indeed, many of the pain management strategies in humans were developed on the basis of animal models. Since animals cannot communicate verbally, the term nociception frequently has been used in animals instead of pain (SANHOURI et al. 1992).

2.1.2 Physiologic (nociceptive) pain

Nociception is the activity generated in the nervous system in areas where it is not processed by the consciousness and it refers to the neurophysiologic processes whereby, noxious stimuli are transduced, transmitted, modulated, projected and perceived (MUIR and WOOLF 2001; ANDERSON and MUIR 2005). Noxious, mechanical ,chemical, or thermal stimuli are transduced into electrical signals (action

potentials) by high threshold pain receptors (nociceptors) located on the peripheral terminals of thin, myelinated C and A delta nerve fibers (JENKINS 1987). The nerve action potentials are transmitted centrally to the superficial layers of the dorsal horn of the spinal cord where they are modified (modulated) by local and descending inhibitory neurons and projected to the brain (perception) (MUIR and WOOLF 2001; ANDERSON and MUIR 2005).

2.1.3 Pathologic pain

Pathologic pain is produced by tissue or nerve damage and involves the development of peripheral sensitization, central sensitization, structural reorganization of neural elements within the central nervous system (CNS) and disinhibition (Fig.1). Injury of tissues releases inflammatory mediators such as prostaglandin's (ZIMMERMANN 1986), leukotriens (LEMKE 2004), histamine and bradykinin (JULIUS and BASBAUM 2001; LEMKE 2004) which stimulate nociceptors in nearby nerve endings (RANG et al. 1991). Therefore, a stimulus affecting a relatively small number of nerve endings stimulates many more, effectively amplifying the sensations. The resulting impulses are conducted via the ventro-lateral part of the spinal cord to the brain stem and thalamus, whereas there is further amplification called wind-up. Conscious perception of pain is a result of activation of certain areas of the cerebral cortex via the thalamus. Theoretically, pain is a central conscious experience that is due to nociception in peripheral nerves (WOOLF and CHONG 1993; OTTO and SHORT 1998).

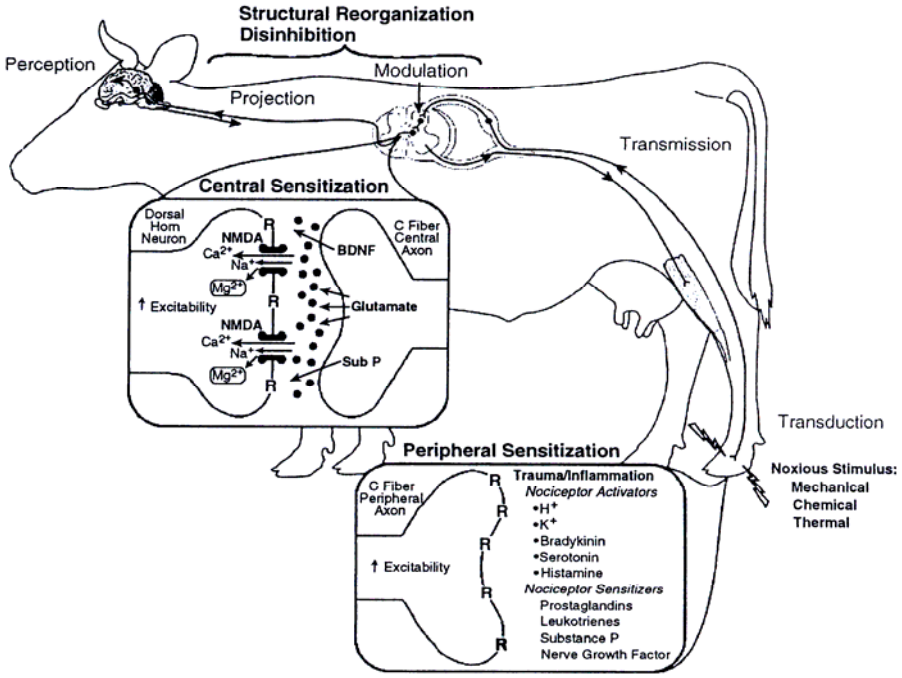

Fig.1: Physiologic (nociceptive) pain. A noxious stimulus is transduced, transmitted, modulated, projected and perceived. The brain also generates responses that travel via descending nerve pathways that facilitate or inhibit (modulate) sensory input to the spinal cord. Pathologic pain peripheral sensitization, central sensitization, structural reorganisation and disinhibition are several of the more prominent mechanisms responsible for the development of moderate, severe and chronic pain conditions. BDNF (brain-derived neurotrophic factor); NMDA (N-METHYL-D-aspartate) (ANDERSON and MUIR 2005).

Tissue injury results in acute pain, which stimulates the muscular action to avoid the noxious stimulus caused by either reflex limb flexion or conscious mechanisms thus activating the autonomic nervous system and leading to a heightened state of arousal. Increased sympathetic tone can become persistent if the insult is prolonged or severe (WOOLF and MAX 1991). Chronic pain is a pain which has persisted beyond normal tissue healing time, the presence of high levels of inflammatory mediators around the site of injury and persistent activation of pain fibre pathways in the spinal cord lead to decrease in pain threshold, however that stimuli are perceived as being more painful than would be normal for the individual concerned, this is termed hyperalgesia (LEY et al. 1995; WHAY et al. 1998). Another phenomenon associated with chronic pain is allodynia, in which similar mechanisms lead to normally non-painful stimuli being perceived as painful. Prevention and modulations

of hyperalgesia and allodynia are some of the main objectives of analgesia (LEY et al. 1991; OWENS et al. 1995), being in particular important, in the chronically lame cow that over time perceives the lesion as being more painful than it was initially (hyperalgesia) and perceives pain in undamaged surrounding tissues in touch (allodynia) (BLEY et al. 1998; OTTO and ADAMS 2005; HUDSON et al. 2008).

2.2 Stress and distress

Pain is responsible for stress and can lead to distress (CLARK et al. 1997). Stress has been defined as "a state that occurs when an animal is required to make abnormal or extreme adjustments in its physiology or behaviour in order to cope with adverse aspects of its environment and management" (FRASER et al. 1975), while CLARK et al. (1997) defined stress as an adaptive pattern of behavioural, neural, endocrine, immune, haematological and metabolic changes directed toward the restoration of homeostasis. The stress response prepares the animals for an emergency reaction (MELLOR et al. 2002). Acute pain induces a significant stress response and leads to changes in the autonomic nervous system and in hormonal secretion. It initiates secretions of glucocorticoides (primarily cortisol) and a catabolic state with changes in plasma metabolites increased sodium and water level and altered carbohydrates and protein metabolism (CHAPMAN and GAVRIN 1999; DESBOROUGH 2000). The maintenance of normal homeostatic balance to pain is by negative feed back mechanism that acts at multiple levels within the brain and sympatho-adrenal and hypothalamic-pituitary adrenal (HPA) axes (MELLOR et al. 2002), thereby elevating catecholamine and glucocorticoids which enhances cardio-respiratory, hormonal, metabolic and all indicators of stress responses (Fig.2; MINTON 1994; CHAPMAN and GAVRIN 1999; ALI et al. 2006).

Increases in corticotrophin-releasing hormone (CRH), plasma cortisol, and vasopressin often directly correlate with the stressful or painful event and help to restore homeostasis (SANFORD and SCHLICHER 1986; ANIL et al. 2002). The major stress alterations are enhanced secretion of glucocorticoids and increased sympathetic nervous system activity. Synchronised control of hypothalamic releasing hormones of ACTH and of catecholamine, results in biochemical and physiologic

manipulations of stress (WOOLF and MAX 2001; ALI et al. 2006). In cattle numerous manipulations including transport (LOCATELLI et al. 1989), therapeutic interventions (NAKAO et al. 1994), and surgery (FISHER et al. 2001) increased secretion of cortisol from the adrenal cortex. Metabolic stress responses included e.g. plasma concentrations of non-esterified fatty acids (NEFA), ß-hydroxybutyrate, glucose, lactate (MUDRON et al. 1994a), triglycerides and cholesterol (TASCHKE and FOLSCH 1997).

Assessment of stress is an essential aspect of animal welfare and management (MOBERG 2000). Nociceptive stimulation caused by surgery initiate an endocrine – metabolic stress response resulting in ACTH release which in turn increased cortisol secretions from adrenal cortex (MARTIN et al. 2001). It has been reported that, the level of cortisol release is influenced by the severity of the surgical trauma (DESBOROUGH 2000).

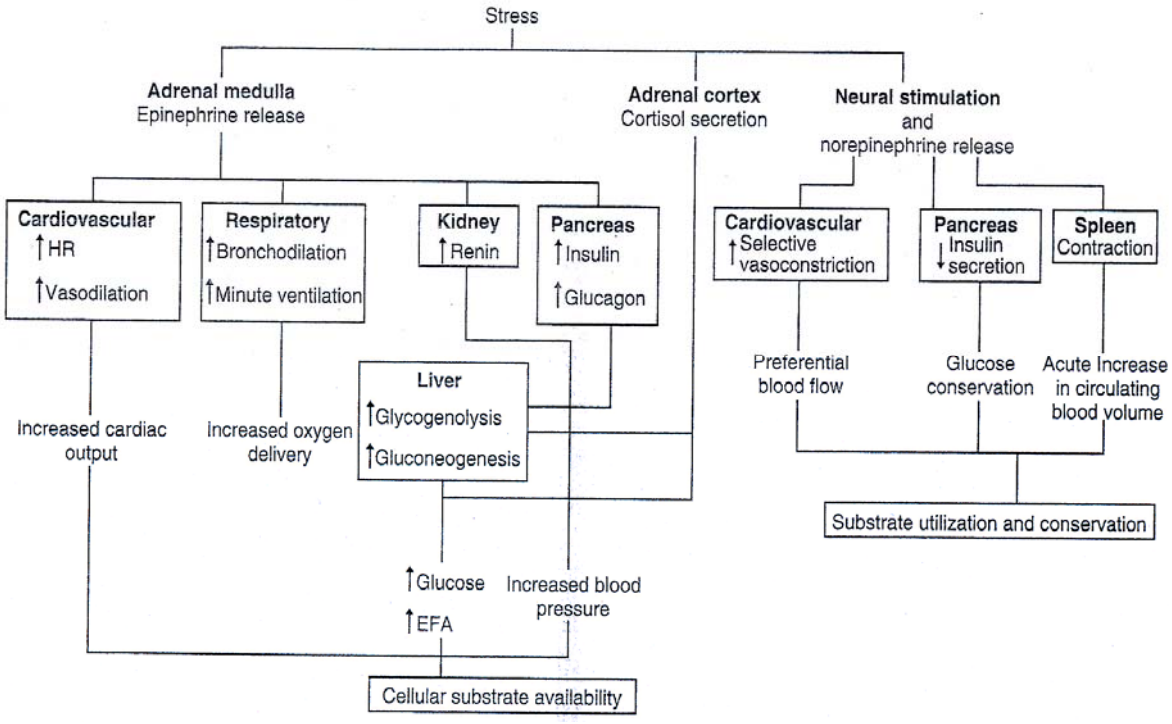

Fig.2: Diagrammatic representation of the stress response according to WAGNER (2009).

2.3 Effects of stress and pain on the body systems

2.3.1 Cardio-respiratory parameters

There is a long history of using physiological variables in assessing pain and distress (GREGORY 2004). Measures include responses of the sympathetic-adrenomeduallary system, such as changes in heart rate due to release of nor epinephrine, and responses of HPA system, such as concentration of cortisol, ACTH and CRF. In principle at least, these measures may be particularly useful in prey species such as cattle that are considered stoic and unlikely to show pronounced behavioural responses to pain until injurious are advanced (WEARY et al. 2006).

Stress and pain response induce activation of the sympathetic autonomic nervous system, thus increased release of catecholamine from adrenal medulla, this leads to increase in respiratory rate, blood pressure, heart rate and myocardial activity with a simultaneous increase in myocardial oxygen consumption as well as peripheral vasoconstriction (MELLOR and STAFFORD 1999; HUDSON et al. 2008). Furthermore, the effect on the respiratory system appears in the form of reduced and shallow breathing as well as polypnea (MORTON and GRIFFITHS 1985).

2.3.2 Endocrine parameter (cortisol)

It is recognised that certain environmental or management conditions termed stressors, to which animals are exposed, induce alterations in the HPA axis and activate the sympathetic nervous system. The secretion of ACTH, the pituitary hormone controlling steroidogenesis in the adrenal cortex, is increased, thereby increasing the secretion of cortisol (DICKSON, 1993; RANHEIM et al. 2000; HUGHAN et al. 2001), while stimulation of the sympathetic nervous system increases the production and release of catecholamine from the central and peripheral nervous system. An increase in cortisol or catecholamine levels has been regarded as being indicative of stressful conditions (MINTON 1994).

Cortisol concentrations in blood are used widely to study HPA activity. This presents some problems related to the invasiveness of blood sampling techniques (STEWART 2008) and alternatives have been used (e.g., urine, saliva, faeces, milk). In addition,

the increase in the cortisol plasma level as a response to a noxious stimulus reflecting the activation of the HPA axis (STAFFORD et al. 2003; DOHERTY et al. 2007) may not persist for the duration of the effects of the stressor and thus may reflect the overall impact of the stressors on the animals (MORMEDE et al. 2007). However, increases in plasma cortisol concentrations that are evident in animals submitted to stressful and painful procedures are considered to be indicative of pain (MORTON and GRIFFITHS 1985; STAFFORD et al.2002; ZULAUF et al. 2003; STAFFORD and MELLOR 2005). Also lameness expressing pain due to orthopaedic disorders in sheep and cows led to increased plasma cortisol levels (LEY et al. 1991; EL-GHOUL and HOFMANN 2002). Although the concentration of cortisol in the plasma is widely used as an indicator of stress, caution is advised because an increase does not occur with every type of stressor and because a wide variety of stressors can activate the HPA axis (BROOM 1991; MOLONY and KENT 1997).

2.3.3 Metabolic stress response

Stress leads to activation of the HPA system and the release of neuroendocrine mediators such as ACTH, cortisol, catecholamine, glucagon, rennin, aldosterone, anti-diuretic hormone (ADH) and growth hormone (GH) and thereby to changes in the whole body metabolism (MELLOR and STAFFORD 1999; ANIL et al. 2002). This includes changes in water and electrolyte balance and catabolic metabolism with increased glycogenolysis, gluconeogenesis, lipolysis and proteolysis that in turn lead to hyperglycemia and increased plasma concentration of lactate, glycerine and non-esterified fatty acids (NEFA) (MUDRON et al. 1994b; MELLOR and STAFFORD 1999; WATERMAN-PEARSON 1999; CAMBRIDGE et al. 2000). An increase in lactate level in blood was found in lame cows due to claw affections (EL-GHOUL and HOFMANN 2002), after abdominal surgery (MUDRON et al. 2005) and during transport of cattle (CHACON et al. 2005). MUDRON et al. (2005) showed that, the blood concentration of NEFA after abdominal surgery in cattle was increased. While, LEY et al. (1993) observed no significant differences in the levels of NEFA between healthy and lame sheep. Release of catecholamine and glucocorticoids in response to stress and painful stimuli will increase gluconeogenesis, induces insulin

resistance, and reduced whole body glucose consumption, which in turn explains the increase in blood glucose levels (CHACON et al. 2005).

2.3.4 Behavioural signs

The assessment of animal pain is more complex and we have to learn to recognize the signs of pain, which involves both behavioural and physiological responses (BUFALARI et al. 2007). Therefore, behavioural changes are possibly the most commonly used indicators of pain or discomfort in animals (LOEFFLER 1993; O'CALLAGHAN 2002). The presence of more than one behavioural indicator of pain (postures, facial expressions and vocalizations) helps to confirm pain (LOEFFLER 1990). Signs of pain in cattle include loss of appetite, loss of weight, sharp decrease in milk yield and cessation of grooming (DOBROMYLSKY et al. 2000; GEORGE 2003) and with severe pain turning the head to look at the affected area and kicking at the affected area, tooth grinding, head pressing as well as a general reluctance to move. Pain specifically associated with the locomotion system may result in obvious lameness. This may not be seen if limb pain is bilateral, however in cattle with bilateral hind limb pain the back may be arched and the gait stilted as the animal attempts to transfer more of its weight to the forelimbs (DOBROMYLSKY et al. 2000; O'CALLAGHAN 2002). Vocalisations in cattle in response to pain include grunting and bellowing (GEORGE 2003). Rapid shallow respiration may be noted in individuals with severe pain. Non-specific signs of chronic pain may include loss of appetite, change in personality, alterations in urinary or defecatory activity and soiling associated with reduced grooming (FLECKNELL 2000; GEORGE 2003). There are many factors that influence the behavioural responses to pain, such as the location of the pain and the type of tissues involved, the severity of pain either acute or chronic pain. However, the measurement and interpretation of behavioural responses can be difficult and the variation in responses can be misleading (STEWART 2008).

2. 4 Pain management strategies

2.4.1 Pre-emptive analgesia

Pre-emptive analgesia refers to the application of analgesics before the patient is exposed to noxious stimuli (ANDERSON and MUIR 2005). It is a useful approach to prevent a peripheral and central sensitization of the CNS during the surgical procedures and consequently, it can prevent the increased experience of animal to pain and negative effects on the body but it cannot eliminate postoperative pain (ACVA 1998; VALVERDE and GUNKEL 2005; HUDSON et al. 2008). Pre-emptive analgesic techniques include the use of anaesthetic pre-medication such as opioids, α2-agonists, nonsteriodal anti-inflammatory drugs (NSAIDs) or the pre-surgical epidural administration of local anaesthetics or opioids (ACVA 1998).

2.4.2 Multimodal (Balanced) analgesia

Multimodal analgesia is currently recommended for effective post-operative pain control, it is achieved by combining different analgesics that act by different mechanisms (opioids, NSAIDs, α2-agonists and local anaesthetics) resulting in additive or synergistic analgesia, lower total doses of analgesics and minimal side effects (BENSON and THURMON 1987; ACVA 1998). The use of multimodal analgesia may decrease the recorded pain score and the requirement for postoperative analgesics in different surgical procedures (BENSON and THURMON 1987; JIN and CHUNG 2001; UNDERWOOD 2002).

2.5 Pain management in cattle

Pain medications can work in various ways depending on the drug being used (FIERHELLER 2009). Pain therapy can decrease pain by acting at the site of injury, the sensory nerve and spinal cord or in the brain (Fig.3; PEARSON 2007; FIERHELLER 2009). Where the activation of nociceptors after transmission of the information is perceived as pain (ANDERSON and MUIR 2005).

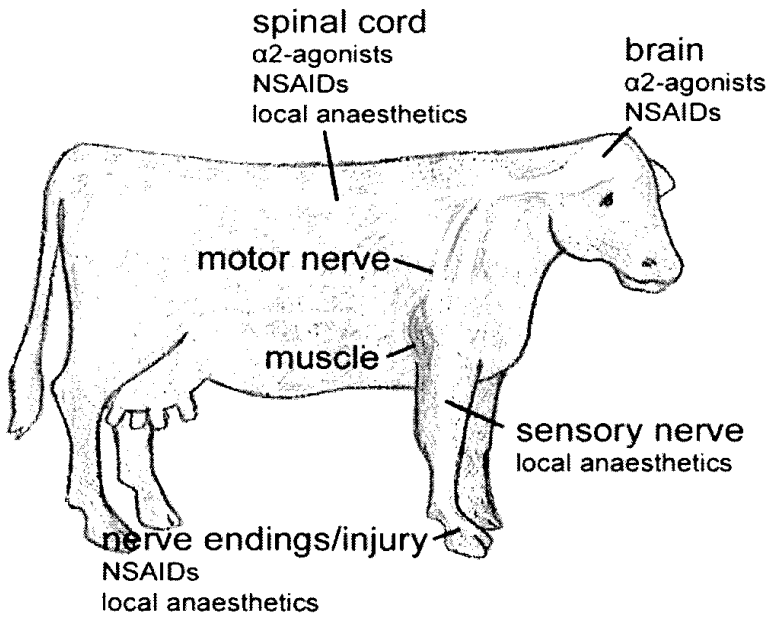

Fig.3: Pain medications in cattle act on different levels of the pain pathway according to (FIERHELLER 2009).

2.5.1 Opioids

Opioids used in veterinary practice are buprenorphine, butorphanol, fentanyl, meperidine, methadone, morphine and oxymorphone (FLECKNELL 2000). They have a short duration of action and in ruminants have disappointing analgesic properties compared to other species, so they are not widely used in food animals as they are mostly not approved (PEARSON 2007; FIERHELLER 2009). Furthermore, they can inhibit rumen-reticular contractions, and some cause abnormal behaviours such as propulsive walking, hypersensitivity and hyper-excitability, which can be dangerous for animals and personnel (FLECKNELL 2000). Butorphanol is the most widely used opioid in food animal practice; the recommended dose is 0.05 $mgkg^{-1}$ subcutaneously every 4-6 hours as well as it may be indicated for short term analgesia for acute and severe post-operative pain (LASCURAIN et al. 2006). It was reported that appetite is increased following its administration (PEARSON and MELLOR 1975; NAVARRE 2006).

2.5.2 Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for their analgesic, antipyretic and anti-inflammatory properties. They produce therapeutic effects through inhibition of cyclo-oxygenase enzymes (COXs) which is necessary for prostaglandin synthesis during the inflammatory response (ANDERSON and MUIR 2005). Two types of COX enzymes are commonly recognised, ideally NSAIDs should be more specific for COX-2 than COX-1 in order to spare most of homeostatic functions, and it produces analgesic effects by central mechanisms (VALVERDE and GUNKEL 2005). Flunixin meglumine is one of the strongest cyclo-oxygenase inhibitors, it has analgesic, antipyretic and anti-inflammatory activity and it is suitable especially for musculoskeletal pain and intestinal spasm (ANDERSON et al. 1990). REID and NOLAN (1991) described that flunixin is an effective analgesic drug for treatment of mild and moderate acute pain caused by trauma and surgery as well as it works synergistically in combinations with opioids. The plasma half-life of flunixin in adult cattle is 8 hours (HARDEE et al. 1985). Meloxicam is a new NSAID due to its binding properties, inhibits COX-2 enzymes, it is used to support the antibiotic therapy in respiratory diseases of calves and cattle (SMITH et al. 2008). Meloxicam reduced the physiological stress response to dehorning until 24h postoperative (HEINRICH et al. 2009). Ketoprofen is used as antipyretic, analgesic and anti-inflammatory drug to treat acute mastitis and diseases of the musculoskeletal system as well as inhibition of both centrally and peripherally effects of pain (WHAY et al. 2005). Carprofen has antipyretic, analgesic and anti-inflammatory properties and it has stronger analgesic effect than phenylbutazone although the mechanism of action in cattle is not clear yet (LUDWIG et al. 1989; CHENG et al. 2002). Recently, carprofen was used in a multimodal protocol for pain management in cattle and it has a positive effect on wellbeing in adult cows (LIST 2009) and calves (SCHULZE 2009) after abdominal surgery.

2.5.3 Local anaesthetics

Local anaesthetics, such as lidocaine and procaine, are the most commonly used pre-emptive analgesic drugs in food animal practice as they are easily administrated, inexpensive and have limited toxicity in cattle (WEARY et al. 2006; WREN 2008).

After dissociation in an alkaline environment they act by inhibiting sodium channels to impede nerve conduction by preventing depolarization of the nerve fiber. In infected tissues, quality of local anaesthesia is often poor because the relatively more acidic environment prevents dissociation (FIERHELLER 2009).

The effects of local anaesthetics (procaine HCL) as regional intravenous analgesia during claw treatment in cattle are supposed to last for about 60 minutes, after this time sensation gradually return to the foot and thus pain (O'CALLAGHAN 2002). Therefore, it is advisable to use NSAIDs in addition to local anaesthetics as it has been shown that its use modulates the level of hyperalgesia after treatment (LIVINGSTON 1994; WHAY et al. 1998).

2.5.4 Alpha 2-adrenoreceptor agonists (α2-agonists)

It is well known that α2-agonists have moderate analgesic, myo-relaxing and strong sedative effects in cattle (CLARKE and HALL 1969; RUCKEBUSCH and ALLAL 1987; PICAVET et al. 2004). They can provide deep sedation and moderate analgesia in cattle. They should not be used for the provision of longer term analgesia (HUDSON et al. 2008). Alpha2-agonists include xylazine, detomidine, romifidine and medetomidine. Xylazine and detomidine are the only licensed drugs in food animals (HUDSON et al. 2008). Dose dependent adverse effects such as cardio-respiratory depression, salivation, reduced rumen motility, ataxia, and increased uterus tonus are described for α2-agonists (CLARKE and HALL 1969; HOPKINS 1972; CAMPBELL et al. 1979; YOUNG 1979; RUCKEBUSCH 1983; RUCKEBUSCH and ALLAL 1987; GREENE 2003; PICAVET et al. 2004).

Alpha-2 agonists produce CNS depression by stimulating both pre-synaptic and post synaptic $\alpha 2$ -adrenoceptors in the CNS and peripherally decreasing nor-epinephrine release centrally and peripherally as well as reducing ascending nociceptive transmission, the net result is a decrease in CNS sympathetic out flow and a decrease in circulating catecholamine and other stress–related substances (MAZE and TRANQUILLI 1991; NANNARONE et al. 2007).

Alpha2-agonists produce dose dependent sedation, analgesia and muscle relaxation, are practical to administer as effective volumes are usually small and can be injected intramuscularly or intravenously (TRANQUILLI and BENSON 1992). In addition, their effects are reversible with specific antagonists, such as yohimbine, tolazoline and atipamezole (HSU et al. 1989). In ruminants, reversal may be indicated to shorten time of recumbency and to minimise the risk of associated complications (HSU et al. 1989).

Alpha2-agonists profoundly alter cardiovascular function by producing bradycardia, hypertension followed by hypotension, decreased myocardial contractility and perfusion and arrhythmias as well as mild respiratory depression (TAYLOR 1999). They decrease gastrointestinal motility, depress thermoregulation and increase myometrial activity of the pregnant uterus and xylazine may cause abortion when given to cattle in late pregnancy (MAZE and TRANQUILLI 1991).

2.5.4.1 Xylazine hydrochloride (Rompun®, Bayer)

Xylazine hydrochloride ([2-(2, 6-dimethylphenylamino)-4H-5, 6-dihydro-1, 3-thiazine hydrochloride], Rompun®; Fig.4) is a thiazine derivative first synthesized in Germany in 1962 for use as an antihypertensive agent. It was used in veterinary medicine for sedation, analgesia, and muscle relaxation and for anaesthetic pre-medication. It's available as 2% solution for intravenous (IV) and intramuscular (IM) injection (CLARKE and HALL 1969; HALL et al. 2001).

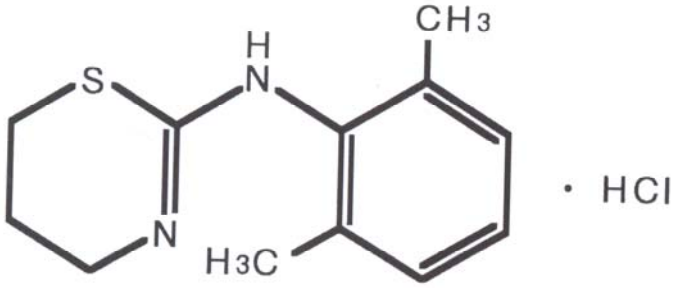

Fig. 4: Chemical structure of xylazine hydrochloride

Xylazine has been used alone or in combination with other agents such as ketamine, acepromazine, pentobarbital, halothane, morphine, butorphanol and guaifenesin in dogs (CLARKE 1982; TRANQUILLI et al. 1984), horses (CLARKE and HALL 1969; MUIR et al. 1977), ruminants (HOPKINS 1972) and swine (THURMON et al. 1986). Among the domestic animals, cattle are most sensitive, whereas the pig is quite resistant to xylazine. In cattle, the degree of sensitivity varies among breeds, Brahmans evidently are the most sensitive breed followed by Hereford, Jerseys, Holsteins and Angus (THURMON et al. 1999).

2.5.4.1.1 Pharmacodynamics

Xylazine HCL is a potent α2-agonist and is structurally related to clonidine, an antihypertensive agent recommended for human therapy. The primary effects following IV or IM administration in mice, rats, rabbits, dogs, and cats as well as in cattle, horses, sheep, goats and pigs were sedation, analgesia, and muscle relaxation (DOHERTY et al. 1986; NOLAN et al. 1986). A single IV or IM administration produced short lived increases of arterial blood pressure followed by a long period of hypotension and bradycardia. Mydriasis, impaired thermoregulation, respiratory depression (cattle, horses, cats, and dogs), hyperglycemia, hypoinsulienmia, polyuria, prolonged intestinal transit time, inhibition of rumen reticular contractions, salivation (sheep and cattle) and emesis (dogs and cats) were also observed. In horses, effects of xylazine persisted for 2 - 4 hours whereas in ruminants some effects (hyperthermia, hyperglycemia, ruminal atony and prostration) persist as long as 12-24 hours or up to 36 hours (GREENE and THURMON 1988). Cattle being the most sensitive species require approximately 1/10 of the dose used to induce an equivalent sedative state in horses, dogs and cats (KUMAR and THURMON 1979; GREENE 2003).

2.5.4.1.2 Pharmacokinetics

Xylazine radioactivity in blood plasma reached its peak in the first 1.5 hours after intramuscular injection of 0.33 mg kg^{-1} BW in three male calves and one dairy cow. Total excretion of radioactivity in urine and faeces was 68, 86, 83 and 100% at 10, 24, 48 and 72 hours respectively (MURPHY and JACOBS 1975). In another study,

five calves and four lactating cows were administered a single intramuscular dose (0.3 or 0.6 mg kg^{-1} BW) of xylazine. Maximum concentrations of xylazine were achieved in blood 20 minutes after dosing. These were 0.04 mg L^{-1} for the first dose (0.3 mg kg^{-1} BW) and 0.06 mg L^{-1} for the second dose (0.6 mg kg^{-1} BW). No xylazine was found in blood 8 hours after administration (TAKASE et al. 1976). Urinary excretion of xylazine was studied in three cows, two cows were administered an IM dose of 0.2 mg kg^{-1} BW and one was administered an IM dose of 0.5 mg kg^{-1} BW. Less than 1% of the dose was excreted unchanged in the urine. Unchanged xylazine was no longer detectable 6 hours following administration. Metabolites were no longer detected in urine 10 hours after administration. The limit of detection for unchanged xylazine was 1-5 µg L^{-1} (PÜTTER and SAGNER 1973).

2.5.4.1.3 Adverse effects

The most common side effect occurring frequently with xylazine is an initial hypertension (due to peripheral postsynaptic adrenoreceptors causing vasoconstriction), which results in a baroreceptor-mediated reflex bradycardia. As the peripheral effects diminish, central α2-actions lead to decreased blood pressure and cardiac output (HALL et al. 2001; EMAMI et al. 2007). Other side effects include short term atrio-ventricular block (most often first or second degree), sinus arrhythmia, decreased respiratory rate, vomiting, increased urine output, transient hyperglycemias (due to inhibition of insulin secretion) and increased myometrial tone and intrauterine pressure (GUARD and SCHWARK 1984; TAKASE et al. 1986).

2.5.4.1.4 Toxicity

Cardiac arrhythmias, hypotension, and profound CNS and respiratory depression may occur as a result of an accidental overdose of xylazine (TAKASE et al. 1986). Seizures have also been reported after overdoses. Tolazoline or atipamezole are alpha-blocking agents and are used as antidotes to reverse xylazine effects (Hall et al. 2001).

2.5.4.1.5 Dosage

The dose range commonly recommended for IM administration in cattle is 0.05 mg kg^{-1} BW for mild sedation with slight decrease in muscle tone, 0.2 mg kg^{-1} BW for moderate sedation with marked decrease in muscle tone and some analgesia (animals mostly remain standing) and 0.3 mg kg^{-1} BW for deep sedation with further decrease in muscle tone and a moderate degree of analgesia (the animal lies down in sternal position). The IV dosages are about ½ of the IM dosage to achieve same effects as with IM administration (GREENE and THURMON 1988; HALL et al. 2001; LIN and RIDDELL 2003).

Cows should not be disturbed until xylazine has taken its full effect. The onset of action develops in approximately 10 to 15 min after intramuscular administration and within 3 to 5 min following intravenous administration and it persists for approximately 45-60 min depending on the dosage rate used. Induction and recovery are commonly smooth (GREENE and THURMON 1988). As xylazine has a wide margin of safety, increasing the dose does not increase the degree of sedation but rather the duration of effect (EMAMI et al. 2007; BANI-ISMAIL et al. 2010).

2.5.4.1.6 Antagonists

It has been known that yohimbine is an effective antagonist for reversing the effects of xylazine induced sedation in a variety of animal species (GUARD and SCHWARK 1984; DOHERTY et al. 1986). Yohimbine improves blood gases and respiratory depression associated with xylazine in sheep (DOHERTY et al. 1986). ROMING (1983) observed that, tolazoline is a proper antagonist of xylazine sedation in cattle without any harmful effect. Additionally, most of adverse effects of xylazine can be minimised by the use of tolazoline as a reversal agent, allowing cattle to be aroused and safely stand within minutes of administration (TAKASE et al. 1986; POWELL et al. 1998).

Atipamezole HCL is the most potent and selective α2-adrenoceptors antagonist currently known (VIRTANEN 1989). Since atipamezole was liciend in farm animals, it is considered effective in reversing xylazine induced sedation in cattle (HALL and

CLARKE 1983), calves (Rioja et al. 2008) dogs (JARVIS and ENGLAND 1991) and ponies (LUNA et al. 1992). It is also reversing the effects of medetomidine and medetomidine–ketamine in cattle and horses (RAEKALLIO et al. 1991).

2.5.4.1.7 Effects of xylazine (Rompun®, Bayer)

2.5.4.1.7.1 Sedative and hypnotic effect

The sedative effects of α2-agonists xylazine in cattle and horses were described by HALL et al. (2001) and LIN and RIDDELL (2003). The behavioural events in cattle are initially, lowering the head, drooping eyelids, salivation, bloat as well as ataxia. Additionally, there is generally a reduced awareness of the environment, although a response may occur to stimulation such as noise or touch. Sedation is consistently achieved when xylazine are given to patients in calm and quiet surroundings with minimal environmental stimuli (SINCLAIR 2003). The onset of sedation is slower following IM administration (ENGLAND et al. 1992; BANI-ISMAIL et al. 2010).

The degree of sedation produced by xylazine in cattle depends on the route of administration, dosage given and the animal temperament (LIN and RIDDELL 2003). Therefore, low doses of xylazine (0.025-0.05 mg kg^{-1} IV or IM) provide sedation without recumbency in cattle while higher doses 0.1 mg kg^{-1} IV or 0.2 mg kg^{-1} IM provide recumbency and light planes of general anaesthesia for approximately one hour (GREENE 2003).

2.5.4.1.7.2 Effects on cardiovascular system

Administration of xylazine may induce a biphasic blood pressure response in animals, that is characterised by an initial transient hypertensive phase which is accompanied by a hypertensive-induced baroceptor reflex decrease in heart rate and followed by a hypertensive phase and a stabilisation of heart rate below base line values (GREENE and THURMON 1988; CONDINO et al. 2010) as xylazine stimulate central and peripheral α2- receptors (LIN and RIDDELL 2003).

Body temperature may decrease in animals sedated with α2-agonists. Hypothermia is attributed to CNS depression of the thermoregulation, in combination with a

reduction in muscular activity (MASSONE et al. 1993). YADAV et al. (2008) reported that, rectal temperature of cows decreased 0.26 and 0.35 C° at 90 and 120 min respectively after low doses IM xylazine administration.

2.5.4.1.7.3 Effects on respiratory system

The effects of xylazine on respiration, acid-base balance and blood gas values vary according to species and anaesthetic combination. In cattle, xylazine induces a decrease of the respiratory rate, which is accompanied by an increase in pH and presumably induces metabolic acidosis (HALL and CLARKE 1983). Hypoxemia is commonly observed after xylazine injections but no significant changes occurred in carbon dioxide tension (GREENE and THURMON 1988). In small animals, respiratory rate is also decreased following xylazine administration, but arterial pH, PO_2 or PCO_2 are not significantly affected (HALL et al. 2001). The PaO_2 was significantly decreased in sheep and goats after IM administration of xylazine (BANI-ISMAIL et al. 2010). HALL and CLARKE (1983) stated that, hypoxaemia induced by xylazine in sheep can be life-threatening. Xylazine induces a considerable pulmonary edema in sheep (CELLY et al. 1998).

2.5.4.1.7.4 Effects on metabolic parameters

Alpha-2 agonists can reduce the stress response to surgery as assessed by the attenuation of catecholamine, ACTH and plasma cortisol levels (EICHNER et al. 1979). Stress is a generalised response of the body to various factors called stressors. Thus, pain, blood loss, excitement and underlying pathological conditions may all act as stressors in the surgical patient. It is well known that, the endocrine - metabolic stress response is characterised by the increase of catecholamine, cortisol, glucose, and non esterified fatty acids (NEFA) blood levels (BENSON et al. 2000). The same authors mentioned that**,** adrenoreceptors play an important role in the co-ordination of these events, therefore α2- adrenergic agents may interfere with the pathophysiology of the stress response during and after anaesthesia (AMBRISKO and HIKASA 2002). SYMONDS (1976) and TOSO et al. (1993) reported that xylazine induced a transient state of hyperglycemia in dairy cattle as hepatic glucose output was increased and plasma insulin concentrations were

reduced. The hyperglycaemic effect of xylazine is thought to be due to its direct effect on α2- adrenoceptors of pancreatic islet beta cells resulting in an inhibition of insulin release (SYMONDS 1976). AMBRISKO and HIKASA (2002) observed that, the plasma level of NEFA was decreased significantly after xylazine injection in dogs.

2.5.4.1.7.5 Effects on stress hormone (cortisol)

Although various studies have been shown that α2- agonists reduce the preoperative levels of stress–related hormones and thus attenuate the stress response of surgery in dogs (BENSON et al. 2000; AMBRISKO and HIKASA 2002), little is known about the effect of α2- agonists on surgical stress response in cattle (WHAY et al. 1998; BENSON et al. 2000). It has been shown in small animals that, xylazine decreases plasma catecholamine and cortisol level, inhibit insulin release and lipolysis and increase plasma glucose and glucagon levels (AMBRISKO and HIKASA 2002). In contrast, STILWELL et al. (2010) studied the effect of hot-iron disbudding on plasma cortisol of calves sedated with xylazine and they found that, all calves given xylazine showed a higher cortisol level at all times compared with the animals not sedated. Cortisol concentrations decreased in sheep after xylazine and ketamine administration and it was close to normal 10 h postoperatively (HUGHAN et al.2001). The author's assumed diminished response to surgical stress and perhaps to pain by α2- agonists or that the HPA axis adapted to a continuing high levels of nociceptive stimulation (HUGHAN et al. 2001).

2.5.4.1.7.6 Effects on insulin level

ABDEL EL MOTAL and SHARP (1985) reported that, xylazine in vitro inhibits the release of insulin by activation of α2-receptors on pancreatic ß-cells. Previous studies in ruminants showed after xylazine administration a significant hypoinsulinemia and hyperglycemia in sheep (BROCKMAN 1981), calves (KASUYA et al. 1996) and cows (HSU et al. 1989) after administration of xylaziine.

2.5.4.1.7.7 Other effects

Xylazine in cattle induced a profuse salivation due to its parasympathomimetic like action (DEROSSI et al. 2005). Profound inhibitory effects of xylazine on rumen

motility due to stimulation of the α2- adrenoreceptrors in the fore stomach musculature (GUARD and SCHWARK 1984) which subsequently may lead to ruminal tympany, especially during lateral recumbency. One of the well known effects of xylazine is the increased frequency of urination in cattle (ROSENBERGER et al. 1968).

2.5.4.2 Other α2- agonists

2.5.4.2.1 Detomidine hydrochloride (Domosedan®, Pfizer)

Detomidine HCL is primarily used as a sedative agent in horses in a dose of 0.01-0.3 mg kg^{-1}. Horses remain standing also with the highest dose. Detomidine induces deep sedation. Side effects are bradycardia, arterial hypertension, ataxia, sweating, piloreaction, muscle tremor and diuresis (CLARKE and TAYLOR 1985). The dose of detomidine in cattle appears to be similar to those in horses (CLARKE and TAYLOR 1986). Low hypnotic effects with detomidine in cattle indicates that they are more likely to remain standing than after xylazine (LIN and RIDDELL 2003). Low doses of detomidine may be used safely in early and late pregnancy in cattle (CLARKE and TAYLOR 1986). Sedation in cows after detomidine lasts longer than after xylazine application (LIN and RIDDELL 2003).

2.5.6.2 Medetomidine hydrochloride (Domitor®, Pfizer)

Medetomidine HCL is commonly used for sedation, hypnosis and analgesia in small animals (CULLEN 1996). It has been used in cattle with IV dose of 10-20 µg kg^{-1} causing sedation similar to that seen after 0.1- 0.2 mg kg^{-1} of xylazine (JALANKA 1989). A high dose of medetomidine doesn't seem to reduce the overall endocrine stress response in cattle and sheep (RANHEIM et al. 2000a; RANHEIM et al. 2000b).

2.5.6.3 Romifidine (Sedivet®, Boehringer Ingelheim)

Romifidine is an alpha2-agonist dug labelled for use in horses (MASSONE et al. 1993). It has been administrated IV, IM and epidurally in horses, dogs, goats and cattle (AMARPAL et al. 2002). When it was given IM (0.02 mg kg^{-1} BW) to adult cattle they became recumbent in 15 min and the sedation period lasted for approximately one hour (MASSONE et al. 1993). It appears to have similar effects to xylazine,

although it may provide long duration of the onset of action and longer duration of analgesia than with xylazine. Romifidine can be used for epidural analgesia in cattle undergoing flank surgery (FIERHELLER et al. 2004).

2.6 Pain in lame cows

The importance of lameness in dairy cattle has been increasingly recognised in the last two decades and is now considered as one of the most urgent health and welfare problems of dairy cattle (WHITAKER et al. 2000; GROHN et al. 2003). Lame cows become hypersensitive to noxious stimuli and in some cases remain hyperalgesic for up to 28 days after treatment and apparent resolution of lameness. Pain experienced by lame cattle has a substantial impact on the welfare of affected cattle (COUNCIL 1997; WHAY et al. 1997; WHAY et al. 1998; O'CALLAGHAN 2002). Pain management in lame cows including adequate and early treatment of orthopaedic disorders and the use of analgesics and sedatives is still a subject of continuing neglect in dairy farming (WHAY et al. 1998; O'CALLAGHAN 2002).

2.6.1 Assessment of pain associated with lameness

Several reasons why adequate pain control might not be used in cattle are at hand. May be the pain experienced by the cows is simply overlooked out of habit, as the main focus lies on the correction of foot shape and the treatment of lesions. May be some veterinarians even hope that the animal will make a full recovery without analgesics (LIVINGSTON 1994; BEUSKER 2007). Moreover, accepting that a cow is suffering from pain would result in additional time and money to be invested. The behaviour of an animal may be misinterpreted, so the observer believes that the animal is not experiencing any pain, even though a similar condition would be very painful for a human being. Some farmers and even veterinarians believe that pain may actually prevent the animal from moving too much and causing further injury (BEUSKER 2007). The ideal and most useful way of controlling pain in this context would be to eliminate the pathological pain while leaving the physiological pain mechanism untouched. It is almost impossible, with the drugs available, to draw this fine line in animals (LIVINGSTON 1994). If a veterinarian uses local anaesthetics during treatment, the effect will only last for a certain time, after which the cow will be

severely lame again. It would be in the cow's interest to prescribe longer-acting analgesics for the recovery period as it is highly unlikely that a normal dose of analgesic would block the pain so completely that the cow would cause further damage to the digit (O'CALLAGHAN 2002). With the drugs available, a veterinarian can and should provide reasonable pain management and make things easier, for the animal as well as for himself (LIVINGSTON 1994; LOGUE et al. 1998).

2.6.1.1 Behavioural monitoring

Pain associated lameness in dairy cows likely influences both individual and social behaviour of affected animals. Lame cows have reduced daily activity levels (O'CALLAGHAN et al. 2003), spending more time lying and less time feeding (GALINDO and BROOM 2002). Moreover, lame cows are less likely to start social interactions with other cows, although they are as likely to be subjected to aggressive behaviour by other animals as sound cows (GALINDO and BROOM 2002).

FEIST (2004) studied the behavioural signs of pain in cows after claws surgery, and found that, behavioural signs for the evidence of pain were the change of mental status of the cow, facial expression, carriage of the ear and expression of the eye.The weight bearing of the affected leg was more pain indicative while standing and less prominent in walking animals. Feed intake and position of the head improved when analgesics were given (SINGH et al. 1994). Disturbed behaviour and vocal expression like teeth grinding were observed less often in animals with analgesics (FEIST 2004). The spine position, the lying behaviour and social interactions in lame cows seem less predictive for signs of pain in tie than in free stalls (O'CALLAGHAN et al. 2003).

2.6.1.2 Pedometer

The pedometer is an electronic device that transmits information about the number of steps that the cow takes over a set time. A pedometer is regularly used for detecting cows in estrus and mastitis (MAZRIER et al. 2006). Pedometers are also useful in the evaluation of the effects of lameness on daily activity (O'CALLAGHAN et al. 2003; EDWARDS and TOZER 2004; MAZRIER et al. 2006). Pedometers were used

for collection 24 hours data for standing, lying and walking as behavioural signs of pain in lame cows (FEIST 2004). Pedometers have also been used to assess weaning stress in calves (HALEY et al. 2005). CURRAH et al. (2009) used pedometers for assessment of postoperative pain associated with castration in beef calves and found that, calves after castration show decreased activity and take fewer steps.

2.7 Restraining cattle

2.7.1 Stress induced by restraining

Ineffective restraint allows more freedom for the cow to resist, and this might be falsely interpreted as pain and stress related behaviour. O'CALLAGHAN LOWE et al. (2004) compared the working practice of claw trimmers and veterinary orthopaedic surgeons. They reported that, most claw trimmers believed that routine foot trimming was neither stressful nor painful. A significantly higher proportion of veterinary surgeons considered both preventive foot trimming and treating claw lesions to be potentially painful and stressful. This perception might have been distorted by the fact that in contrast to professional foot trimmer 88% of the veterinary respondents relied on farm facilities only and probably the use of local anaesthesia to restrain the cattle for treatment. Many farms however lack special facilities designed to restrain lame cows for claws trimming. Well designed restraining devices have the potential to reduce stress experienced by the cow (GRANDIN 1998; PESENHOFER et al. 2006). The animal must be held tightly enough to provide a feeling of restraint, while avoiding pain caused by excessive pressure. Restraint can be a very strong source of stress, training cattle to accept handling procedures and facilities can help to reduce this type of stress (ZAVY et al. 1992; GRANDIN 1998).

2.7.2 Cardio-respiratory depression during recumbency

WAGNER et al. (1990); TAGAWA et al. (1994) studied the effects of change in body position on cardiopulmonary function and plasma cortisol in cattle. In these studies plasma cortisol concentration increased with the change in body position. In a supine position, cortisol values were increased to more than three times the control

value.The arterial oxygen tension and oxygen saturation were significantly decreased with change in body position. The decrease was most pronounced when cattle were restraint in a supine position (TAGAWA et al. 1994). Arterial carbon dioxide tension, heart rate, mean arterial pressure and central venous pressure did not change significantly with changes in body position. Thus, restraining of cattle in a lateral recumbency or supine position without introducing anaesthesia was found to exert a strong stress.

It is well known that also lateral recumbency in dairy cows leads to substantial respiratory depression due to impaired ventilation and perfusion/ventilation mismatching (WATNEY 1986a; KLEIN and FISHER 1988; WAGNER et al. 1990; TAGAWA et al. 1994). Cattle placed in lateral or dorsal recumbency experience a shift of abdominal viscera that places massive weight and pressure on major abdominal vessels and the diaghram. The increased pressure may impede blood return to the heart and cause a decrease in cardiac output, blood pressure, and tissue perfusion. The cardiopulmonary effects of four positions (standing, right lateral, left lateral and dorsal recumbency) maintained for 30 minutes were evaluated in conscious cattle to which no sedatives or anaesthetic drugs were given. This study revealed no significant changes in heart rate, respiratory rate, mean arterial blood pressure, arterial pH, P_aCo_2, arterial base excess or venous blood values. However, significant decreases in P_aO_2 developed when cattle were placed in lateral or dorsal recumbency (WAGNER et al. 1990).

In adult cows the ventilation of some lung areas was reduced in dorsal recumbency (HORNOF et al. 1986; WATNEY 1986a). Large animals such as horses and cattle are particularly susceptible to compression of the lung because of the weight of abdominal viscera pressing against the diaphragm (MCDONELL et al. 1979; WAGNER et al. 1990). This hypoventilation and ventilation–perfusion mismatch may lead to insufficient oxygenation of the blood in these areas, to shunt the blood into well ventilated areas of the lung, vasoconstriction will occur in the hypoxic lung areas (HORNOF et al. 1986). Hypoxic pulmonary vasoconstriction is very pronounced in cattle (BISGARD et al. 1975). If the compression of the lung becomes severe in adult

animals, a ventilation–perfusion mismatch can result in mixing of un-oxygenated blood from hypoxic lung areas and well-oxygenated blood from well-ventilated lung areas in the left side of the heart (WATNEY 1986b; WAGNER et al. 1990). As a result, the PaO2 can decrease by 35 % in adult cows positioned in dorsal or lateral recumbency (KLEIN and FISHER 1988; WAGNER et al.1990).

2.8 Functional claw trimming in cattle

Functional claw trimming is a simple, repeatable method to maintain physiological biomechanical function of the bovine digit and can avoid the onset of lameness in cattle and preventing claw horn lesions from evolving from a sub-clinical to the clinical stage (TOUSSAINT-RAVEN 1989; SHEARER and AMSTEL 2001; TOL et al 2004; PESENHOFER et al 2006). It is carried out at least once or better twice a year according to Dutch standards (TOUSSAINT-RAVEN 1989) and it considered now an important part of any lameness management and control as well as claw health prophylaxis programme (SHEARER and AMSTEL 2001).

Modern claw trimming requires proper restraint systems for cattle such as walk-in crush or tilt tables (SHEARER and AMSTEL 2001). This is particularly important when grinding discs are applied, which are preferred by many veterinarians and professional claw trimmers as they work fast and effectively (SHEARER and AMSTEL 2001). Hydraulic tilt tables are used frequently in Europe, where the cow is placed in lateral recumbency and all 4 feet can be trimmed easily. The mobile walk-in crush is used also, where the cow remains standing (SHEARER and AMSTEL 2001). The claw trimming procedures itself induce stress and defence reactions in cows. Thus, claw trimming using tilt tables required significantly less time than using the walk-in crush due to better restraint (PESENHOFER et al. 2006).

PESENHOFER et al. (2006) studied the effect of claw trimming in cattle and their restraint in either a mobile walk-in crush or a tilt table on animal's stress reactions and they found that, the tilt table appeared preferable, because animals were fixed more quite with less evasion movements and fixation required less time than in the walk-in crush.

3 Chapter 1: Effects of xylazine hydrochloride (Rompun®) during lateral recumbency and claw trimming on hormonal, metabolic and cardio-respiratory stress-response in dairy cows

3.1 Summary

The aim of the study was to investigate the effects of lateral recumbency (LR) and xylazine treatment in combination on stress response and cardio-respiratory depression in cows undergoing painless claw trimming. In a blinded experimental study, six healthy, non-pregnant, non-lactating, German Holstein Frisian cows weighing 610±87.9 kg (mean± SD) and 4.3±3.3 years old were used in a cross over design with two weeks intervals between treatments. The treatments were Xyl-LR: xylazine (Rompun®, 0.05 mg kg^{-1} BW, IM) or Plac-LR: an equal volume of placebo 15 minutes before LR (30 minutes) for claw trimming or Xly-St: xylazine in the same dose and route of administration without further manipulation (cows remained standing). In short term intervals (30 minutes before until 2 hours after drug application) heart rate (HR), respiratory rate (RR), mean arterial blood pressure (MAP), venous and arterial blood gases and venous plasma levels of cortisol, insulin, glucose, lactate and non-esterified fatty acids (NEFA) were measured and clinical signs of sedation and free gas bloat recorded.

In placebo treated cows (Plac-LR), LR induced a significant ($P < 0.05$) increase in MAP, RR, plasma level of cortisol, lactate and NEFA, a significant ($P < 0.05$) decrease in mean arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) and arterial oxygen saturation (SaO_2). Xylazine induced (Xyl-St) a significant ($P < 0.05$) reduction in HR, RR, MAP, plasma insulin, and NEFA, PaO_2, SaO_2, and a significant increase in plasma glucose and $PaCO_2$. After sedation with xylazine (Xyl-LR) during LR, HR, RR, MAP, PaO_2, SaO_2 and plasma levels of cortisol, lactate and NEFA, were significantly ($P < 0.05$) reduced whereas $PaCO_2$ was increased compared to Plac-LR. Lowest SaO_2 found during LR after xylazine treatment were about 90%. All xylazine treated cows showed mild to moderate signs of sedation, mild and clinically irrelevant free gas bloat and were able to stand and

walk before and after restraining in LR. We concluded that, xylazine administered in a low dose in adult cows preceding LR and claws trimming on a surgical tipping table can reduce hormonal and metabolic stress responses but has some additional effect on respiratory depression of LR. When xylazine is applied before cows are turned into LR the desired effects of sedation and analgesia need to be balanced against the adverse effect of cardio-respiratory depression of xylazine. Higher doses of xylazine than the used 0.05 mg kg^{-1} BW may induce clinically relevant cardio-respiratory depression during LR in cows.

3.2 Introduction

Surgical interventions are commonly necessary for treatment of lame cows (VANMETRE et al. 2000; STARKE et al. 2007). They require proper analgesia and restraining to ensure adequate and safe surgical conditions for the surgeon and the treated animals. For restraint cows are frequently laid down and fixed in lateral recumbency (LR) on a surgical tipping table. Restraining is perceived by the treated animal as a considerable stress (TAGAWA et al. 1994; PESENHOFER et al. 2006).

Although animal welfare is a growing subject of concern in farm animals, the need for adequate analgesic protocols including sedation for alleviation of stress in general and in particular for orthopaedic surgical interventions are still are not adequately considered (CLUTTON et al. 2007a; 2007b; HUDSON et al. 2008). This appears under animal welfare aspects intolerable (GALINDO and BROOM 2002; WHAY et al. 2005; CLUTTON et al. 2007a; 2007b).

Alpha2- agonists, such as xylazine, have dose dependent strong sedative, moderate analgesic and myo-relaxing effects in cattle. As adverse effect of alpha2- agonists cardio-respiratory depression such as bradycardia, bradypnoe, hypotension, hypoxemia and hypercapnia is reported (CLARKE and HALL 1969; HOPKINS 1972; CAMPBELL et al. 1979; YOUNG 1979; RUCKEBUSCH 1983; RUCKEBUSCH and ALLAL 1987; GREENE and THURMON 1988; PICAVET et al. 2004). Also LR leads to cardiopulmonary depression in dairy cows (KLEIN and FISHER 1988; WAGNER et al. 1990; TAGAWA et al. 1994). It is unknown, if the application of alpha2- agonists

may exaggerate cardio-respiratory depression to an intolerable state in cows fixed in LR (STARKE et al. 2006; CLUTTON et al. 2007a; 2007b; STARKE et al. 2007a; 2007b).

Thus, the aim of our study was to investigate the effect of sedation with xylazine on cardio-respiratory, endocrine-metabolic and behavioural stress response in dairy cows undergoing painless claw trimming in LR on a surgical tipping table.

3.3 Material and methods

The study was approved by the Ethical Animal Care and Use Committee of the Federal State of Lower Saxony, Germany (research permit number 33.9-42502-05-08A573).

3.3.1 Experimental animals, housing and feeding

Six healthy, non-pregnant, non-lactating, German Holstein Frisian cows (age: 4.37±3.27 years; body weight (BW): 610±87.9 kg) were included in this study. All cows were kept in open free stalls with straw bedding at the Clinic for Cattle-University of Veterinary Medicine Hannover, Germany and were fed a diet based on hay and one kg of concentrate according to maintenance with free access of water.

3.3.2 Study design and treatments

The study was carried out in a controlled cross-over design where the six cows were grouped in pairs and each pair of cows received three different treatments in different sequence. Intervals between treatments were two weeks. Allocation of cows to cow pairs and sequence of treatments were randomized.

To allow differentiation between effects of xylazine, LR and the combination of xylazine and LR the following treatment protocols were chosen: Cows received 15 min before claw trimming in left LR on a hydraulic tipping table (Werner GmbH, Höhenkirchen, Siegertsbrunn, Germany; duration of LR 30 min) either xylazine (group Xyl-LR; 0.05 mg kg^{-1}IM; Rompun® 2%, Bayer, Leverkusen, Germany) or an equal volume of placebo (group: Plac-LR; sodium chloride solution; B. Braun, Melsungen AG, Germany). In the third group, xylazine was administrated in the same

dose and route of application but cows remained standing without further manipulations (group: Xyl-St). The right anconaeus muscle was used for IM injections. All treatments were carried out by a second person so that the examiner remained blinded against treatments in group Xyl-LR and Plac-LR.

3.3.2.1 Lateral recumbency on a surgical table

Food was not withheld before cows were treated. Cows had access to water and hay at all times and the last concentrate feeding (1 kg) was about two hours before. All cows carried a halter with a 1.5m long strong rope which was used to guide the cows. With the hydraulic tipping table in perpendicular position cows were led to the table with one person at the head and a second person in the pelvic region. While the person at the head fixed the head with a neck belt to the head pad of the table, the second person pushed the cow where necessary at the tuber coxae in a position parallel and close to the table unless the cow took this position voluntarily. Then the abdominal and thoracic belts were tightened thoroughly but not so tight, that thoracic movements were significantly inhibited. Thereafter cows were turned into left LR in horizontal position and the two hind legs were fixed tightly on leg pads in middle of the metatarsus. After legs were fixed the abdominal and thoracic belts were loosened so that the animal remained safely fixed but the compression of the belts on the abdomen and thorax were reduced and the animals could breathe freely. To let cows stand up, the procedure was performed inversely. Abdominal and thoracic belts were tightened, the legs released, the table was turned in perpendicular position and the belts (first thoracic, abdominal and then neck belt) removed when the feet had safe contact to the ground.

When cows moved very slowly and reluctantly to the tipping table no more than gentle and patient pushing and encouragement by voice was used to direct cows into the correct position for fixation.

The whole table was padded with a rubber mat and an extra soft rubber cushion was put under the whole left front limb in contact with the table to avoid muscular and nerve damage during LR.

All cows in this study were used to human contact and handling and had already experience with claw trimming in LR on the surgical tipping table.

3.3.3 Instrumentation

All cows received indwelling catheters in the right caudal auricular artery and right jugular vein after surgical preparation of the skin and local anaesthesia (Procain 2%, Selectavet) two hours before the experimental procedure started. The arterial catheter (Vygon®, 20G-L. 6cm-ø 0.9mm-27ml / min; Leader-Flex, Ecouen, France) was inserted according to (MULLER and GOETZE 1987) using the Seldinger technique (SELDINGER 1953), fixed with tape (Fig.14 appendix), and immediately connected to a calibrated electro-mechanical transducer via a fluid filled extension set. The jugular vein catheter (WVI Jugular catheter®, ø 2.4mm, length 20cm with Teflon catheter, Walter, Veterinär-instrumente e.K., Baruth /Mark, Germany) was sutured to the skin and additionally fixed with tape (Fig.15 appendix). The auricular artery catheter was permanently flushed with heparinised 0.9% saline solution (3ml h^{-1}; Braun GmbH, Melsungen; 10.000 IU heparin L^{-1}, Heparin-Calcium, Ratiopharm, Germany), while the jugular vein catheter was flushed after insertion and each blood sampling. Both catheters were equipped with three way-stop-cocks and were removed after the experiment was finished.

3.3.4 Monitoring

3.3.4.1 Cardio-respiratory parameters

Mean arterial blood pressure (MAP) was continuously recorded by means of a fluid filled extension connected to the arterial catheter and a mechanical-electrical transducer (Supermon Module 7272, Kontron instruments, Watford, England). The zero pressure point was taken at the level of the scapulo-humeral joint in the standing animal and the centre of the thorax in LR (WAGNER et al.1990; AMORY et al.1992). Characteristic pressure waves were used to confirm correct catheter positioning. A bipolar ECG (EKG-Module 7271, Kontron instruments, Watford England) was used to monitor ***heart rate*** (HR, beat min^{-1}; SPRUNG et al. 1983).

Rectal body temperatures were recorded using a digital thermometer (Besrcare, Model DT-K01A, Germany).

Respiratory rates (RR) were measured by counting thoracic excursions for a period of 1 minute.

Blood gases analysis in a venous and arterial blood which was collected anaerobically in heparinised syringes (Heparin Lithium Salt, Sigma Aldrich-Chemicals GmbH, Germany) from catheters and placed on ice after collection, was performed within 15 min after collection *(*Rapid lab.™ 348, Bayer health care Diagnostic). Results were corrected to body temperature and haemoglobin level (BURNETT and NOONAN 1974) which was measured automatically (Cell tac MEK -6108G, Nihon-Kohdan). Arterial and venous partial pressure of oxygen (P_aO_2, P_vO_2; mmHg) and carbon dioxide tension (P_aCO_2, P_vCO_2; mmHg) were recorded as well as arterial bicarbonate concentration (HCO_3s, mmol L^{-1}), base excess (BE; mmol L^{-1}), oxygen saturation (S_aO_2 %), and blood pH values were analysed.

Cardiopulmonary parameters were calculated by using the following standard formulas:

- **arterial oxygen content**: C_aO_2 [ml/dl] according to (SPRUNG et al. 1983)
  Hb x S_aO_2 x 1.36 + (P_aO_2 x 0.003)
- **venous oxygen content**: C_vO_2 [ml/dl] according to (SKARDA and MUIR1996)
  Hb x O_2SAT_v x 1.36 + (P_vO_2 x 0.003)
- **Alveolar oxygen tension***:* PAO_2 [mmHg] according to (SKARDA and MUIR 1996)

PAO2 = PiO2 - PaCO2

PiO2 = FiO2 x (BP- partial pressure H2O Vapor; 47 mmHg)

BP = Barometric pressure [760 mmHg at sea level], FIO_2 = fraction of O_2 at room temperature (20.95%)

- **Pulmonary capillary oxygen content**: CcO2 [ml dl^{-1}] (SKARDA and MUIR 1996)
  CcO2 = Hb x 1 x 1.36 + (PAO_2 x 0.003)

In the EDTA tubes, the white blood cells (WBCs), erythrocytes (RBCs), packed cell volume (PCV), haemoglobin (Hb) and platelets (PLT) were measured on a flow cytometer automatically (Nihon Kohden, Celltac alpha, MEK 61086).

3.3.4.2 Hormonal and metabolic parameters

Heparinised blood samples were collected in short term intervals and centrifuged at 1.500g (10 min, 4C°) immediately. Plasma was stored at -20 C° until analysis of cortisol (chemiluminescent enzyme immunoassay, Siemens diagnostics, Eschborn, Germany) and insulin (Insulin RIA, the DSL-1600 insulin Radioimmunoassay kits, Texas, USA). Plasma glucose, nonesterified fatty acids (NEFA), Beta-hydroxybutrate (B-HBS), lactate, sodium (Na), potassium (K) and chloride (Cl) were measured using ion-sensitive electrodes on an automated analyser (ABX Pentra 400, Horiba ABX Diagnostics) using commercial test kits. The strong ion Difference (SID) was calculated according to CONSTABLE (1999); SID= $[Na^+] + [K^+] - [CL^-]$.

3.3.4.3 Clinical signs

The sedation score was achieved by summing up the recorded scores (0 to 3) for five clinical signs (change from initial attitude, head lowering, ptosis, ptyalism and ability to stand; Tab.1). Accordingly the sedation score could range from 0 to 15. A sedation score of 0 was given when the sum of clinical scores was 0 and 1, 1 for a sum of scores of 2 and 3, 2 for a sum of scores of 4 and 5, 3 for a sum of scores of 6 and 7, 4 for a sum of scores of 8 and 9, 5 for a sum of scores of 10 and 11, 6 for a sum of scores of 12 and 13 and 7 for a sum of scores of 14 and 15. Also the occurrence of free gas bloat was recorded (Score 0: no, 1: mild, 2: moderate, 3: severe, 4: life threatening).

Table 1: Clinical signs for assessment of sedation score in cows after treatment with xylazine or placebo modified according to FIERHELLER et al. (2004).

Clinical signs	Score			
	0	**1**	**2**	**3**
Change from initial attitude	no	mild	moderate	severe
Head lowering	no	mild	moderate	severe
Ptosis	no	mild	moderate	severe
Ptyalism	no	mild	moderate	severe
Ability to stand	unchanged	mild swaying in hind legs	moderate swaying in hind legs	try or do lay down

3.3.5 Experimental protocol

The application of drugs was set as time 0 (t0). Baseline values were determined 15 min before drug application. In group Xyl-LR and Plac-LR, MAP, HR, RR and rectal body temperature were measured at 15, 30 (start of LR), 45, 55, 60 (end of LR), 75, 90, 105, 135, and 195 min after drug treatment, whereas blood samples were drawn from catheters for blood gases and endocrine-metabolic parameters at 15, 30, 45, 60, 75, 105,135 and 195 min after drug treatment. Clinical signs were recorded at -15, 10, 15, 75, 105, 135, and 195 min after drug application. Recordings for cows of the group Xyl-St were performed accordingly.

3.3.6 Statistical analysis

Data were analysed using the statistical analysis system (SAS version 9.1 for Windows, SAS institute Inc, Cary, NC, USA). Continuous data were analysed by two-factorial analysis of variance for repeated measurements (Proc GLM, Repeated statement; factor: group, time and time x group). At each time point multiple comparisons of group means were performed by the LSMEANS statement (pdiff/tdiff option). Within groups means at different time points were compared with baseline values using the paired t-test. Clinical signs scores were analyzed with the wilcoxon signed rank test for statistical differences. The level of significance was set at P <

0.05. Data were presented as mean ± SD. The program Sigma Plot® 2001 (Systat Software Inc., Chicago, USA) was used to create graphs.

3.4 Results

3.4.1 Clinical signs

Mean scores for sedation were significantly ($P < 0.05$) higher in the Xyl-LR and Xyl-St group than in Plac-LR. Free gas bloat was mild (score 1) in both groups treated with xylazine. After xylazine treatment all cows were able to stand and to walk at all times. However, one cow treated with xylazine lay down for one minute after she was turned back from LR on the hydraulic tipping table to standing position. The cow stood up without support and walked back to the stable without signs of ataxia (Tab.2).

No matter if cows were treated with xylazine or placebo the procedure of turning cows into LR and back into standing position was always uncomplicated and was performed by not more than two people, one at the head and one in the pelvic region. To get cows into the correct position close and parallel to the surgery table needed never more than gentle and patient encouragement by voice and pushing at the tuber coxae towards the table. No cow led to the tipping table tried to escape or showed defence reactions like leg or head kicking or pushing. Behaviour of slow and reluctant moving to the table appeared less as expression of fear and discomfort but more as uncertainty of cows about what was expected and where they should go and stand. After LR all cows moved calmly and consistently back to the stable.

3.4.2 Endocrine stress response

Mean plasma cortisol concentration were significantly higher during LR in placebo treated cows compared to cows in group Xyl-LR and Xyl-St and plasma cortisol in Xyl-LR were higher ($P < 0.05$) than in Xyl-St during LR (Fig.5; Tab.10 appendix). Mean insulin plasma concentrations remained in Plac-LR almost unchanged during the experimental period while xylazine induced hypoinulinemia ($P < 0.05$) in cows in groups Xyl-LR and Xyl-St (Fig.8; Tab.10 appendix).

3.4.3 Cardio-respiratory stress response

Mean HR was not affected by LR but xylazine treatment induced bradycardia ($P < 0.05$) in groups Xyl-LR and Xyl-St (Fig.6; Tab.9 appendix). During LR, MAP was significantly ($P < 0.05$) higher in group Plac-LR than in groups Xyl-LR and Xyl-St (Fig. 6; Tab.9 appendix). In groups Xyl-St and Xyl-LR, xylazine application induced a significant ($P < 0.05$) but mild reduction of MAP. In group Xyl-LR, MAP increased to baseline values during LR.

In xylazine treated animals (group Xyl-LR and Xyl-St) mean rectal body temperature decreased ($P < 0.05$) by about 0.2°C in almost all animals post-injection followed by a rise in body temperature to baseline values within the experimental period. In Plac-LR mean body temperature remained unchanged (Tab.9 appendix).

Mean RR increased significantly ($P < 0.05$) in group Plac-LR and was significantly higher than in groups Xyl-LR and Xyl-St during LR (Fig.7; Tab.9 appendix). Xylazine application induced a significant ($P < 0.05$) reduction in mean RR in both xylazine treated groups.

During LR mean P_aO_2 and S_aO_2 dropped significantly ($P < 0.05$) in group Plac-LR. After xylazine treatment P_aO_2 and S_aO_2 were significantly ($P < 0.05$) reduced. Mean $P_aO_2$and S_aO_2 were significantly ($P < 0.05$) lower during the period of LR in group Xyl-LR than in group Plac-LR and Xyl-St. While no animals of groups Xyl-St and Plac-LR showed S_aO_2 values less than 92% during the experimental period, four out of six cows were found with $S_aO_2 < 92\%$ during LR in group Xyl-LR (Fig.7; Tab.11 appendix). While mean P_aCO_2 remained almost unchanged during LR in group Plac-LR, The P_aCO_2 increased by about 10% in both xylazine treated groups and were significantly ($P < 0.05$) higher than in Plac-LR (Fig.7; Tab.11 appendix).

While no group differences were found for arterial oxygen tension (PAO2) and the mean pulmonary capillary O2 content (CcO2) significantly ($P < 0.05$) increased during LR in group Xyl-LR and Plac-LR (Tab. 13 appendix).

No significant differences in the mean arterial pH were found between experimental groups. Mean arterial pH remained nearly unchanged in all experimental groups throughout the experimental period. On average LR induced no change in the mean arterial BE and HCO_3^- in the Plac-LR group, whereas xylazine treatment induced a significant ($P < 0.05$) increase in BE, HCO_3^- and SID in standing cows (Xyl-St) or during LR (Xyl-LR) (Tab.10 & 11 appendix). Together with P_aCO_2, xylazine treated cows exhibited a fully metabolically compensated respiratory acidosis.

There was no statistically significant change in WBC_S and total protein in all groups. While RBC_s, Hb and PCV showed a significant ($P < 0.05$) decrease in cows of xylazine treated groups. The PLT was significantly ($P < 0.05$) decreased in Xyl-St group (Tab.14; appendix).

3.4.4 Metabolic stress response

While mean plasma concentrations of glucose and NEFA remained unchanged during LR in group Plac-LR, glucose concentrations were significantly higher and NEFA significantly ($P < 0.05$) lower compared to Plac-LR after xylazine treatment in groups Xyl-LR and Xyl-St (Fig.8; Tab.10 appendix).

Significantly higher ($P < 0.05$) mean plasma lactate concentrations were seen in the Plac-LR group compared to the Xyl-LR and Xyl-St group during LR. In both xylazine treated groups mean lactate levels remained nearly unchanged (Fig.8; Tab.10 appendix). The B-HBS showed a significant decrease ($P < 0.05$) in cows of both xylazine treated groups (Tab.10, appendix).

3.5 Discussion

It is well known that, LR in dairy cows leads to respiratory depression due to impaired ventilation and pulmonary perfusion/ventilation mismatching (WATNEY1986a; KLEIN and FISHER 1988; WAGNER et al. 1990; TAGAWA et al. 1994). When alpha2-agonists such as xylazine are used for sedation before LR it appears possible that respiratory depression may be aggravated by xylazine in cows when turned into LR (STARKE et al. 2007a). Therefore, we performed this study to evaluate the effects of pre-emptive use of xylazine in dairy cows undergoing painless claw trimming in LR on stress and cardio-respiratory response during LR.

In accordance with previous reports (MASSONE et al. 1993; HOQUE 1994; LIN and RIDDELL 2003; DEROSSI 2005; YADAV et al. 2008), the low xylazine dose (0.05 mg kg^{-1} BW IM) induced in cows of the Xyl-LR and Xyl-St groups in average 15 minutes after application mild to moderate sedation lasting for about one to two hours (Tab.2). With the low xylazine dose as in this study cows are still able and willing to stand and to walk and show only mild signs of free gas bloat. The mild free gas bloat was attributed to a prompt and profound inhibitory effect of xylazine on rumen motility by stimulation of the α2-adrenergic receptors in the fore-stomach musculature (GUARD and SCHWARK 1984; RUCKEBUSCH and ALLAL 1987) and to sympathetic blockade and reduction in norepinephrine release (HABIB 2002). The mild free gas tympany seen in cows of all groups disappeared shortly after the cows were moved back to the stable without further intervention.

It was reported earlier (ABILAY et al. 1975; ALAM and DOBSON 1986; BOANDL et al. 1989; BREARLEY et al. 1990; TAGAWA et al. 1994) and is also demonstrated in this study, the rise in plasma cortisol levels in placebo treated cows that being turned into lateral recumbency on a surgical tipping table for claw trimming, which itself is a painless procedure, is perceived as considerable stress by cows. In cows sedated with xylazine the cortisol increase was significantly reduced during LR compared to placebo treated cows. The release of cortisol from the adrenal cortex is triggered by the release of corticotropin–releasing factor (CRF) and ACTH in the brain

(AMBRISKO and HIKASA 2002). A significant decrease in plasma cortisol concentration in cows treated with xylazine and turned into LR, might be due to a centrally acting $\alpha 2$-adrenergic inhibition of CRF release by xylazine as mentioned in goats (SANHOURI et al. 1992) and dogs (AMBRISKO and HIKASA 2002). However, xylazine treatment per se left plasma cortisol levels unchanged in cows which remained standing. Thus, we assume that the reduced plasma cortisol levels in cows of the Xyl-LR group are caused mainly by reduced stress perception due to the sedative effect of xylazine. Also (BREARLEY et al. 1990; STAFFORD et al. 2003) attributed the lower plasma cortisol levels in xylazine treated calves during and after disbudding to the sedative and analgesic effects of xylazine.

FLECKNELL (2000) and STILWELL et al. (2010) studied the effects of different protocols of analgesia for amputation dehorning in calves on plasma cortisol and found that plasma cortisol increased in xylazine–sedated (1 ml approx. 0.2mg Kg^{-1} IM) calves before any procedures were carried out. The authors assumed that the effect of muscle relaxation by xylazine induced stress to the calves by limiting their ability to react to human proximity and contact. In contrast to (FLECKNELL 2000; STILWELL et al. 2010) in this study plasma cortisol concentrations even decreased after xylazine treatment. The divergent results may be due to the low dose of xylazine we used in our study which allowed cows to stand, walk and respond to human contact even though restricted by mild sedation. Also cows are more used to human contact and handling and may perceive this less stressful than calves.

The technique of the arterial blood sampling in large ruminants is still difficult due to the small lumen of the auricular artery (MULLER and GOETZE 1987; MUYLLE et al. 1996). In animals with poor temperament or limited handling experiences in arterial sampling may generally not be possible or may require undue restraint, such restraint will lead to stress induced abnormalities of blood gases values (FISHER et al. 2001; NAGY et al. 2002). Several authors consider collection of arterial blood from caudal auricular arteries are useful, reliable and suitable in calves and adult cattle (FISHER et al. 2001). Rates of successful samplings of arterial blood from catheter connected to caudal auricular artery as well as measurement of arterial blood pressure obtained

in this study indicated that blood collection from this peripheral artery with a relatively small lumen is possible without any serious problems in dairy cattle. These findings were in agreement with that reported by ADAMS et al. (1991). The authors explicate that, the advantages of selection of caudal auricular artery for measurement of arterial blood pressure, acid-base balance and arterial blood gases in cattle are sampling in the standing animal, good visibility of the artery and less obvious defence reactions of animals.

In the present study, the placebo treated cows showed on average almost unchanged HR but a significant increase in MAP during LR. We attribute the mild hypertension to stress induced secretion of catecholamine during restraining in LR inducing peripheral vasoconstriction and an increase in systemic vascular resistance (ABILAY et al. 1975; ALAM and DOBSON 1986; BOANDL et al. 1989; BREARLEY et al. 1990; TAGAWA et al. 1994). Similar observations have been reported in sheep (DOHERTY et al. 1986) while in adult cattle MAP was slightly lowered (WAGNER et al. 1990). The authors assumed as possible cause for the reduced MAP impeded venous blood return to the heart caused by compression of large abdominal vessels by the weight of visceral organs in particular the rumen during LR. The cows in this study were on a hay diet and not starved and thus had a completely filled rumen when the experimental procedures started. However, the divergent results in MAP in our study and in the one of WAGNER et al. (1990) may be due to the fact that we always turn cows into left LR to avoid or at least to reduce the compression of large abdominal vessels. Another explanation may be the additional claw trimming performed to our cows during LR. Possibly the manipulation at the claw, although per se painless, may have provoked a stronger stress response in our cows compared to those in the study of WAGNER et al. (1990).

In cows of both xylazine treated groups, no matter if cows remained standing or were turned into LR, mean HR decreased significantly by about 30% for more than three hours compared to baseline values and placebo treated cows. A decrease in HR is considered a classical response after administration of α2-agonists in cattle (CLARKE and HALL 1969; HOPKINS 1972; HSU et al. 1989; RUFFOLO et al. 1993;

SINGH et al. 1994) which can be caused by a central decrease in sympathetic activity leading to a relative increase in vagal tone (ANTONACCIO et al. 1973). Similar findings are reported after systemic and epidural administration of xylazine in sheep (AZIZ and CARLYLE 1978; WATERMAN et al. 1987; KÄSTNER 2006), mares (SKRADA and MUIR 1996), goats (AMARPAL et al. 2002; BANI ISMAIL et al. 2010) calves (ANTONACCIO et al. 1973; MEYER et al. 2009) and dogs (HSU et al. 1989).

Administration of xylazine induces in cattle, sheep and horses mild hypotension after a short period of hypertension (AZIZ and CARLYLE 1978; TRACHSEL and SCHATZMANN 1984; CLARKE and PATON 1988). The initial transient systemic hypertension may have been missed in our cows because the first measurement took place 15 min after application of xylazine. Despite the considerably reduced HR after xylazine treatment MAP increased slightly after cows were turned into LR which is presumably mediated by a stress induced release of catecholamine and thereby increased systemic vascular resistance.

Restraining in LR induced an increase in RR in cows of this study. The polypnea may be related to excitement, discomfort or struggling during restraining as previously reported for cattle (KLEIN and FISHER 1988) and ponies (HALL 1984). In contrast, WAGNER et al. (1990) recorded a non significant decrease in RR during dorsal and lateral recumbency in conscious cattle. Despite polypnea in placebo treated cows during LR, P_aO_2 and S_aO_2 fell whereas the P_aCO_2 remained almost unchanged. Large animals such as horses and cattle are particularly susceptible to compression of the lung because of the weight of abdominal viscera pressing against the diaphragm during LR (HORNOF et al. 1986; WATNEY 1986a, WATNEY 1986b; WAGNER et al. 1990; RIOJA et al. 2008). Hypoventilation of compressed lung areas and ventilation–perfusion mismatch during LR may lead to insufficient oxygenation of the blood. To shunt the blood into well ventilated areas of the lung, vasoconstriction will occur in the hypoxic lung areas (BISGARD et al. 1975; HORNOF et al. 1986) which may result in mixing un-oxygenated blood from hypoxic lung areas and well-oxygenated blood from well-ventilated lung areas in the left side of the heart (STEGMANN and LITTLEJOHN 1987; KLEIN and FISHER 1988; WAGNER et al.

1990). As a result, the P_aO_2 can decrease by 35 % in adult cows positioned in dorsal or lateral recumbency (KLEIN and FISHER 1988; WAGNER et al. 1990).

The decrease in RR following treatment with xylazine either in standing position of the cows or in LR might be attributed to the direct depression of xylazine on respiratory centres in the brain (RINGS and MUIR 1982). Similar observations had been obtained before in cattle (MUIR et al. 1977; CAMPBELL et al. 1979; PICAVET et al. 2004), horse (MUIR et al. 1977), sheep (STRAUB 1971; AZIZ and CARLYLE 1978), calves (MEYER et al. 2009) and goats (AMARPAL et al. 2002). Xylazine induced hypoventilation and led per se to mild hypoxemia and hypocapnia in cows of the Xyl-St group. When xylazine treated cows were turned into LR a further decrease of P_aO_2 and increases of P_aCO_2 were observed. The mean S_aO_2 was with about 93% during LR slightly but significantly lower than in the Plac-LR and the Xyl-St group. To maintain adequate oxygenation of peripheral tissues in cattle S_aO_2 values greater than 92% are seen to be sufficient (WAGNER et al. 1990). However, while no cow in the Plac-LR and the Xyl-St group showed S_aO_2 values of less than 92%, in four out of six cows the SaO_2 was found slightly below 92% with a minimum of 89.5%. Thus, xylazine treatment even in a low dose (0.05 mg kg^{-1} BW) does slightly aggravate respiratory depression of LR getting close to a clinically relevant level. We therefore discourage to use xylazine in higher doses in cows turned into LR without providing extra oxygen to avoid insufficient tissue oxygenation.

However, plasma lactate level remained almost unchanged in cows of both xylazine treated groups. Lactate is a metabolic product of anaerobic glycolysis and arises from pyruvate (CHACON et al. 2005). Thus, lactate level in the Xyl-LR group indicates no increased anaerobic glycolysis and thus sufficient even though marginal oxygenation in peripheral muscular tissues. On the other hand hypoinsulinemia was observed in this study in all cows after xylazine treatment. This is a well known effect of xylazine and due to inhibition of the insulin release by activation of α2-receptors on pancreatic ß-cells (ABDEL EL MOTAL and SHARP 1985; HSU et al. 1989). In accordance with literature (SINGH et al. 2006) in consequence with decreasing insulin plasma levels hyperglycemias occurred in all cows treated with xylazine.

Thus, the unchanged mean plasma lactate level in the Xyl-LR group may also be due to reduced glucose utilization in peripheral tissues.

Although S_aO_2 was significantly higher in the Plac-LR than in the Xyl-LR group during LR, in the Plac-LR group mean lactate level increased significantly indicating anaerobic glycolysis and reduced oxygenation in peripheral tissues. We assume that the higher plasma lactate level were caused by stress induced catecholamine release (BICKHARDT and CARSTENSEN 1992; CHACON et al. 2005) in cows of the Plac-LR group provoking vasoconstriction and thereby reduced oxygen delivery to peripheral tissues. Another explanation could be the stress during LR inducing a higher tonicity and thereby metabolism of muscles with enhanced metabolic glucose consumption.

Hyperglycaemic effects of α2-adrenoreceptor agonists are well known (SINGH et al. 2006). Administration of xylazine alone may cause hyperglycaemia associated with a reduction in plasma insulin levels without any change in glucagons concentration in dogs and cattle (GOLDFINE and ARIEFF 1979; TOSO et al. 1993). It has been shown that the administration of xylazine to cows produced a prolonged hyperglycemia by increasing hepatic glucose production (SYMONDS 1976). The prolonged hyperglycemias after xylazine treatment in our cows in standing or LR were associated with reduced plasma insulin matches with previous reports (SYMONDS 1976; EICHNER et al. 1979; HSU et al. 1989). However, LR without sedation induced no change in plasma glucose, despite stress perceived by cows during restraining in LR induced a significant increase in plasma cortisol level. Thus, plasma glucose is not reliable indicators of the stress intensity in animals (SCHOLZ 1990). In contrast to our results in a previous study TAGAWA et al. (1994) plasma glucose increased gradually with the changes of body position and was significantly higher in the supine position than lateral recumbent position in cattle.

In the present study, LR induced a slight rise in plasma NEFA level. This might be due to the previously mentioned increased secretion of catecholamine. MCQUAY et al. (1999) attributed the increases in plasma NEFA level after stress perceived by

horses during laparoscopic surgery to hypoxemia, such explanation might be correlated to our observations, as hypoxemia during LR was recorded. To our knowledge, this is the first report of suppression of plasma NEFA (lipolysis) level in cows after xylazine treatment, this may be mediated by both central and peripheral $\alpha 2$-adrenoceptors, as previously reported for dogs (TAOUIS et al. 1988; AMBRISKO and HIKASA 2002) human (VIKMAN et al. 1996) calves (SCHOLTYSIK et al. 1998) and cat (KANDA and HIKASA 2008).

In cows of both xylazine treated groups, no matter if cows remained standing or were turned into LR, we found that xylazine induced a fully metabolically compensated respiratory acidosis as indicated by unchanged blood pH and increase in P_aCO_2, HCO_3, BE and SID. No disturbance in the acid base balance was observed in placebo treated cows and turned into LR (Tab.10 &11 Appendix).

In the present study, RBCs, Hb and PCV significantly decreased after treatment with xylazine, this may be explained by pooling of the circulating reservoirs due to the decreased sympathetic activity (WAGNER et al. 1991). On the other hand, there was a mild decrease in the body temperature in cows of both xylazine treated groups which may be attributed to the depression of the hypothalamic–thermoregulatory centre (MACDONALD et al. 1989; BANI ISMAIL et al. 2010).

The procedure of turning cows into LR in the xylazine and placebo treated group was always uncomplicated and possible without any force. In our experience in cows used to human contact and handling significant force will provoke disturbance, fear, and defence reactions in other words stress slowing down the whole process of turning cows into LR. Calm, gentle and patient handling, supported by a comfortive voice and clear guidance, giving cows some time to explore the unfamiliar environment (here surgery theatre with tipping table) and let them understand and learn where they are expected to go and stand will reduce the stress for the cows and will thereby improve their cooperation and handling. We agree with WAIBLINGER et al. (2004) that fair handling and the strict avoidance of fear and painful situations is not only in the interest of the well-being of cows but will also

simplify the daily work with cows. Nevertheless, according to the observed increase in plasma cortisol, LR is experienced as stress by cows, even when handled appropriately as in this study. This stress can be eased by pre-treatment with xylazine. We see the use of xylazine for sedation certainly indicated in excited or aggressive cattle or those which express significant fear or discomfort. However, the desired effects of sedation and analgesia need to be balanced against the adverse effect of cardio-respiratory depression of xylazine. In our opinion in cows being trained to close human contact by calm and gentle handling avoiding pain and discomfort from an early age on as suggested by WAIBLINGER et al. (2004) the use of xylazine is not implicitly indicated before cows are turned into LR.

Conclusions

Pre-emptive low dose xylazine treatment (0.05 mg kg^{-1} BW) of adult dairy cows can alleviate experienced stress during lateral recumbency on a hydraulic tipping table and claw trimming according to results of cardiovascular (MAP), hormonal (cortisol) and metabolic (lactate, NEFA) stress response. Xylazine treatment in a low dose has some additional effect on respiratory depression of lateral recumbency in dairy cows. The use of higher doses of xylazine before cows are turned into lateral recumbency may lead to clinically relevant respiratory depression when no extra oxygen is administered. When xylazine is applied before cows are turned into LR the desired effects of sedation and analgesia need to be balanced against the adverse effect of cardio-respiratory depression of xylazine.

3.6 Tables and figures

Table 2: Mean scores for sedation and free gas bloat (mean ± SEM) in cows which were turned into lateral recumbency (LR; time 30 – 60 min) following IM injection (time 0) of placebo (Plac-LR; n = 6) or xylazine (Xyl-LR; n= 6) or which remained standing after xylazine application (Xyl-St; n = 6).

		Time after treatment [min]					
	Treatment	**-15**	**15**	**75**	**105**	**135**	**195**
Sedation score	P - LR	0.0±0.0	1.0±0.4^{b*}	1.0±0.7^{b*}	0.7±0.4^{b*}	0.3±0.4^{b}	0.3±0.3^{b}
	X - LR	0.0±0.0	3.2±1.5^{a*}	3.8±1.8^{a*}	3.5±1.8^{a*}	2.3±0.9^{a*}	1.7±0.4^{a*}
	X - ST	0.0±0.0	4.7±1.0^{a*}	4.5±1.0^{a*}	3.7±0.8^{a*}	2.8±1.1^{a*}	2.0±0.9^{a*}
Bloat score	P – LR	0.0±0.0	1.0±0.6*	0.5±0.4^{b}	0.0±0.0^{b}	0.3±0.3	0.0±0.0
	X – LR	0.0±0.0	1.0±0.6*	1.0±0.6^{ab*}	0.3±0.3^{ab}	0.5±0.4	0.0±0.0
	X – ST	0.0±0.0	1.3±0.4*	1.3±0.4^{a*}	0.7±0.4^{a*}	0.3±0.3	0.0±0.0

Corresponding means with different superscripts differ significantly (P < 0.05) among groups.

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.

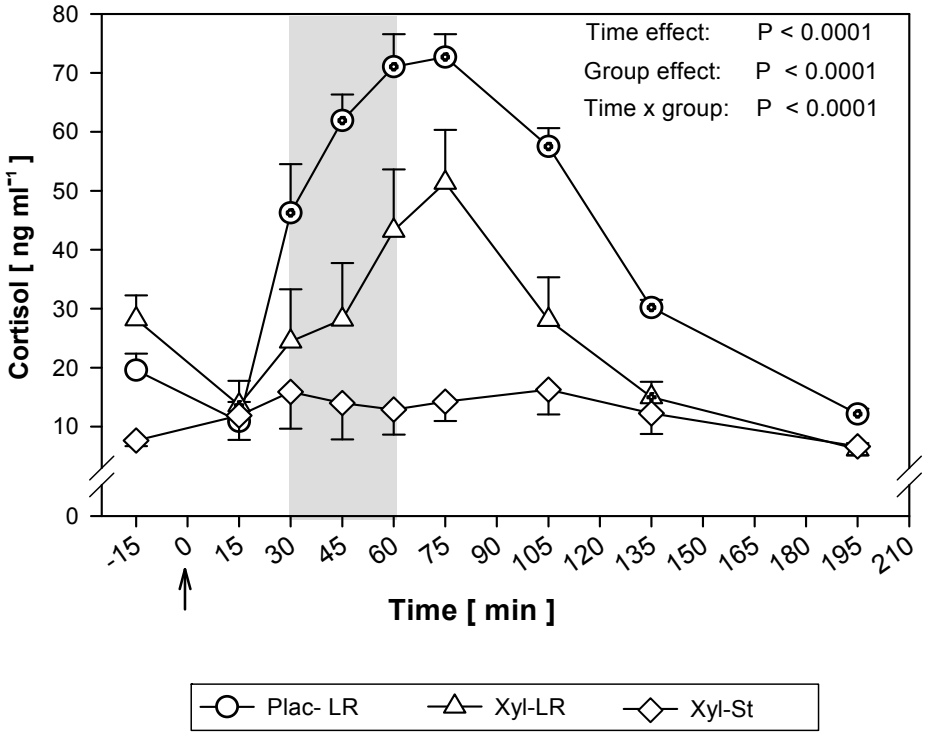

Fig.5: Plasma cortisol concentrations (mean ± SEM) in cows which were turned into lateral recumbency (LR; time 30 – 60 min; grey underlay) following IM injection (time 0) of placebo (Plac-LR; n = 6) or xylazine (Xyl-LR; n= 6) or which remained standing after xylazine application (Xyl-St; n = 6). Symbols with a cross differ significantly (P < 0.05) from baseline.

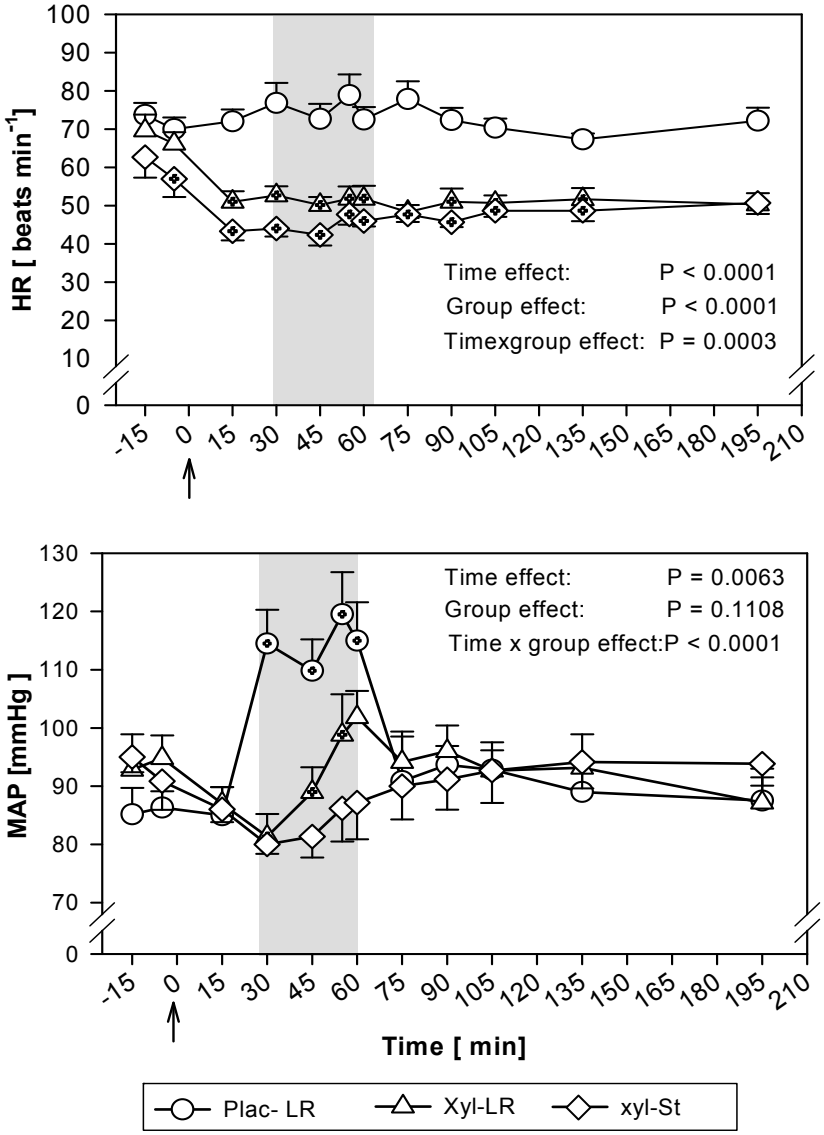

Fig.6: Heart rate (HR) and Mean arterial blood pressure (MAP) (mean ± SEM) in cows which were turned into lateral recumbency (LR; time 30 – 60 min; grey underlay) following IM injection (time 0) of placebo (Plac-LR; n = 6) or xylazine (Xyl-LR; n= 6) or which remained standing after xylazine application (Xyl-St; n = 6). Symbols with a cross differ significantly (P < 0.05) from baseline.

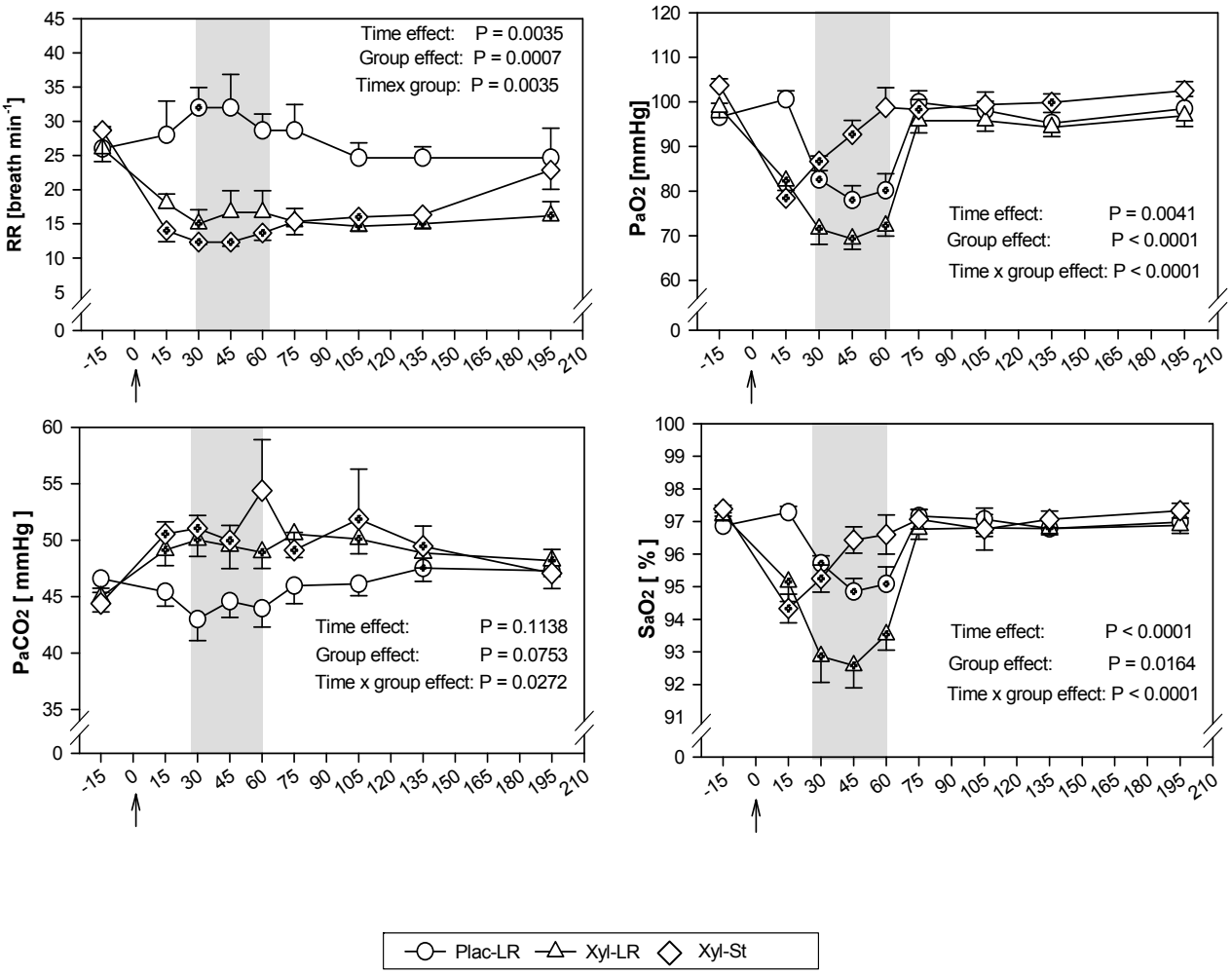

Fig.7: Respiratory rate (RR), mean arterial partial pressure of oxygen (PaO2), carbon dioxide (PaCO2)and arterial oxygen saturation (SaO2)(mean ± SEM) in cows which were turned into lateral recumbency (LR; time 30 – 60 min; grey underlay) following IM injection (time 0) of placebo (Plac-LR; n = 6) or xylazine (Xyl-LR; n= 6) or which remained standing after xylazine application (Xyl-St; n = 6). Symbols with a cross differ significantly (P < 0.05) from baseline.

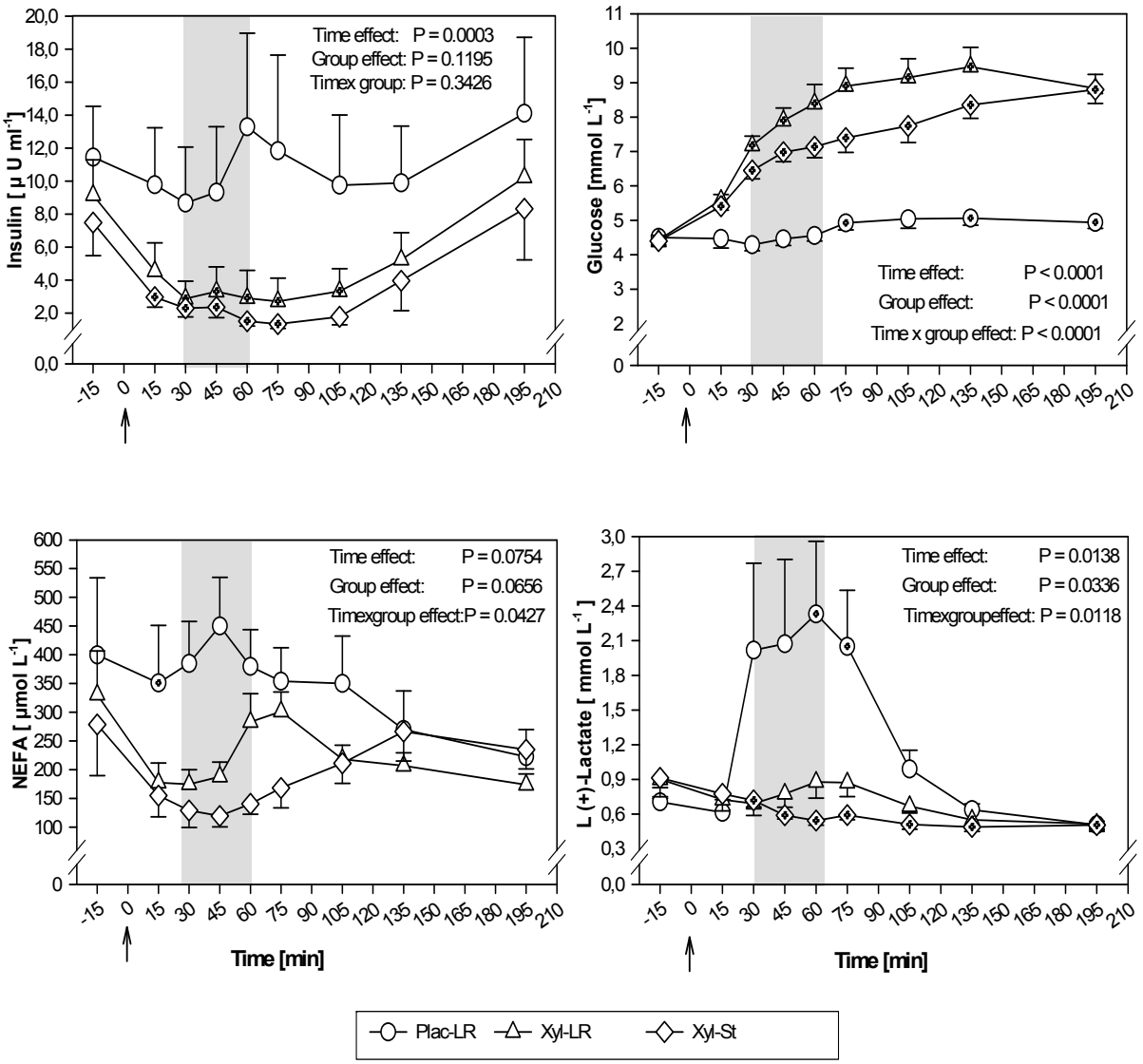

Fig.8: Plasma insulin, glucose, non-estrified fatty acid (NEFA) and lactate levels (mean ± SEM) in cows which were turned into lateral recumbency (LR; time 30 – 60 min; grey underlay) following IM injection (time 0) of placebo (Plac-LR; n = 6) or xylazine (Xyl-LR; n= 6) or which remained standing after xylazine application (Xyl-St; n = 6). Symbols with a cross differ significantly (P < 0.05) from baseline.

4 Chapter 2: The use of xylazine hydrochloride (Rompun®) in the analgesic protocol for claw treatment in lateral recumbency on a surgical tipping table in lame dairy cows

4.1 Summary

The aim of this study was to investigate the effect of pre-emptive xylazine treatment in the analgesic protocol for claw treatment in lateral recumbency (LR) in lame cows on the stress and pain response. In a prospective, blinded, placebo-controlled clinical case study, 24 lame, German Holstein Frisian cows (not more than four month pregnant), weighing 531±85.5 kg and aged 4.4 ±1.5 (mean ± SD) years old were used. Cows were randomly allocated into two groups of 12 cows each and either treated with Xylazine (Rompun®; 0.05 mg kg^{-1} BW, IM) or an equal volume of sterile saline (controls) 15 minutes before LR for claw treatment. After initial claw examination in each cow a retrograde intravenous local anaesthesia (LA) with 20 ml of 2% procaine was performed. At regular preset time intervals over an observation period of 6 hours (30 min before drug application to 6 hours post-operative) heart rate (HR), respiratory rate (RR), plasma levels of cortisol, glucose, lactate and non-esterified fatty acids (NEFA) were determined and signs of behaviour monitored (via video recording and pedometer). All cows treated with xylazine showed mild signs of sedation for about one to two hours, and were able to walk and to stand at all times. In the evaluation of behavioural signs xylazine demonstrated significantly ($P < 0.05$) additional analgesic effects to LA by reduced pain response on the insertion of the needle for LA, reduced ear flicking during claw treatment, reduced lameness score and longer standing periods in the first hour after claw treatment and improved appetite after claw treatment in LR. No significant ($P < 0.05$) difference was found for rumen motility in both groups. In both groups rumen motility ceased during the surgical intervention in LR. Mean HR and RR as well as plasma levels of lactate and NEFA were significantly ($P < 0.05$) reduced after xylazine treatment compared to baseline and controls. Mean plasma concentration of cortisol was significantly ($P < 0.05$) lower in xylazine treated cows after being turned into LR but raised to levels in

controls during the surgical treatment. In conclusion, according to the signs of behaviour monitoring, plasma cortisol, NEFA and lactate, the use of low dose xylazine in the analgesic protocol for treatment of lame cows in LR on a surgical tipping table can alleviate stress and has additional analgesic effects to LA. Due to cardio-respiratory depression by xylazine we discourage to use higher doses of xylazine in cows receiving claw treatment in lateral recumbency.

4.2 Introduction

Lameness in cattle has a great economic impact on the dairy industry, it is ranked as the third most important problem after mastitis and reproduction disorders (CLARKSON et al. 1996; RAJALA-SCHULTZ and GROHN 1999; WHITAKER et al. 2000). Ninety percent of all limb affections in cattle result from claw disorders (MURRAY et al. 1996; VAN AMSTEL and SHEARER 2006). The incidence of claw horn lesions associated with lameness is a major concern in managing modern intensive dairy herds. The claw health disorders lead to traumatisation of tissue which is perceived by the affected animal as a pain. The pain experienced by lame dairy cows is an important animal welfare issue and results in decreasing productivity (feed intake, fertility, milk yield)(CLARKSON et al. 1996; RAJALA-SCHULTZ and GROHN 1999; WHITAKER et al. 2000; MELENDEZ et al. 2003) and alters behaviour (HASSALL et al. 1993; GALINDO and BROOM 2002).

Pain management in farm animals including dairy cows has not progressed to the same degree as in companion animal practice and remains as a challenge for the veterinary profession (SHORT et al. 1992). Pain due to claw lesions is a serious component of lameness often masked by the stoical nature of cattle, this leads to delayed detection of lameness by farmers and often results in treatment without consideration of the pain experienced by the cow (MURRAY et al. 1996). Pain in cattle may be alleviated by pre-emptive, reactive or multimodal analgesia which is currently recommended for effective pain control for surgical interventions. Where pain is predictable like when conducting surgical procedures, it is preferable to provide pre-emptive analgesia by ensuring that effective analgesia is in place before

the onset of pain. In humans and animal medicine, it is well recognised that the most effective analgesia is provided using a combination of agents which act on different pathways, resulting in additive or synergistic analgesia and lower total doses of analgesics and minimal side effects. However, this strategy is often neglected in cattle (JIN and CHUNG 2001; VALVERDE and GUNKEL 2005; HUDSON et al. 2008). Thus, multimodal pain management may be essential for all operative procedures of the bovine digit, it allows not only the surgeon to operate safely but also prevents the sensitisation to pain and enhance convalescence by decreasing inflammation (ANDERSON and MUIR 2005; STARKE et al. 2008).

The clinical use of analgesics for the alleviation of post-operative pain is increasingly being applied to animals (KEHLET and DAHL 1993). The choice of the analgesic regimen in a particular setting is a complex matter, dependent on factors such as the animals species involved, the type and duration of surgery, the severity of the pain and the efficacy of the analgesics (KEHLET and DAHL 1993). The low use of analgesics in farm animals is attributed to economical and practical considerations such as the low cost of individual animals relative to the cost of treatments and the current paucity of licensed analgesic agents for the use in animals intended for human consumption (O'CALLAGHAN 2002) as well as concern about drug residues in foods of animal origin and the need to observe withdrawal time. As cattle are food producing animals, the drugs available for using as analgesics are restricted by licensing regulations, thus the classes of drugs that have analgesic activity suitable for cattle include local anaesthetics, non steroidal anti-inflammatory drugs (NSAIDs) , α2-agonists and partial opiods agonists (WHAY et al. 2003).

The alpha2-agonist xylazine in cattle has a dose dependent effect, it has both analgesic and sedative effects (GREENE and THURMON 1988). The analgesic effects are very short lived (< 1 hour) but the sedative effects can last more than 24 hour, which makes it poor choices for long term pain management (VALVERDE and GUNKEL 2005 ; NAVARRE 2006). Thus, it can be administrated to relieve stress, keep the animal calm on the operating table and provide a short term analgesic effect (THURMON and KO 1997; PESENHOFER et al. 2006). Furthermore, it acts on α2-

receptors located throughout the body to produce the opposite response to the noradrenaline. Pain relief occurs by activating α2-receptors in the brain and spinal cord, reducing the transmission of pain (FIERHELLER et al. 2004).

Intravenous regional analgesia (IVRA) has a rapid onset, is easy to perform and is more reliable compared to nerve blocks. It is the technique of choice for surgery of the distal bovine limb (ANTALOVSKY 1965; PRENTICE et al. 1974; NUSS 2004). It also allows intravenous administration of a water soluble antibiotic together with the local anaesthetic (STANEK 1994; NAVARRE et al. 1999). Furthermore, there is little risk of thrombosis of the digital veins with repeated injections or when high doses of antibiotics are administrated (PESENHOFER et al. 2006). It desensitises the limb distal to the tourniquet and is very useful for painful procedures in the foot. Nevertheless, it is underused in the treatment of lame cows (WHAY et al. 2003; STARKE et al. 2007c).

Many reports have highlighted the importance of behaviour for assessment of pain in animals, in particular in lame dairy cows (MORTON and GRIFFITHS 1985; O'CALLAGHAN 2002; FEIST 2004). Changes of the mental status of the cow, facial expression, carriage of the head and ear, locomotion score, feed intake, rumination, standing and lying behaviours as well as vocal expression like vocalisation and teeth grinding were observed as pain indicators (MORTON and GRIFFITHS 1985; STAFFORD et al. 2002). Pedometers are a valuable tool for detecting lameness for early discovery and treatment of developing foot affections through the recording of the duration of periods of activity, standing and lying. Also feed intake and rumination behaviour may indicate pain (MAATJE et al. 1997; MAZRIER et al. 2006; MCGOWAN et al. 2007). FEIST (2004) found that, the activity of lame cows undergoing pain therapy was higher than in controls.

We have recently reported (chapter 1) that, pre-emptive low dose application of xylazine can alleviate stress when cows are turned into LR for painless claw trimming with little additional effect on cardio-respiratory function. The aim of this study is to investigate the effect of pre-emptive application of xylazine in the analgesic protocol

for surgical treatment of claw disorders in lame dairy cows in lateral recumbency on stress and pain response.

4.3 Material and methods

The study was approved by Animal Care and Committee of the Federal State of Lower Saxony for consumer protection and food safety, Germany (research permit number 33.9-42502-05-08A573).

4.3.1 Selection of animals, housing and feeding

The study was conducted on 24 clinically lame German Holstein Frisian cows, non-pregnant or not pregnant for more than 3 – 4 months with an average weight of 531 ± 85.5 kg (mean± SD) [range 370 to 743 kg] and an average age of 4.4± 1.5 years [range 2 to 7.4 years]. All cows suffered from lameness due to claw lesions and were referred to the Clinic for Cattle, University of Veterinary Medicine Hannover, Germany, for adequate surgical claw treatment. The type of claw alterations is listed in table 17 in the appendix. No other noteworthy diseases such as displacement of the abomasum, ketosis, acute stages of mastitis or endometeritis, or extreme fever were detected. No analgesics were given within one week before admission to the clinic. Cows were kept in individual free stalls on straw bedding and were fed a diet based on hay and concentrates according to maintenance.

4.3.2 Study design and experimental procedures

The present trial was performed as blinded, randomized, prospective and placebo-controlled clinical study. An indwelling venous catheter (WVI Jugular catheter® ,ø 2.4mm, length 20cm with Teflon catheter, Walter, Veterinär-instrumente e.K., Baruth /Mark, Germany) was introduced into the right jugular vein for repeated blood sampling (Fig.15, appendix) and it was removed after the last blood sample was taken on the same day.

Lame cows were randomly allocated before the diagnosis of the claw disease into either the xylazine group (X; n= 12) or controls group (C; n= 12) and received an

intramuscular injection of xylazine (Rompun®, Bayer, Leverkusen, Germany) in a low dose of 0.05 mg kg^{-1} BW or an equal volume of sterile isotonic saline solution (0.9% sodium chloride solution, ad.us.vet. B.Braun, Germany) 15 minutes before LR for claw treatment. After initial claw examination on the surgical hydraulic tipping table in each cow of both groups a retrograde intravenous local anaesthesia (ANTALOVSKY 1965) with 20 ml of 2% procaine (Procosal 2%, Selectavet, GmBH, Holzolling, Germany) was performed.

Xylazine or placebo treatments were performed in the stable. Fifteen minutes after drug application cows were moved to the surgery theatre and turned into LR for initial claw examination, then (20 min after start of LR) the regional anaesthesia was applied and thereafter the surgical claw treatment performed according to the type of the claw affection. After turning into standing position cows were moved back to the stable.

4.3.3 Cardio-respiratory parameters

Heart rate was recorded by counting the heart beats in one min by auscultation, RR by counting thoracic excursions in 1 minute and rectal temperature was recorded by a digital thermometer.

4.3.4 Endocrine- metabolic parameters

Heparinised blood samples were collected in short term intervals and centrifuged at 1.500g (10 min, 4C°) immediately. Plasma was stored at -20 C° until analysis of cortisol (chemiluminescent enzyme immunoassay from Siemens diagnostics, Eschborn, Germany). Plasma glucose, nonesterified fatty acids (NEFA), Beta-hydroxybutrate (B-HBS) and lactate were measured on an automated analyser (ABX Pentra 400, Horiba ABX Diagnostics) using commercial test kits. Sodium and Potassium were measured by ion-sensitive electrodes and chloride by coulomb titration in serum. The strong ion Difference (SID) was calculated according to CONSTABLE (1999); SID= $[Na^+] + [K^+] - [CL^-]$.

4.3.5 Behavioural monitoring

4.3.5.1 Video recording

Behavioural signs were recorded using a portable digital video camera (Samsung, VP-MX 20, VPMX 20/EDC; China). The video camera was hand held (recording from behind and from lateral) or placed approximately 3 meter in front and 2 meter above the tipping table. For recording right before, during, and right after LR, so that one ear, the head, all four legs and the tail were visible on the recording. Signs of behaviour were camera recorded from 10 min before treatment while they were moved to the surgery theatre, during turning cows into LR, on the surgical tipping table, during turning back into standing position and on the way back to the stable. In addition to video recordings, a trained observer recorded the behaviour of cows using standard recording sheets. All behavioural recordings were analysed in frequency of occurrence in 10 minutes and scores according to table 3 and 4.

Sedation and free gas bloat were documented by a score system which was used in the first study (see chapter 1). For rumen motility the number of rumen contractions per 3 min was determined by auscultation. Food consumption was recorded as time in % of one hour.

Table 3: Lameness scoring system (SPRECHER et al. 1997).

LS	Clinical description
1	Stands and walks normally.
2	Mildly lame, stands with flat back, but arches when walks, gait is slightly abnormal.
3	Moderately lame-stands and walks with an arched back, and short strides with one or more legs.
4	Lame-arched back standing and walking, with one or more limbs favoured but at least partially weight was bearing.
5	Severely lame-arched back refuses to bear weight on one limb, may refuse or have great difficulty moving from lying position.

Table 4: Clinical description of behavioural signs included in the entire study modified after (MOLONY and KENT 1997; GALINDO and BROOM 2002).

Behaviour	*Description (Definition)*
During LR	***before, during and after LA***
Ear flicking	Vigorous movement of one or both of the ears independent of a head shaking (n/10min)
Head shaking	All exaggerated movements of the head without any discernible reason (n/10min).
Kicking	Attempts to use front or hind limbs during lying on a tipping table (n/10min).
Tail wagging	Rapid tail movement from side to side (n/10min).
Vocalisation	Occurrence of each vocal sound as a pain related behaviour (n/10min).
Defecation	Number of times of defecation (n/10min).
Urination	Number of times of urination (n/10min).
Tooth grinding	Score of teeth crushing as a pain indicators (score1: no to 5: highly severe).
During LA	
Response to needle insertion	Determination of pain response on venous needle insertion (score 1(no) to score 4 (severe defence reaction).

4.3.5.2 Pedometry

Pedometers (Ice Tag™ System, versions 2.004 and 2.009, Ice Robotics, Scotland, UK) were used to determine the proportion of time (in %) an animal is standing, lying or active (which total 100% for each time period) and also generate a count of steps taken in a given period. Each device weights 190 gm and is contained in a plastic housing (96 x 81 x 31 mm). The device is strapped to a cow's right or left hind metatarsus just above the fetlock joint for 24 hours (Fig.16 appendix). The data can be exported in time periods of minutes.

The Ice Tag™ analyses classify activity as follows: Lying = the animal is lying down in sternal or lateral position, Standing = the animal is still standing, and active = the animal is standing and moving. Where the animal is active, a step count indicates the number of steps taken by the animal during that time. Recordings were performed while the cow was in the individual pen during a period of 1 hour before treatment and over a period of 4 hours after LR and claw treatment. Results are expressed in average percentage of 1 hour (lying, active) and as mean number of steps per min during recorded active periods.

4.3.6 Examination and sampling schedule

Blood samples were collected from the jugular vein in heparin and fluoride coated tubes before drug treatment (baseline), 15 min after cows were turned into LR (before regional anaesthesia), at the end of LR, and one, three, and six hours after LR. Blood samples were centrifuged and plasma was stored at -20°C until analysis. At same time points HR, RR, lameness and sedation scores and rumen motility were determined.

4.3.7 Statistical analysis

Data were analysed using the statistical analysis system (SAS version 9.1 for Windows, SAS institute Inc, Cary, NC, USA). Data were presented as mean ±SD. To estimate the main effects and interactions of groups and time, a two way analysis of variance for repeated measurements (SAS-procedure GLM, Repeated measurement) was used. Additionally, the groups were compared at each time point using Bonferoni test while differences between each measurement and the baseline were tested using the paired t-test. Behaviour frequencies were tested for group differences by Fisher's test and scores by Wilcoxon rank-sum test. The level of significance was set at $P < 0.05$. The program Sigma Plot® 2001 (Systat Software Inc., Chicago, USA) was used to create graphs.

4.4 Results

4.4.1 Cardio- respiratory parameters

In the control group, in average HR and RR remained almost unchanged, while HR and RR were significantly ($P < 0.05$) decreased after xylazine treatment during and after LR compared to control cows and baseline (Fig. 9; Tab.16 appendix). Xylazine treatment induced a mild increase in body temperature (not significant; Tab.16 appendix).

4.4.2 Endocrine - metabolic parameters

Mean plasma level of cortisol was significantly ($P < 0.05$) lower in xylazine treated cows after being turned into LR but raised to levels in controls during surgical

treatment on the tipping table. No differences between groups were seen in plasma cortisol postoperatively (Fig.10; Tab.15 appendix). In each of both groups one cow exhibited with 2 ng ml^{-1} plasma cortisol concentrations which were close to the detection limit. In both cows plasma cortisol concentrations stayed at this low level, even during the entire period of LR.

After xylazine treatment mean plasma glucose was significantly ($P < 0.05$) higher compared to controls. In placebo treated control cows plasma glucose increased significantly ($P < 0.05$) compared to baseline during LR (Tab.5). Mean plasma NEFA concentrations remained significantly ($P < 0.05$) lower in xylazine treated cows during LR and in the period thereafter compared to controls (Fig.11; Tab.15 appendix). However, mean B-HBS showed no statistically difference between both groups (Tab.5). In both groups B-HBS rose significantly compared to baseline. Mean plasma lactate concentration showed little increase (n.s.) during LR in both groups and decreased significantly ($P < 0.05$) below baseline levels after LR. The mean SID was significantly ($P < 0.05$) increased in both groups during LR compared to baseline and showed greater values in the xylazine treated cows than in controls ($P < 0.05$; Tab.5).

4.4.3 Behavioural monitoring

The mean score of sedation was significantly ($P < 0.05$) greater before LR in the xylazine treated cows compared to controls but no group differences were seen after LR (Fig.13). For mean rumen motility no statistically significant ($P < 0.05$) group differences were found. In both groups rumen motility ceased during LR. Cows of both groups showed mild signs of ruminal free gas bloat after LR (Tab.7).

During the insertion of the needle for regional intravenous anaesthesia (LA), control cows exhibited in average on the visuell analogue scale (VAS) significantly greater values than xylazine treated cows (Tab.6). After the LA (during claw treatment) ear flicking was significantly more often seen in controls than in cows of the xylazine group. Other signs of behaviour during the period cows were in LR did not differ significantly (Tab.6). The recorded mean lameness score was in the xylazine group significantly lower one hour after claw treatment than in controls (Fig.12).

In xylazine treated cows the evaluation of the pedometer recordings revealed in the first hour after claw treatment in LR a significantly ($P < 0.05$) higher percentage of standing than in controls. The average period of activity (standing and active) was not significantly different between both groups and generally with about 2 – 3 % per hour low. Also the mean number of steps per min during active periods was not significantly different (Tab.8).

Coming back from claw treatment in LR to the free stall, xylazine treated cows started to consume offered feed mostly immediately, while controls preferred to lie down. On average the time period in % of one hour cows spent feeding was longer in xylazine treated cows than in controls (n.s.; Tab.7).

4.5 Discussion

In this study the alpha2- agonist xylazine demonstrated again (LIN and RIDDELL 2003) its sedative effect in cows. According to our score system and in agreement with the literature (HALL et al. 2001; LIN and RIDDELL 2003; chapter 1) sedation lasted in the low dose we used for about 1 – 1.5 hours just long enough to cover the period of claw treatment in our cows. Cows experience being turned into and being fixed in LR as stress (chapter 1; GRANDIN 1998; PESENHOFER et al. 2006) which can be seen also in the typical stress responses here on of plasma cortisol and glucose concentrations (CHACON et al. 2005; MUDRON et al. 2005) in the cows of this study. As in a previous study (chapter 1) cows which were sedated with xylazine exhibited on average lower plasma cortisol responses as controls when they were fixed on the tipping surgery table in LR. However, at the end of the claw treatment mean plasma cortisol concentrations reached the same level as in controls. We have to distinguish between handling stress leading to fear or discomfort and stress provoked by pain (PARROTT et al. 1987; LEY et al. 1991; SYLVESTER et al.1998a; 1998b; HEINRICH et al.2009). During LR but before the LA claws were trimmed and the affected claw of our cows were examined by paling off lose horn and using probes and in some cases taking x-rays, so without producing pain. In contrast after the LA claw defects were treated and nociceptors were stimulated in surgical area.

Thus, we assume that the HPA-axis was activated despite xylazine pre-treatment by strong stimulation of nociceptors during the surgical claw treatment, although all cows received regional intravenous anaesthesia, which is seen as the most effective analgesic regime in surgeries on the distal limb in cattle (ANTALOVSKY 1965; PRENTICE et al. 1974; NUSS 2004).

In both groups we found one cow which exhibited during the entire experimental period extremely low plasma cortisol concentrations (< 2 ng L^{-1}) with even absolutely no increase in plasma cortisol during LR indicating no stress induced response of the HPA axis. We did no further investigations in these two cows but we assume that prior to admission to the clinic the animals received glucocorticoids which can suppress the response of the HPA axis (MINTON 1994; SYLVESTER et al.1998a; HEINRICH et al.2009) although the owner of the animals affirmed the converse. If there was an elsewise acquired or congenital insufficiency of the HPA-axis can not be answered from this study.

The stress provoked cortisol and catecholamine release commonly induces lipolysis and thereby increased plasma NEFA concentrations (EL-GHOUL and HOFFMANN 2002; CHACON et al. 2005; MUDRON et al. 2005). In agreement with a previous study, where cows also experienced a stress challenge by fixation in LR, plasma NEFA concentrations remained significantly lower during and after LR in consequence of the xylazine pre-treatment. The suppression of stress induced lipolysis may be mediated by both central and peripheral α2-adrenoceptors as previously reported for dogs (TAOUIS et al. 1988; AMBRISKO and HIKASA 2002) human (VIKMAN et al. 1996) calves (SCHOLTYSIK et al. 1998) and cats (KANDA and HIKASA 2008). This xylazine effect may be important in early lactation when cows exhibit frequently enhanced lipomobilisation and are prone to the development of considerable fatty liver (DRACKLEY 1999).

The alpha-2 agonist xylazine has shown analgesic effects in horses (OHNESORGE et al. 1991), goats (DEROSSI et al. 2005), buffaloes calves (SARRAFZADEH-REZAEI et al. 2007) and in cattle (LEE and YAMADA 2005). Due to a synergism

(MCQUAY et al. 1999) analgesic effects of local anaesthetics are prolonged when used in combination with xylazine in horses (BENSON et al. 2000) and sheep undergoing orthopaedic surgery (SCOTT and GESSERT 1996; 1997). As in other studies (SHUTT et al. 1989; MOLONY et al. 1993; GRAF and SENN 1999; GRONDAHL-NIELSEN et al. 1999; GALINDO and BROOM 2002) changes in cow's behaviour such as lameness scoring, activity, appetite and changes on pain induced behaviour changes were used to evaluate the stress and pain perceived by lame cows during claw treatment in LR after xylazine pre-treatment. However, the use of behavioural parameters to assess pain and to determine the analgesic effectiveness of drugs presents a challenging task (MOLONY and KENT 1997). In comparison to controls xylazine pre-treated cows exhibited in this study significantly I) reduced pain response during the insertion of the needle for intravenous regional anaesthesia, II) less frequent ear flicking during the surgical intervention at the claws, III) lower lameness scores and IV) longer standing periods during the first hour after claw treatment, and V) more frequently immediate start of feed consumption when cows arrived back in their individual free stall from claw treatment.

All these behaviour alterations may indicate less pain perception and improved well-being of the xylazine treated cows (FRASER and BROOM 1990, WHAY et al. 1998; FAULKNER and WEAVY 2000; LAVEN et al. 2008). No group differences were found for other behaviours such as teeth grinding, head shaking, kicking, tail wagging, vocalisation, defecation and urination. However, in our experience these behaviour responses are provoked in cows which are used to human contact and handling due to their stoic nature only when very painful procedures are performed or cows feel significant fear or discomfort. The dairy cows used in this study came from relatively small regional dairy herds (less than 100 cows / herd) and were therefore in majority used to close human contact, including claw trimming and claw treatment, so that we do not assume the stimulation of extreme fear or discomfort on handling in cows of both groups. Also extreme pain during claw surgery can be excluded because all cows received LA. Thus, severe defence reactions during the period of LR and claw treatment could not be expected because the stress and pain challenge was relatively low in our cows. However, considerable defence reactions as head

shaking, vocalisation, kicking, or tail wagging were seen little more frequently in cows of both groups compared to period of LR before LA and little, but not significantly, more frequently in control than in xylazine treated cows. We conclude therefore that the pre-treatment with xylazine in combination with LA improved and prolonged to some extent analgesia compared to LA alone.

Although α2-agonists have been reported to cause prolonged depression of thermoregulation (HALL et al. 2001), we found no significant alterations of rectal body temperature after xylazine treatment. This may be due to the low xylazine dose we used, that claw treatments in LA were performed in a surgery theatre at room temperature. Also in a previous study (chapter 1), where healthy cows were turned into LR, no changes in body temperature were found after xylazine application.

Alpha2-agonists such as xylazine inhibit rumen motility by stimulation of α2-adrenergic receptors in the fore-stomach musculature (GUARD and SCHWARK 1984; RUCKEBUSCH and ALLAL 1987). In consequence, rumen motility ceased and mild free gas bloat was frequently seen in cows after xylazine treatment. In agreement with ROSENBERGER et al. (1968) also in placebo treated control cows no rumen contractions were detected by auscultation during LR and claw treatment and mild free gas bloat was observed after the cows were turned back into a standing position. Since stress hormones such as catecholamine also have the potential to inhibit rumen motility we suppose that stress by LR and claw treatment induced the observed rumen atony in controls. Thus as in a previous study (chapter 1) we conclude that, the use of xylazine in a low dose poses no additional risk of bloat in cows turned into LR for claw treatment and that the observed mild free gas bloat in both groups is of little clinical relevance.

In this study cows treated with xylazine showed significantly lower mean HR and RR during LR compared to baseline and controls. This is in accordance with other studies where the depressive effect of α2-agonists on cardio-respiratory function was already shown (CLARKE and HALL 1969; CAMPBELL et al. 1979; BREST 1980; HODGSON et al. 2002; PICAVET et al. 2004; NANDI et al. 2008; chapter 1). Alpha2-

agonists can lead also to dose dependent, reduced cardiac output, reduced mean arterial blood pressure, increased vascular resistance, increased pulmonary shunt volume, decreased arterial oxygen partial pressure and saturation and to increased arterial carbon dioxide partial pressure (MEYER et al 2009; chapter 1). Similar effects on cardio-respiratory depression are provoked by lateral recumbency (WATNEY 1986a; KLEIN and FISHER 1988; WAGNER et al. 1990; TAGAWA et al. 1994; chapter 1). In a previous study it was demonstrated that xylazine already in a low dose aggravates the effects of LR on respiratory depression and that arterial oxygen saturation can fall below 92%. An arterial oxygen saturation of 92% is seen as just sufficient for tissue oxygenation (WAGNER et al. 1990).

Lactate is a metabolic product of anaerobic glycolysis and arises from pyruvate (CHACON et al. 2005). A mild increase in blood lactate concentrations were reported in cattle during different surgical interventions such as claw treatments and abdominal surgeries and were attributed to stress induced vasoconstriction and reduced tissue oxygenation (EL-GHOUL and HOFFMANN 2002; CHACON et al. 2005; MUDRON et al. 2005). In contrast to our previous study (chapter 1) in this study mean plasma lactate concentrations did not increase significantly during the stress induced by LR and claw treatment. Plasma lactate remained on average lower in cows of the xylazine group than in controls which are in agreement with previous presented results (chapter 1). Thus, we do not have an indication for reduced tissue oxygenation in cows of both groups in this study. Nevertheless, because of the significant aggravation of LR induced respiratory depression by xylazine treatment already in a low dose (chapter 1). We conclude that xylazine should not be given in higher doses when cows are turned into LR.

Conclusions

The pre-emptive xylazine treatment in a low dose (0.05 mg kg^{-1} BW) can reduce hormonal and metabolic stress response and has short term additional mild analgesic effects to LA in lame cows receiving claw treatment in LR. Thus, xylazine appears to be an appropriate sedative for stress alleviation in cows turned into LR and can be used as an analgesic in a multimodal analgesic protocol for short term

pain management during claw surgeries. Since xylazine has as lateral recumbency depressive effects on cardio-respiratory function the desired effects of sedation and analgesia have to balanced against the possibility of reduced tissue oxygenation before the use of xylazine in cows turned in LR, at least we discourage to use higher doses of xylazine under field conditions.

4.6. Figures and tables

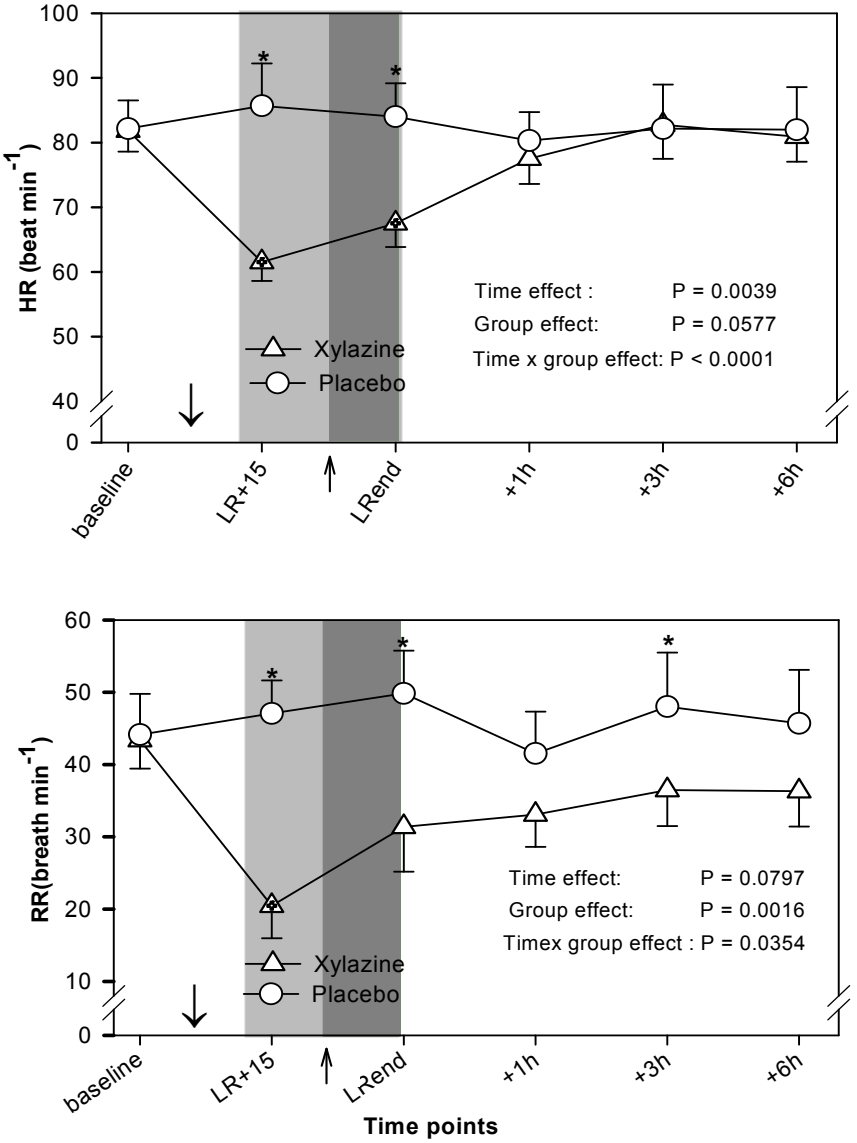

Fig.9: Heart rate (HR) and respiratory rate (RR; mean ± SEM) of cows following treatment (↓) with xylazine (n= 12; 0.05 mg kg^{-1} BW IM) or placebo (controls; n= 12; equal volume IM) measured at baseline in the standing cow at the stable, during lateral recumbency [LR] (after 15 min and at the end) and at 1, 3, 6 hours post-operative. The period of LR [grey underlay] includes the time before local anaesthesia (↑; light grey) and after local anaesthesia (dark grey). Symbols with a cross differ significantly ($P < 0.05$) from baseline. Significant ($P < 0.05$) differences between group means are indicated by an asterisk (*).

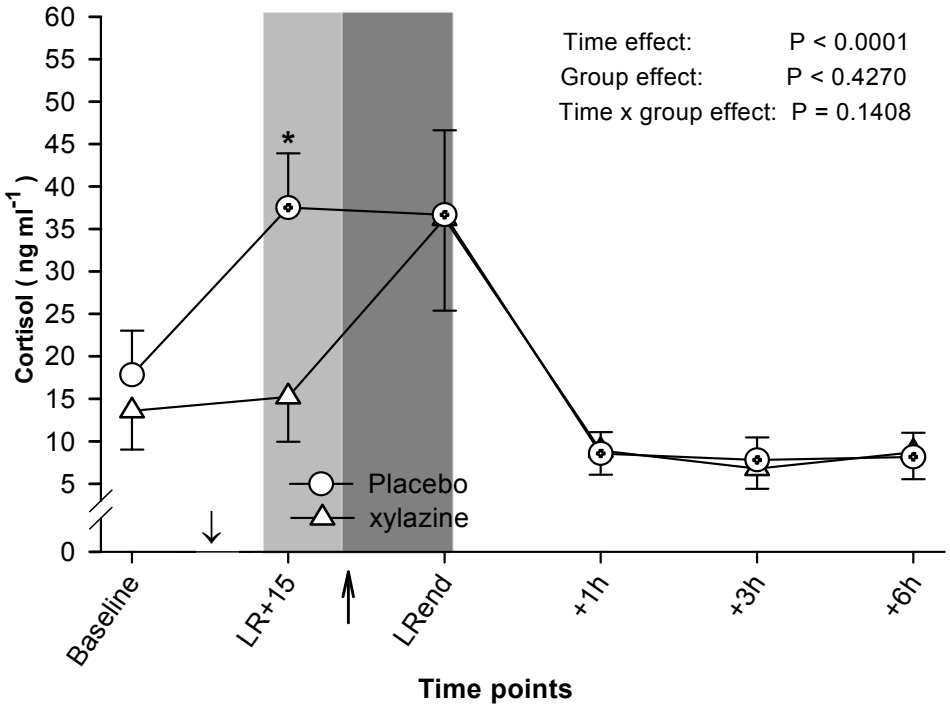

Fig.10: Plasma cortisol concentration (mean ± SEM) of cows following treatment (↓) with xylazine (n= 12; 0.05 mg kg^{-1} BW IM) or placebo (controls; n= 12; equal volume IM) measured at baseline in the standing cow at the stable, during lateral recumbency [LR] (after 15 min and at the end) and at 1, 3, 6 hours post-operative. The period of LR [grey underlay] includes the time before local anaesthesia (↑; light grey) and after local anaesthesia (dark grey). Symbols with a cross differ significantly (P < 0.05) from baseline. Significant (P < 0.05) differences between group means are indicated by an asterisk (*).

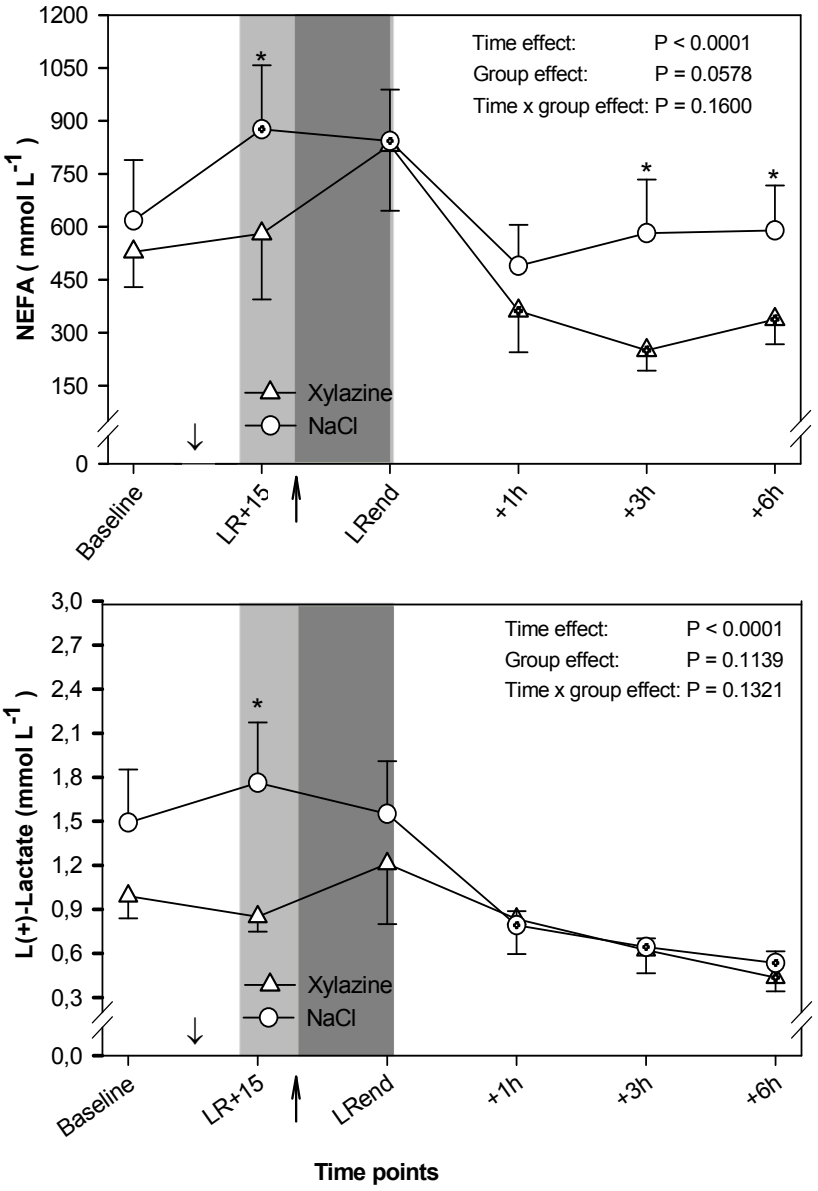

Fig.11: Plasma non-estrified fatty acid (NEFA) and plasma lactate levels (mean ± SEM) of cows following treatment (↓) with xylazine (n= 12; 0.05 mg kg^{-1} BW IM) or placebo (controls; n= 12; equal volume IM) measured at baseline in the standing cow at the stable, during lateral recumbency [LR] (after 15 min and at the end) and at 1, 3, 6 hours post-operative. The period of LR [grey underlay] includes the time before local anaesthesia (↑; light grey) and after local anaesthesia (dark grey). Symbols with a cross differ significantly ($P < 0.05$) from baseline. Significant ($P < 0.05$) differences between group means are indicated by an asterisk (*).

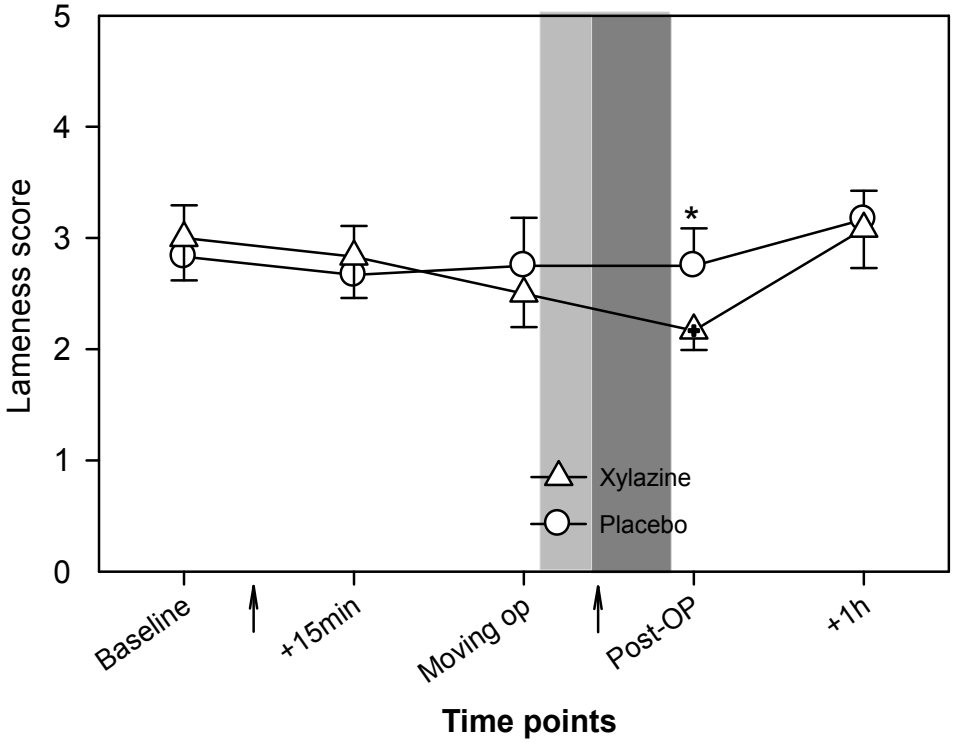

Fig.12: Lameness scores (mean ± SEM) of cows following treatment (↑) with xylazine (n= 12 ; 0.05 mg kg^{-1} BW IM) or placebo (controls; n= 12 ; equal volume IM) measured at baseline in the standing cow at the stable, 15 min after drug administration, during moving of the cow to operation threatre for claw treatment in lateral recumbency (LR; grey underlay) which include the time before local anaesthesia [↑; light grey] and after local anaesthesia [dark grey] then direct after standing and at one hour postoperative. Symbols with a cross differ significantly ($P < 0.05$) from baseline. Significant ($P < 0.05$) differences between group means are indicated by an asterisk (*).

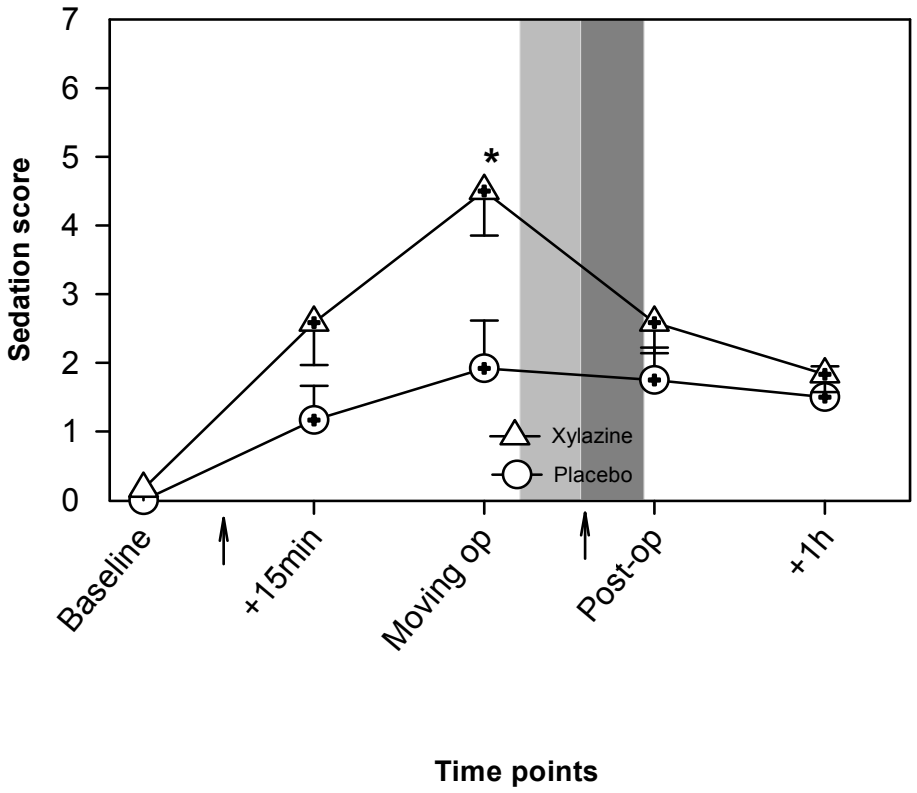

Fig. 13: Sedation score (mean ± SEM) of cows following treatment (↑) with xylazine (n= 12; 0.05 mg kg^{-1} BW IM) or placebo (controls; n= 12 ; equal volume IM) measured at baseline in the standing cow at the stable, 15 min after drug administration, during moving of the cow to operation room for claw treatment in lateral recumbency (LR; grey underlay) which include the time before local anaesthesia [↑; light grey] and after local anaesthesia [dark grey] then direct after standing and at one hour postoperative. Symbols with a cross differ significantly ($P < 0.05$) from baseline. Significant ($P < 0.05$) differences between group means are indicated by an asterisk (*).

Table 5: Plasma glucose, beta-hydroxybutirates (B-HBS) and strong ion difference (SID) (Mean± SD) of cows following treatment with placebo (controls; C; n= 12) and xylazine (X; n= 12) measured at baseline in the standing cow at the stable, during lateral recumbency [LR; grey underlay] (after 15 min and at the end) and at 1, 3, 6 hours post-operative.

Variables		LR			Post-OP		
		Baseline	**LR+15min**	**LR end**	**+1h**	**+3h**	**+6h**
Glucose	**C**	4.47±0.87	4.59±0.98[a]	**5.00±1.18[a]**	5.20±1.75[a]	4.50±1.02	**4.20±0.63**
[mmol L^{-1}]	**X**	4.92±1.25	**8.41±2.08[b]**	**8.95±3.18[b]**	**8.44±3.17[b]**	5.88±2.51	4.65±1.63
B-HBS	**C**	0.57±0.37	0.61±0.38	**0.85±0.55**	0.55±0.31	0.55±0.32	0.59±0.43
[mmol L^{-1}]	**X**	0.54±0.63	0.55±0.75	**0.84±0.41**	0.71±1.13	0.66±0.94	0.54±0.65
SID	**C**	40.4±2.08	**41.5±2.18[a]**	**42.4±1.56**	39.9±2.07	40.1±1.80	39.5±2.50
[mEq L^{-1}]	**X**	41.7±1.74	**43.9±1.51[b]**	**43.3±2.29**	41.5±2.39	**40.3±2.54**	40.3±2.50

Bold means differs significantly ($P < 0.05$) from base line within groups.

Corresponding means with different superscripts differ significantly ($P < 0.05$) among groups.

Table 6: Behavioural signs in frequencies and scores (Mean± SD) of placebo (controls; C; n= 12) and xylazine (X; n= 12) treated cows observed during lateral recumbency (LR; before, during and after local anaesthesia) on a surgical tipping table.

		During LR		
Variables		***Before LA***	***During LA***	***After LA***
Response to needle insertion (score) **#**	**C**	2.33±0.49	2.17±1.03[a]	1.83±1.50
	X	1.83±0.83	1.33±0.49[b]	1.50±0.67
Ear flicking [n / 10min]	**C**	1.04±0.71	2.26±1.76	1.79±2.07[a]
	X	0.99±0.62	1.78±1.96	1.29±0.88[b]
Head shaking [n / 10min]	**C**	1.08±0.87	2.23±2.17	1.51±1.18
	X	0.90±0.67	2.35±1.95	1.53±0.70
Kicking [n / 10min]	**C**	0.18±0.29	0.52±0.73	0.21±0.68
	X	0.26±0.59	0.18±0.49	0.31±0.40
Tail wagging [n / 10min]	**C**	0.41±0.47	1.52±1.62	0.56±0.92
	X	0.24±0.33	0.99±0.80	0.62±0.93
Vocalisation [n / 10min]	**C**	0.07±0.13	0.63±1.01	0.18±0.20
	X	0.37±0.61	0.58±1.25	0.46±0.42
Teeth grinding* [score]	**C**	1.25±0.45	1.42±0.67	1.25±0.62
	X	1.25±0.45	1.17±0.39	1.42±0.67
Defecation [n / 10min]	**C**	0.07±0.18	0.04±0.14	0.10±0.15
	X	0.07±0.19	0.00±0.00	0.06±0.14
Urination [n / 10min]	**C**	0.17±0.28	0.04±0.14	0.07±0.09
	X	0.08±0.15	0.19±0.31	0.08±0.09

LA = local anaesthesia; Corresponding means with different superscripts (a or b) differ significantly ($P < 0.05$) among groups. **# Response to needle insertion:** Score 1: no, 2: mild, 3: moderate, 4: severe and 5: highly severe. *** Teeth grinding:** Score 1: no, 2: mild, 3: moderate, 4: severe and 5: highly severe.

Table 7: Rumen motility and feed consumption (Mean± SD) of placebo (controls; C; n= 12) and xylazine (X; n= 12) treated cows observed for one hour after claw treatment in lateral recumbency (LR; grey underlay) on a surgical tipping table.

Variables				During LR			Post-OP
		Baseline	***AT***	***Before LA***	***During LA***	***After LA***	***+1 h***
Rumen motility	**C**	2.33±0.49	2.17±0.39	0.00±0.00	0.00±0.00	0.00±0.00	1.92±0.67
[contraction / 3min]	**X**	2.25±0.75	2.00±0.60	0.00±0.00	0.00±0.00	0.00±0.00	2.25±0.62
Feed consumption	**C**	ND	ND	ND	ND	ND	24,4± 14,4
[time in % of 1 hour]	**X**						38,6± 21,3

ND = not determined; LA= local anaesthesia ; AT= after treatment

Corresponding means with different superscripts differ significantly (P < 0.05) among groups.

Table 8: Pedometer results (Mean± SD) of placebo (controls; C; n= 12) and xylazine (X; n= 12) treated cows recorded for 4 hours after claw treatment in LR on a surgical tipping table (period of standing , lying [not shown] and activity sum up to 100%; Mean numbers of steps per minute during recorded active periods).

Parameter		Postoperative period [hour]				
		BL(-1h)	**+1h**	**+2h**	**+3h**	**+4h**
Standing	**C**	50.4±40.0	29.6±22.8[a]	23.3±21.1	32.8±32.9	**17.4±14.8**
[%]	**X**	59.5±42.2	61.4±24.6[b]	**36.8±27.3**	41.5±29.9	**35.5±29.4**
Activity	**C**	1.60±1.82	2.46±1.96	2.01±1.85	1.92±1.85	1.02±0.98
[%]	**X**	2.50±2.62	3.84±2.50	2.85±2.03	2.81±2.52	2.47±2.47
Nr. of steps	**C**	0.42±0.51	**1.01±0.92**	0.69±0.61	0.80±0.82	0.43±0.43
(per min)	**X**	0.98±1.18	1.58±1.06	1.15±0.96	1.07±0.91	0.93±0.87

BL = baseline (1 hour before treatment).

Bold means differs significantly ($P < 0.05$) from base line within groups

Corresponding means with different superscripts differ significantly ($P < 0.05$) among groups.

5 General discussion

The first aim of this study was to investigate the effects of pre-emptive xylazine application and lateral recumbency (LR) together on cardiovascular, respiratory and endocrine-metabolic stress responses in healthy dairy cows subjected to painless claw trimming on a surgical tipping tables in LR, while the second goal was to evaluate the effectiveness of xylazine in a multimodal analgesic protocol for alleviation of stress and relief of pain perception by lame cows undergoing claw treatment on a surgical tipping table in LR using the fore-mentioned variables, in addition to monitoring of behavioural signs via video recording and pedometers.

Results of this study confirm that IM administration of xylazine at a low dose 0.05mg kg^{-1} BW is relatively safe and induces effective moderate sedation in the treated animals, as high dose of xylazine may induce residual sedation after the claw treatment is finished which may hamper handling of the cows after restraining in LR. Thus, further investigations should be carried out to evaluate the effects of a moderate dose of xylazine.

In study 1, cows received the different treatments in a cross-over design in an experimental study. This design has the advantage of reducing individual subject variation from the overall treatment effects, and thus enhancing statistical power. Accordingly, only six animals were used in this study, which received in 2 weeks intervals (wash out period) alternating the three treatments a) placebo (control 1) or b) xylazine before restraining in LR or c) xylazine but without further manipulation (control 2). The three treatments gave the opportunity to distinguish clearly between the pure effects of LR and xylazine and the interaction of LR and xylazine. Beside cardio-respiratory function we wanted to investigate the effect of xylazine on the hormonal, metabolic and behavioural stress response of cows when turned into LR. From our daily work and a study of PESENHOFER et al. (2006) we knew that cows used to close human contact and fair handling tolerate fixation in LR generally without any noteworthy defence reactions indicating that such situations do not create a situation of considerable fear or discomfort and therefore most likely with

little stress. In order to create a scenario with a realistic stress challenge comparable to a situation in the field, we turned cows not only into LR but performed also claw trimming as a defined type of painless manipulation at the claws. With this study set-up we could investigate the effects of xylazine on an expected moderate but significant stress challenge without inducing pain and the expected cardio-respiratory depression of LR.

The cows in this study were used to human contact and handling so that without sedation all cows were led easily to and fixed in LR on the hydraulic tipping table with only two people, one at the head (with holster and rope) and one in the pelvic region. Guidance by low voice and gentle pushing in combination with patience to allow cows to explore the unfamiliar environment and to learn where and how they are expected to move were sufficient to bring the cow from the stable in a close and parallel position next to the tipping table. All cows had experience with claw trimming in LR. Thus, we conclude that previous experiences of cows were only little stressful and were not associated with considerable fear or pain. In pervious studies it was repeatedly shown that gentle and considerable handling from an early age will not only reduce cattle reactions during typical husbandry procedures but also stress and thereby well-being in cattle (HEMSWORTH et al. 1989; GRANDIN 1993; BOIVIN et al. 1994; DE PASSILLE et al. 1996). According to our experience from this study and in agreement with PESENHOFER et al. (2006) cows used to considerable human handling will well tolerate fixation in LR without pre-emptive sedative medication without showing signs of considerable fear such as attempts to escape, reluctance to move, leg or head kicking or pushing. However, LR does produce some stress as indicated by the release of the stress hormone cortisol during LR. This stress can be reduced by xylazine treatment. So we do not see necessarily the need for pre-emptive sedation of all cows before being turned into LR but it appears helpful in excited or aggressive cows to alleviate experienced stress and to make handling easier and safer for the handler.

Stressed cows respond with elevated plasma NEFA level indicating enhanced lipolysis mediated by the release of the stress hormones cortisol and catecholamine

(CHACON et al. 2005; MUDRON et al. 2005). Interestingly pre-treatment with xylazine does reduce significantly the increase of plasma NEFA, may be mediated by both central and peripheral α2-adrenoceptors as previously reported for dogs (TAOUIS et al. 1988; AMBRISKO and HIKASA 2002) human (VIKMAN et al. 1996) calves (SCHOLTYSIK et al. 1998) and cats (KANDA and HIKASA 2008). This xylazine effect may be important in early lactation when cows exhibit frequently enhanced lipomobilisation and are prone to the development of considerable fatty liver (DRACKLEY 1999).

In the second study we could also demonstrate by the evaluation of behaviour responses despite the stoic nature of cattle (FRASER and BROOM 1990) that xylazine will improve and extent the duration of local regional anaesthesia to some extent in cows undergoing surgical claw treatment, which was according to our knowledge not shown before.

However, xylazine pre-treatment will aggravate the depression of respiratory function by LR. We found in four out of six cows treated with xylazine before LR arterial SaO_2 values below 92%, which is just seen as sufficient for adequate tissue oxygenation (WAGNER et al. 1990). Lowest SaO_2 values we found were with about 90% just below the mentioned threshold. However the low plasma lactate level found in cows after xylazine treatment during LR did not give an indication for considerably increased anaerobic glycolysis. Thus, in our opinion xylazine still appears in a low dose despite the recorded respiratory depression to be an adequate sedative in cattle before LR but we discourage to use higher doses than the one used in this study (0.05 mg kg^{-1} BW). Xylazine has a dose dependent effect on respiratory depression and although not studied we suspect it may develop substantial clinical relevance. In order to avoid inappropriate use of xylazine we also discourage to give xylazine into the hands of non-professionals for cows before LR since the range between low (0.05 mg kg^{-1} BW) and high doses (0.1 mg kg^{-1} BW) is small and only very small volumes are used.

Although not studied xylazine should not be applied to cows in advanced stages of pregnancy since alpha2-agonists will increase uterus muscular tone and may cause abortion (GUARD and SCHWARK 1984; TAKASE et al. 1986). When the application of alpha2-agonists in pregnant cows is unavoidable cows should at least be pre-medicated with uterus relaxant drugs to reduce the risk of abortion.

In conclusion, the use of the alpha2-agonist xylazine in the analgesic protocol of cows for claw treatment in lateral recumbency will reduce the behavioural, hormonal and metabolic stress response of cows and will thereby improve welfare. In accordance with STARKE et al. (2007) when xylazine is used in cows before LR the desired effects of sedation and analgesia need to be balanced against the adverse effect of cardio-respiratory depression. As others (HEMSWORTH et al. 1989; GRANDIN 1993; BOIVIN et al. 1994; DE PASSILLE et al. 1996) we also encourage the training of cattle to close human contact and handling from the day of birth on. This will reduce handling stress and improve thereby welfare of cows and it will also reduce stress and will improve welfare of animal handlers by making handling of cows much easier.

6 Summary

Awad Zaboulla Hassan Rizk

The use of the alpha2- agonist xylazine (Rompun®) in a multimodal analgesic protocol for orthopaedic intervention in lateral recumbency on a surgical tipping table in dairy cows

Since animal welfare is a growing subject of concern, recently members of the European College of Veterinary Anaesthesiology demanded generally in order to avoid or at least to alleviate stress the pre-emptive use of sedatives before fixation of dairy cows in lateral recumbency (LR) for orthopaedic surgery. Thus, we performed two studies, an experimental study aimed to investigate the effects of pre-emptive xylazine treatment and LR in combination on cardio-respiratory, endocrine-metabolic and behavioural stress responses in healthy dairy cows undergoing painless claw trimming on surgical tipping tables. A clinical study was performed in which we hypothesized that the sedative, analgesic and myo-relaxant effects of xylazine in a pre-emptive treatment would improve the analgesic protocol for claw treatment in LR on a surgical tipping table in lame dairy cows.

Study 1

The aim of the study was to investigate the effects of lateral recumbency (LR) and xylazine treatment in combination on stress response and cardio-respiratory depression in cows undergoing pain-less claw trimming. In a blinded experimental study, six healthy, non-pregnant, non-lactating, German Holstein Frisian cows weighing 610±87.9 kg (mean± SD) and aged 4.3±3.3 years old were used in a cross over design with two weeks intervals between treatments. The treatments were Xyl-LR: xylazine (Rompun®, 0.05 mg kg^{-1} BW, IM) or Plac-LR: an equal volume of placebo 15 minutes before LR (30 minutes) for claw trimming or Xyl-St: xylazine in the same dose and route of administration without further manipulation (cows remained standing). In short term intervals (30 minutes before until 2 hours after drug application) heart rate (HR), respiratory rate (RR), mean arterial blood pressure (MAP), venous and arterial blood gases and venous plasma levels of cortisol, insulin,

glucose, lactate and non-esterified fatty acids (NEFA) were measured and clinical signs of sedation and free gas bloat recorded.

In placebo treated cows (Plac-LR) LR induced on average a significant ($P < 0.05$) increase in MAP, RR, plasma levels of cortisol, lactate and NEFA, a significant ($P < 0.05$) decrease in mean arterial partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) and arterial oxygen saturation (SaO_2). Xylazine induced in average (Xyl-St) a significant ($P < 0.05$) reduction in HR, RR, MAP, plasma insulin, and NEFA, PaO_2, SaO_2, and a significant increase in plasma glucose and $PaCO_2$.

After pre-emptive sedation with xylazine (Xyl-LR) during LR HR, RR, MAP, PaO_2, SaO_2 and plasma levels of cortisol, lactate and NEFA, were significantly ($P < 0.05$) reduced whereas $PaCO_2$ was increased compared to Plac-LR. Lowest SaO_2 found during LR after xylazine treatment were about 90%. All xylazine treated cows showed mild to moderate signs of sedation, mild and clinically irrelevant free gas bloat and were able to stand and walk before and after restraining in LR. We concluded that, xylazine administered in a low dose in adult cows preceding LR and claws trimming on a surgical tipping table can reduce hormonal and metabolic stress responses but has some additional effect on respiratory depression of LR. When xylazine is applied before cows are turned into LR the desired effects of sedation and analgesia need to be balanced against the adverse effect of cardio-respiratory depression of xylazine. Higher doses of xylazine than the used 0.05 mg kg^{-1} BW may induce clinically relevant cardio-respiratory depression during LR in cows.

Study 2

The aim of this study was to investigate the effect of pre-emptive xylazine treatment in the analgesic protocol for claw treatment in lateral recumbency (LR) in lame cows on the stress and pain response. In a prospective, blinded, placebo-controlled clinical case study, 24 lame, German Holstein Frisian cows (not more than four month pregnant), weighing 531±85.5 kg and aged 4.4 ±1.5 (mean ± SD) years old were used. Cows were randomly allocated into two groups of 12 cows each and either treated with Xylazine (Rompun®; 0.05 mg kg^{-1} BW, IM) or an equal volume of sterile

saline (controls) 15 minutes before LR for claw treatment. After initial claw examination in each cow a retrograde intravenous local anaesthesia (LA) with 20 ml of 2% procaine was performed. At regular preset time intervals over an observation period of 6 hours (30 min before drug application to 6 hours post-operative) heart rate (HR), respiratory rate (RR), plasma levels of cortisol, glucose, lactate and non-esterified fatty acids (NEFA) were determined and signs of behaviour monitored (via video recording and pedometer).

All cows treated with xylazine showed mild signs of sedation for about one to two hours, and were able to walk and to stand at all times. In the evaluation of behavioural signs xylazine demonstrated significantly additional analgesic effects to LA by reduced pain response on the insertion of the needle for LA, reduced ear flicking during claw treatment, reduced lameness score and longer standing periods in the first hour after claw treatment and improved appetite after claw treatment in LR. No significant ($P < 0.05$) difference was found for rumen motility in both groups. In both groups rumen motility ceased during the surgical intervention in LR. Mean HR and RR as well as plasma levels of lactate and NEFA were significantly ($P < 0.05$) reduced after xylazine treatment compared to baseline and controls. Mean plasma level of cortisol was significantly ($P < 0.05$) lower in xylazine treated cows after being turned into LR but raised to levels in controls during the surgical treatment. In conclusion, according to the signs of behaviour monitoring, plasma cortisol, NEFA and lactate, the use of low dose xylazine in the analgesic protocol for treatment of lame cows in LR on a surgical tipping table can alleviate stress and has additional analgesic effects to LA. Due to cardio-respiratory depression by xylazine we discourage to use higher doses of xylazine in cows receiving claw treatment in lateral recumbency.

From the results of both studies it was conclude that low dose xylazine treatment in healthy or clinically lame dairy cows preceding LR for painless claws trimming or surgical treatment of the claw disorders on a surgical tipping table can reduce behavioural, hormonal, and metabolic stress response. Xylazine provides additional analgesic effects to local anaesthesia in lame cows undergoing surgical claw

treatment. Although xylazine pre-treatment will aggravate slightly cardio-respiratory depression of LR xylazine appears to be a suitable sedative and analgesic in combination with LA in dairy cows turned into LR for orthopaedic treatment. However, since cows used to close human contact and handling tolerate the fixation in LR on surgical tipping tables well without sedation the desired effects of sedation and analgesia need to be balanced against the cardio-respiratory depression of xylazine.

7 ZUSAMMENFASSUNG

Awad Zaboulla Hassan Rizk

Untersuchungen zur Anwendung des alpha-2-Agonisten Xylazin (Rompun®) zur orthopädischen Behandlung von Kühen in Seitenlage auf einem chirurgischen Klauenkippstand

Im Rahmen des *Animal Welfare* Gedanken haben kürzlich Mitglieder des European College of Veterinary Anaesthesiology eine vorbeugende Gabe von Sedativa bei der Fixation von Milchkühen zur orthopädischen Klauenbehandlung in Seitenlage (SL) am Klauenkippwagen gefordert, um Stress der Tiere zu vermeiden oder zumindest zu reduzieren. Ziel dieser Arbeit war es zu prüfen, ob der alpha2-Agonist Xylazin, ein Sedativum und Analgetikum, die kardio-respiratorische Depression bei Verbringung von Kühen in Seitenlage zur orthopädischen Behandlung verstärkt (Studie 1) und ob durch Xylazin die hormonell-metabolische (Studie 1 und 2) sowie die Stressantwort im Verhalten (Studie 2) von Kühen auf Verbringung in Seitenlage vermindert sowie die Analgesie einer Lokalanästhesie zur orthopädischen Behandlung verbessert wird (Studie 2).

Studie 1

Es wurde eine experimentelle Blindstudie mit sechs gesunden, nicht tragenden, nicht laktierenden Milchkühen der Rasse Holstein Friesian (610±87.9 kg; 4.3±3.3 Jahre) in einem cross-over Design mit zwei Wochen Abstand zwischen den Experimenten durchgeführt. Die Tiere wurden jeweils drei Gruppen zugewiesen. A) Xylazin (Rompun®) in einer Dosierung von 0,05 mg kg^{-1} oder B) eine entsprechende Menge steriler, isotonischer Kochsalzlösung (Placebo) jeweils intramuskulär 15 Minuten vor Verbringung in Seitenlage (30 Min.) am Klauenkippwagen oder C) Xylazin in selber Dosierung ohne weitere Manipulation des Tieres. Ein Katheter wurde in die A. *auricularis caudalis* gelegt, zur invasiven Messung des arteriellen Blutdrucks sowie der Entnahme von arteriellem Blut, sowie ein Venenverweilkatheter in die *v. jugularis* zur stressfreien, regelmäßigen Blutentnahme. In kurzen Abständen (30 Minuten

vorher bis 4 Stunden nach Medikamentengabe) wurden neben klinischen Anzeichen von Sedation Herzfrequenz (HF), Atemfrequenz (AF), mittlerer arterieller Blutdruck (MAP), arterielle und venöse Blutgase sowie Plasmakonzentration von Cortisol, Glukose, Laktat, und freien Fettsäuren (NEFA) bestimmt.

Mit Placebo behandelte Tiere zeigten in Seitenlage eine signifikante ($P<0,05$) Erhöhung von MAP, AF, Plasmakortisol, Laktat und NEFA und eine signifikante ($P<0,05$) Erniedrigung des mittleren arteriellen Sauerstoffpartialdrucks und Kohlendioxidpartialdrucks sowie der arteriellen Sauerstoffsättigung. Vorhergehende Gabe von Xylazin verursachte eine signifikante ($P<0,05$) Reduktion der Plasmakonzentrationen von Kortisol, Laktat und NEFA. Mit Xylazin behandelte Tiere mit oder ohne Verbringung in Seitenlage zeigten eine signifikant ($P<0,05$) niedrigere AF und HF. Nach einer kurzen Phase der Hypotension zeigten die mit Xylazin vorbehandelten Kühe in Seitelage einen signifikant geringeren Anstieg des MAP als die placebo-behandelten Tiere. Eine Xylazin Behandlung induzierte signifikant Hypoxie und Hyperkapnie, welche durch die Seitenlage noch verstärkt wurde. Von sechs mit Xylazin behandelten und in Seitenlage verbrachten Kühen wiesen vier Kühe arterielle Sauerstoffsättigungen unter 92% auf. Mit Xylazin behandelte Kühe wiesen leichte bis mittlere Anzeichen einer Sedation auf und entwickelten eine leichte Pansentympanie, die direkt nach dem Aufstehen aus Seitenlage wieder verschwand. Mit Xylazin behandelte Kühe konnte nach dem Aufstehen aus der Seitenlage selbstständig stehen. Aus den Ergebnissen wird geschlossen, dass eine niedrig dosierte Xylazinbehandlung zu leichter bis mittlerer Sedation bei erhaltenem Stand- und Laufvermögen führt und bei einer Anwendung vor Seitenlage die hormonell-metabolische Stressantwort vermindert und die kardio-respiratorische Depression durch die Seitenlage der Kühe moderat verstärkt wird.

Studie 2

In einer prospektiven, randomisierten, kontrollierten Blindstudie wurden 24 lahme Kühe der Rasse Deutsch Holstein Friesian (nicht länger als 4 Monate tragend) mit einem Körpergewicht von 531±85.5 kg (370-743 kg) und im Alter von 4.4±1.5 Jahren (2-7,4 Jahre) untersucht. Die Tiere wurden randomisiert einer Gruppe 1) Xylazin oder

2) Placebo zugeordnet und bekamen eine intramuskuläre Injektion mit Xylazin (0,05 mg/kg) oder einer entsprechenden Menge steriler Kochsalzlösung 15 Minuten vor der Fixierung in Seitenlage zur Klauenbehandlung. Nach initialer Klauenpflege und erster Untersuchung wurde bei den Tieren beider Gruppen eine retrograde, regionale Stauungsanästhesie mit 20 ml 2%igem Procain an der betroffenen Klaue durchgeführt. In kurzen Abständen (30 Minuten vorher bis 6 Stunden nach dem Aufstehen) wurden Herzfrequenz (HF), Atemfrequenz (AF), sowie Plasmakonzentration von Cortisol, Glukose, Laktat, und freien Fettsäuren (NEFA) bestimmt. Das Verhalten wurde beobachtet, sowie über Videoaufzeichnung und Pedometer überwacht. Alle Tiere der Xylazin-Gruppe zeigten leichte klinische Anzeichen einer Sedation und waren zu jeder Zeit in der Lage zu laufen und zu Stehen. Die mittleren Plasmacortisolkonzentrationen blieben in der Xylazingruppe unter denen der Placebogruppe ($p<0,05$) als die Kühe in Seitenlage verbracht wurden, stiegen aber auf gleiches Niveau während der chirurgischen Klauenbehandlung. Plasma-NEFA Konzentrationen waren im Mittel während und nach der Klauenbehandlung in Seitenlage im Vergleich zur Placebogruppe nach Xylazinvorbehandlung signifikant vermindert ($p<0,05$). Die analgetische Wirkung des Xylazins über die der lokalen Anästhesie hinaus zeigte sich ($p<0,05$) in I) einer verminderten Abwehrreaktion auf die Nadeleinführung für die Lokalanästhesie, II) vermindertes Ohrenschlagen während der chirurgischen Klauenbehandlung, III) geringeren Lahmheitsgrad und IV) verlängerte Standperioden in der ersten Stunde nach der Klauenbehandlung sowie V) in besserem Appetit als die Kühe zurück in den Stall kehrten. Keine Unterschiede zwischen den Gruppen fanden sich in der Vormagenmotorik, die bei beiden Gruppen während der Seitenlage aussetzte aber 1 Stunde anschließend wieder einsetzte. Die mittlere HF und AF waren signifikant ($P<0,05$) erniedrigt bei Gabe von Xylazin im Vergleich zur Placebogruppe.

Aus den Ergebnissen beider Studien wird geschlossen, dass niedrige Dosierungen von Xylazin bei gesunden oder lahmen Milchkühen vor der Verbringung in Seitenlage zur Klauenpflege oder chirurgischen Klauenbehandlung die hormonelle und metabolische Stressantwort verminderen und die analgetische Wirkung einer Lokalanästhesie zur Klauenbehandlung steigern. Obwohl Xylazin in niedriger

Dosierung die kardio-respiratorische Depression durch Seitenlage bei Kühen moderat steigert, erscheint Xylazin als ein geeignetes Sedativum und Analgetikum für ein Schmerzprotokoll zur Klauenbehandlung von Kühen. Allerdings wird ohne zusätzliche wissenschaftliche Untersuchungen derzeit abgeraten, höhere Dosierungen als die hier verwendete (0,05 mg/kg KGW) einzusetzen, da dies möglicherweise zu klinisch relevanter respiratorischer Insuffizienz bei Kühen in Seitenlage führen mag. Kühe, die an die Nähe des und den Umgang mit dem Menschen gewöhnt sind, tolerieren eine Verbringung in Seitenlage ohne Anzeichen der Abwehr, sind allerdings dennoch gestresst. Bei Anwendung von Xylazin bei Kühen vor Verbringung in Seitenlage sind die positiven zu erwartenden Effekte der Sedation und Analgesie gegen die unerwünschte Wirkung der kardio-respiratorischen Depression abzuwägen.

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9. APPENDIX

9.1 First study

Table 9: Cardio-respiratory parameters (mean ± SD) measured for 195 min following IM injection of xylazine (Xyl-LR; n= 6) or placebo (Plac- LR; n= 6) and cows were positioned in lateral recumbency (LR; grey underlay) 15 min after drug administration for 30 min and xylazine (Xyl-St; n= 6) but cows remained standing.

	Baseline			LR			Time after treatment [min]						
	-15	10	15	30	45	55	60	75	90	105	135	195	Effects
MAP [mmHg]													
Plac-LR	107±11.0	106±8.41	106±10.9	134±13.6*^{a}	132±12.2*^{a}	138±15.5*^{a}	135±15.2*^{a}	113±16.4	114±7.67	114±10.0	110±11.5	107±12.9	T: P = 0.0063
Xyl-LR	116±17.0	107±6.22	106±7.46	100±10.2^{b}	107±12.1^{b}	118±17.9^{a,b}	120±12.4^{a,b}	113±12.8	113±11.3	111±12.5	111±12.6	105±10.6	G: P = 0.1108
Xyl-St	115±9.05	106±7.54*	104±6.7*	99.2±7.05*^{b}	98.2±7.39*^{b}	101±15.1*^{b}	103±15.0^{b}	107±13.2	110±10.7	110±13.0	111±10.9	109±6.83	TxG:P < 0.0001
HR [beats min^{-1}]													
Plac-LR	73.7±7.70	70.0±8.94*^{a}	72.0±7.59^{a}	76.,8±12.8^{a}	72.7±9.69^{a}	78.8±13.4^{a}	72.5±7.94^{a}	77.8±11.4^{a}	72.3±7.83^{a}	70.3±5.99^{a}	67.3±3.72^{a}	72.2±8.40^{a}	T: P < 0.0001
Xyl-LR	69.7±10.1	52.7±7.34*^{b}	51.0±6.66*^{b}	52.7±5.89*^{b}	50.2±5.08*^{b}	51.8±7.86*^{b}	51.8±8.16*^{b}	48.3±4.46*^{b}	51.0±8.56*^{b}	50.7±4.84*^{b}	51.7±7.20*^{b}	50.3±7.2*^{b}	G: P < 0.0001
Xyl-St	62.7±13.1	44.7±7.76*^{b}	43.3±5.89*^{b}	44.0±5.06*^{b}	42.3±6.74*^{b}	47.7±6.50*^{b}	46.0±3.58*^{b}	47.7±4.80*^{b}	45.7±3.20*^{b}	48.7±3.93*^{b}	48.7±6.77*^{b}	50.7±7.0*^{b}	TxG:P = 0.0003
RR [breaths min^{-1}]													
Plac-LR	26.0±7.00	25.3±5.47^{a}	28.0±12.1^{a}	32.0±7.16*^{a}	32.0±11.8^{a}	32.0±8.76^{a}	28.7±5.89^{a}	28.7±9.27^{a}	26.0±8.29^{a}	24.7±5.32^{a}	24.7±3.93^{a}	24.7±10.6	T: P < 0.0035
Xyl-LR	26.0±7.90	18.3±3.2^{a,b}	18.0±3.40^{a,b}	15.0±5.02*^{b}	16.7±7.76^{b}	15.7±8.04^{b}	16.7±7.76^{b}	15.3±4.68*^{b}	14.0±3.35*^{b}	14.7±3.27*^{b}	15.0±3.52*^{b}	16.2±5.08*	G: P = 0.0007
Xyl-St	28.7±11.2	16.0±6.20^{b}	14.0±4.00*^{b}	12.3±0.82*^{b}	12.3±1.51*^{b}	13.0±2.76*^{b}	13.7±2.66*^{b}	15.3±4.68^{b}	17.0±5.59^{a,b}	16.0±5.06*^{b}	16.3±4.80^{b}	22.8±6.82	TxG:P = 0.0035
Rectal Temperature [C°]													
Plac-LR	38.7±0.20	38.7±0.19	39.0±0.23	38.7±0.17	38.8±0.19	38.7±0.20	38.8±0.19	38.7±0.31	38.8±0.28	38.8±0.19	38.8±0.16	38.8±0.23	T: P < 0.0067
Xyl-LR	38.8±0.30	38.7±0.22	38.8±0.24	38.9±0.21	38.8±0.28	38.8±0.27	38.7±0.27	38.7±0.30	38.7±0.34	38.8±0.32	38.7±0.27	39.0±0.33*	G: P = 0.0007
Xyl-St	39.0±0.20	38.8±0.14*	38.8±0.19*	38.8±0.21	38.9±0.19	38.8±0.18*	38.8±0.24	38.8±0.24	38.8±0.18*	38.9±0.18	38.9±0.18	39.1±0.34	TxG:P =0.2546

T = time effect ; **G =** group effect ; **T** X **G =** time x group effect

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.

Corresponding means with different superscripts (a or b) are significantly (P < 0.05) different.

Table 10: Endocrine-metabolic parameters (mean ± SD) measured for 195 min following IM injection of xylazine (Xyl-LR; n= 6) or placebo (Plac-LR; n = 6) and cows were positioned in lateral recumbency (LR; greyunderlay) 15 min after drug administration for 30 min and xylazine (Xyl-St; n= 6) but cows remained standing.

	BL	LR				Time [min]				
	-15	15	30	45	60	75	105	135	195	Effects
Glucose [mmol L^{-1}]										
Plac-LR	4.50 ± 0.28	4.47 ± 0.68^{b}	4.29 ± 0.44^{b}	4.46 ± 0.46^{b}	4.56 ± 0.40^{b}	4.92 ± 0.48*^{b}	5.04 ± 0.67^{b}	5.09 ± 0.51*^{b}	4.94 ± 0.43*^{b}	T : P < 0.0001
Xyl-LR	4.43 ± 0.33	5.59 ± 0.38*^{a}	7.18 ± 0.67*^{a}	7.90 ± 0.90*^{a}	8.39 ± 1.35*^{a}	8.90 ± 1.28*^{a}	9.15 ± 1.34*^{a}	9.46 ± 1.37*^{a}	8.84 ± 1.00*^{a}	G: P < 0.0001
Xyl-St	4.40 ± 0.39	5.41 ± 0.28*^{a}	6.45 ± 0.59*^{a}	6.48 ± 0.68*^{a}	7.14 ± 0.78*^{a}	7.39 ± 1.02*^{a}	7.74 ± 1.17*^{a}	8.35 ± 0.96*^{a}	8.80 ± 0.98*^{a}	TxG:P < 0.0001
NEFA [µmol L^{-1}]										
Plac-LR	400 ± 329	351 ± 246	385 ± 178.5^{a}	450 ± 208^{a}	380 ± 157.6^{a}	354 ± 143^{a}	350 ± 203	270 ± 165	223 ± 115	T: P = 0.0754
Xyl-LR	331 ± 187	178 ± 83.8*	175 ± 62.2^{b}	188 ± 62.2^{b}	283 ± 121.3^{a,b}	301 ± 83.7^{a,b}	218 ± 60	207 ± 55	174 ± 46	G: P = 0.0656
Xyl-St	279 ± 218	155 ± 90	129 ± 72.1^{b}	119 ± 45.8^{b}	140 ± 44.4^{b}	168 ± 84.5^{b}	211 ± 85	266 ± 125	235 ± 83	TxG:P = 0.0427
SID [mEq L^{-1}]										
Plac-LR	49.0 ± 2.13	51.0 ± 2.69	53.1 ± 2.18*	53.5 ± 1.63*	54.3 ± 1.72*	51.7 ± 1.72*	51.6 ± 0.83*^{b}	51.3 ± 1.91*	51.0 ± 2.49	T: P = 0.0001
Xyl-LR	50.2 ± 2.33	52.1 ± 1.59*	52.8 ± 1.82*	53.1 ± 2.03*	54.1 ± 2.31*	52.9 ± 2.44*	52.1 ± 2.22^{b}	51.5 ± 1.26*	51.4 ± 2.44	G: P = 0.3478
Xyl-St	48.6 ± 2.88	52.8 ± 1.60*	53.6 ± 1.19*	55.0 ± 2.36*	55.5 ± 2.16*	54.8 ± 3.15*	55.6 ± 2.65*^{a}	53.9 ± 4.00*	51.4 ± 4.03*	TxG:P = 0.0160
L (+)-Lactate[mmol L^{-1}]										
Plac-LR	0.71 ± 0.1	0.62 ± 0.06	2.02 ± 1.84	2.07 ± 1.79	2.33 ± 1.54*^{a}	2.05 ± 1.19*^{a}	0.99 ± 0.40^{a}	0.60 ± 0.13	0.51 ± 0.08*	T: P = 0.0138
Xyl-LR	0.90 ± 0.4	0.73 ± 0.24*	0.69 ± 0.25*	0.78 ± 0.29	0.88 ± 0.34^{b}	0.87 ± 0.30^{b}	0.67 ± 0.14^{ab}	0.60 ± 0.18*	0.52 ± 0.16*	G: P = 0.0336
Xyl-St	0.91 ± 0.2	0.78 ± 0.12	0.72 ± 0.14*	0.59 ± 0.08*	0.54 ± 0.09*^{b}	0.59 ± 0.10*^{b}	0.51 ± 0.10*^{b}	0.50 ± 0.09*	0.51 ± 0.10*	TxG:P = 0.0118
B-HBS[mmol L^{-1}]										
Plac-LR	0.32 ± 0.1	0.32 ± 0.10	0.28 ± 0.04^{a}	0.29 ± 0.06^{a}	0.29 ± 0.09^{a}	0.24 ± 0.07	0.23 ± 0.07	0.30 ± 0.03	0.22 ± 0.06	T : P = 0.0427
Xyl-LR	0.32 ± 0.1	0.30 ± 0.08	0.23 ± 0.07*^{ab}	0.22 ± 0.06*^{ab}	0.27 ± 0.10^{ab}	0.28 ± 0.12	0.33 ± 0.08	0.30 ± 0.10	0.25 ± 0.07	G: P = 0.0030
Xyl-St	0.32 ± 0.1	0.24 ± 0.08*	0.17 ± 0.05*^{b}	0.15 ± 0.03*^{b}	0.15 ± 0.0*^{b}	0.18 ± 0.05*	0.26 ± 0.09	0.30 ± 0.08	0.30 ± 0.08	TxG:P = 0.2724
Cortisol [ng ml^{-1}]										
Plac-LR	19.6 ± 6.92	10.9 ± 8.00*	46.3 ± 20.2*^{a}	61.9 ± 10.8*^{a}	71.0 ± 13.6*^{a}	72.7 ± 9.45*^{a}	57.5 ± 7.60*^{a}	30.2 ± 3.21*^{a}	12.1 ± 2.25*^{a}	T: P < 0.0001
Xyl-LR	28.2 ± 9.89	13.7 ± 10.1*	24.5 ± 21.7^{a,b}	28.2 ± 23.5^{b}	43.2 ± 25.4^{b}	51.4 ± 21.96^{a}	28.1 ± 17.7^{b}	15.0 ± 6.45*^{b}	6.18 ± 2.58*^{b}	G: P < 0.0001
Xyl-St	7.65 ± 2.32	11.9 ± 10.1	15.9 ± 15.3^{b}	14.0 ± 15.1^{b}	12.9 ± 10.4^{c}	14.3 ± 8.09^{b}	16.3 ± 10.3^{b}	12.3 ± 8.54^{b}	6.65 ± 3.77^{b}	TxG:P < 0.0001
Insulin [µU ml^{-1}]										
Plac-LR	11.5 ± 7.54	9.77 ± 8.49	8.67 ± 8.32	9.32 ± 9.74	13.3 ± 13.9	11.8 ± 14.2	9.75 ± 10.4	9.88 ± 8.45	14.1 ± 11.3	T : P = 0.0003
Xyl-LR	9.15 ± 5.22	4.53 ± 4.21	2.87 ± 2.61*	3.30 ± 3.66*	2.90 ± 4.13*	2.70 ± 3.46*	3.32 ± 3.34*	5.23 ± 3.99	10.2 ± 5.64	G: P = 0.1195
Xyl-St	7.48 ± 4.92	2.97 ± 1.52*	2.28 ± 1.28*	2.35 ± 1.56*	1.50 ± 0.70*	1.33 ± 0.68*	1.77 ± 1.18	3.95 ± 4.45	8.32 ± 7.59	TxG:P = 0.3426

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.

Corresponding means with different superscripts (a or b) are significantly (P < 0.05) different.

Table 11: Arterial blood gases (mean ± SD) measured for 195 min following IM injection of xylazine (Xyl-LR; n= 6) or placebo (Plac-LR; n= 6) and cows were positioned in lateral recumbency (LR; greyunderlay) 15 min after drug administration for 30 min and xylazine (Xyl-St; n= 6) but cows remained standing.

	BL	LR				Time [min]				
	-15	15	30	45	60	75	105	135	195	Effects
Arterial pH										
Plac-LR	7.44 ± 0.02	7.44 ± 0.03	7.46 ± 0.03	7.46 ± 0.02	7.46 ± 0.02	7.44 ± 0.02	7.44 ± 0.02	7.44 ± 0.03	7.43 ± 0.01	T: P = 0.5855
Xyl-LR	7.45 ± 0.03	7.44 ± 0.02	7.45 ± 0.03	7.45 ± 0.04	7.46 ± 0.02	7.44 ± 0.03	7.44 ± 0.02	7.44 ± 0.02	7.45 ± 0.04	G: P = 0.6443
Xyl-St	7.43 ± 0.01	7.42 ± 0.02	7.43 ± 0.02	7.44 ± 0.03	7.42 ± 0.06	7.46 ± 0.03	7.43 ± 0.06	7.44 ± 0.03	7.45 ± 0.03*	TxG:P = 0.1944
PaCO2 [mmHg]										
Plac-LR	46.6 ± 3.02	45.4 ± 3.13^{b}	43.0 ± 4.67^{b}	44.6 ± 3.49	44.0 ± 4.07	46.0 ± 3.91	46.1 ± 2.6	47.5 ± 2.89*	47.3 ± 3.81	T: P = 0.1138
Xyl-LR	45.0 ± 3.20	49.1 ± 3.35*^{a,b}	50.0 ± 3.57*^{a}	49.5 ± 4.92*	48.9 ± 3.47*	50.5 ± 5.06	50.1 ± 3.17*	48.9 ± 3.26*	48.2 ± 3.43*	G: P = 0.0753
Xyl-St	44.4 ± 3.33	50.6 ± 2.65*^{a}	51.1 ± 2.82*^{a}	50.0 ± 3.29*	54.4 ± 11.1	49.1 ± 3.88*	51.9 ± 10.8	49.5 ± 4.40*	47.1 ± 5.18	TxG:P = 0.0272
PaO2 [mmHg]										
Plac-LR	96.6 ± 7.56	101 ± 4.65^{a}	82.6 ± 4.95*^{a}	78.0 ± 7.85*^{b}	80.2 ± 9.18*^{b}	99.9 ± 6.42	98.1 ± 5.06	95.2 ± 5.90	98.5 ± 6.77	T: P = 0.0041
Xyl-LR	98.9 ± 5.79	82.3 ± 9.22*^{b}	71.5 ± 8.49*^{b}	69.3 ± 5.80*^{b}	72.2 ± 5.56*^{b}	95.8 ± 6.59	95.8 ± 5.70	94.3 ± 5.14	96.9 ± 5.95	G: P < 0.0001
Xyl-St	104 ± 3.59	78.4 ± 4.25*^{b}	86.7 ± 2.98*^{a}	92.7 ± 7.72*^{a}	98.8 ± 10.8^{a}	98.3 ± 5.49*	99.4 ± 6.93	99.9 ± 4.65*	103 ± 5.03	TxG:P < 0.0001
Arterial HCO3s[mmol L^{-1}]										
Plac-LR	29.7 ± 0.68	29.7 ± 1.25	29.5 ± 1.77^{b}	32.3 ± 1.62	29.8 ± 1.30^{b}	29.6 ± 1.29^{b}	29.8 ± 1.22	30.3 ± 1.39	29.6 ± 1.37	T: P = 0.0002
Xyl-LR	29.8 ± 1.47	31.3 ± 1.89*	32.3 ± 1.66*^{a}	32.2 ± 1.96*	32.0 ± 2.04*^{a,b}	32.0 ± 1.14*^{a}	31.9 ± 1.16*	31.6 ± 1.62*	31.8 ± 2.65	G: P = 0.0180
Xyl-St	28.2 ± 1.64	30.1 ± 0.85*	31.2 ± 1.40*^{a,b}	31.9 ± 1.22*	32.4 ± 1.02*^{a}	32.8 ± 0.97*^{a}	32.2 ± 1.03*	31.9 ± 1.76*	31.4 ± 2.10*	TxG:P = 0.0317
Arterial BE [mmol L^{-1}]										
Plac-LR	5.77 ± 0.73	5.72 ± 1.35	5.57 ± 1.91^{b}	6.38 ± 1.74	5.95 ± 1.39^{b}	5.60 ± 1.41^{b}	5.90 ± 1.31^{b}	6.38 ± 1.49	5.60 ± 1.47	T: P = 0.0002
Xyl-LR	5.93 ± 1.58	7.52 ± 2.00*	8.58 ± 1.74*^{a}	8.48 ± 2.08*	8.35 ± 2.06*^{a,b}	8.22 ± 1.20*^{a}	8.15 ± 1.24*^{a}	7.80 ± 1.72*	7.98 ± 2.84*	G: P = 0.0170
Xyl-St	4.13 ± 1.81	6.40 ± 0.99*	7.42 ± 1.51*^{a,b}	8.10 ± 1.28*	8.65 ± 1.08*^{a}	9.05 ± 1.00*^{a}	8.22 ± 1.02*^{a}	8.02 ± 1.85*	7.53 ± 2.23*	TxG:P = 0.0412
SaO2 [%]										
Plac-LR	96.9 ± 0.73	97.3 ± 0.44^{a}	95.7 ± 0.57*^{a}	94.9 ± 0.99*^{a}	95.1 ± 1.29*^{a,b}	97.2 ± 0.51	97.1 ± 0.36	96.8 ± 0.66	97.0 ± 0.67	T: P < 0.0001
Xyl-LR	97.2 ± 0.43	95.2 ± 1.47*^{b}	92.9 ± 1.96*^{b}	92.6 ± 1.68*^{b}	93.5 ± 1.18*^{b}	96.8 ± 0.76	96.8 ± 0.63	96.8 ± 0.47*	96.9 ± 0.60	G: P = 0.0164
Xyl-St	97.4 ± 0.28	94.3 ± 1.08*^{b}	95.3 ± 1.02*^{a}	96.4 ± 1.00^{a}	96.6 ± 1.47^{a}	97.1 ± 0.72	96.8 ± 1.58	97.1 ± 0.62	97.3 ± 0.56	TxG:P < 0.0001

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.
Corresponding means with different superscripts (a or b) are significantly (P < 0.05) different.

Table 12: Venous blood gases (mean ± SD) measured for 195 min following IM injection of xylazine (Xyl-LR; n= 6) or placebo (Plac-LR; n= 6) and cows were positioned in lateral recumbency (LR; greyunderlay) 15 min after drug administration for 30 min and xylazine (Xyl-St; n= 6) but cows remained standing.

	Time [min]								
Parameter	**-15**	**15**	**30**	**45**	**60**	**75**	**105**	**135**	**195**	**Effects**
Venous PH										
Plac-LR	7.41 ± 0.03	7.41 ± 0.04	7.43 ± 0.03	7.44 ± 0.02^{a}	7.43 ± 0.03	7.41 ± 0.03	7.39 ± 0.04*	7.38 ± 0.03*	7.39 ± 0.02	T: P = 0.0009
Xyl-LR	7.42 ± 0.02	7.41 ± 0.02	7.41 ± 0.03	7.43 ± 0.02^{ab}	7.43 ± 0.02	7.42 ± 0.02	7.41 ± 0.02	7.41 ± 0.02	7.40 ± 0.03	G: P = 0.3159
Xyl-St	7.39 ± 0.03	7.39 ± 0.01	7.40 ± 0.02	7.40 ± 0.02^{b}	7.40 ± 0.02	7.41 ± 0.02	7.40 ± 0.02	7.40 ± 0.02	7.40 ± 0.03	TxG:P = 0.0141
PvCO2 [mmHg]										
Plac-LR	47.6 ± 5.03	49.0 ± 2.85^{b}	48.7 ± 3.21^{b}	48.0 ± 3.46^{b}	48.4 ± 4.34^{b}	48.2 ± 3.63^{b}	51.1 ± 5.54	49.8 ± 4.70	48.7 ± 4.49	T: P < 0.0001
Xyl-LR	47.2 ± 2.53	53.3 ± 3.03*^{a}	54.9 ± 3.25*^{a}	54.8 ± 2.35*^{a}	54.0 ± 3.21*^{a}	53.1 ± 3.10*^{ab}	53.9 ± 4.47*	49.3 ± 4.36	50.5 ± 4.85*	G: P = 0.0425
Xyl-St	44.3 ± 3.32	52.9 ± 2.16*^{ab}	54.4 ± 2.26*^{a}	55.7 ± 2.74*^{a}	56.9 ± 2.13*^{a}	55.4 ± 2.72*^{a}	56.2 ± 3.84*	53.4 ± 3.93*	48.8 ± 2.47*	TxG:P = 0.0002
PvO2 [mmHg]										
Plac-LR	36.8 ± 5.56	37.4 ± 3.20	38.1 ± 3.22	39.9 ± 3.38	40.5 ± 1.76^{a}	41.4 ± 3.57	38.0 ± 7.21	37.6 ± 6.13	40.3 ± 1.80	T: P = 0.0490
Xyl-LR	38.4 ± 3.71	36.4 ± 2.85	36.6 ± 2.38	35.5 ± 3.28	36.3 ± 3,23^{b}	38.5 ± 1.98	37.6 ± 2.70	38.4 ± 2.38	37.7 ± 3.64	G: P = 0.4759
Xyl-St	40.9 ± 4.15	35.2 ± 2.44*	37.0 ± 2.76	37.6 ± 2.10	38.9 ± 2.51^{ab}	39.6 ± 3.31	39.0 ± 2.14	38.7 ± 2.12	39.0 ± 4.30	TxG:P = 0.2353
HCO3s [mmol L^{-1}]										
Plac-LR	27.6 ± 2.26	28.5 ± 2.46	29.8 ± 2.08	29.9 ± 1.82^{b}	29.9 ± 1.03^{b}	28.3 ± 1.90^{b}	28.0 ± 1.94^{b}	27.0 ± 1.81^{b}	27.3 ± 2.22	T: P < 0.0001
Xyl-LR	28.7 ± 1.83	30.5 ± 1.37*	31.8 ± 1.78*	32.8 ± 2.42*^{a}	32.3 ± 1.71*^{a}	31.3 ± 1.25*^{a}	30.8 ± 1.80*^{a}	28.6 ± 1.74^{ab}	28.5 ± 2.43	G: P = 0.0229
Xyl-St	24.9 ± 2.82	28.8 ± 0.84*	30.0 ± 0.78*	31.3 ± 0.83*^{ab}	31.8 ± 1.28*^{ab}	31.7 ± 1.08*^{a}	31.5 ± 1.22*^{a}	30.3 ± 2.48*^{a}	27.9 ± 2.73	TxG:P = 0.0157
BE [mmol L^{-1}]										
Plac-LR	4.10 ± 2.49	5.05 ± 2.66	6.50 ± 2.21	6.58 ± 2.00^{b}	6.55 ± 1.05^{b}	4.78 ± 2.16^{b}	4.57 ± 2.27^{b}	3.47 ± 2.07^{b}	3.60 ± 2.50	T: P < 0.0001
Xyl-LR	4.68 ± 2.05	7.20 ± 1.43	8.60 ± 1.89*	9.62 ± 2.52*^{a}	9.13 ± 1.83*^{a}	8.05 ± 1.31*^{a}	7.52 ± 1.96*^{a}	5.12 ± 1.90^{ab}	5.10 ± 2.79	G: P = 0.0256
Xyl-St	1.03 ± 3.21	5.50 ± 0.93*	6.72 ± 0.85*	8.02 ± 0.88*^{ab}	8.53 ± 1.38*^{ab}	8.38 ± 1.16*^{a}	8.27 ± 1.22*^{a}	6.93 ± 2.59*^{a}	4.33 ± 2.89	TxG:P = 0.0148
SvO2 [%]										
Plac-LR	65.4 ± 8.88	64.7 ± 6.25	67.2 ± 5.40	70.4 ± 4.34^{a}	70.9 ± 3.68^{a}	71.2 ± 4.41	63.2 ± 13.3	62.9 ± 12.3	68.6 ± 3.96	T: P = 0.0255
Xyl-LR	66.9 ± 6.11	62.5 ± 3.70	62.7 ± 2.76	61.6 ± 5.53^{b}	63.4 ± 5.22^{b}	67.0 ± 3.76	64.7 ± 3.90	67.1 ± 3.68	63.5 ± 6.42*	G: P = 0.3754
Xyl-St	68.8 ± 6.84	59.8 ± 4.26	57.5 ± 9.91*	64.0 ± 3.75^{ab}	65.9 ± 4.58^{ab}	67.4 ± 5.28	66.0 ± 3.78	65.6 ± 3.03	66.0 ± 6.22	TxG:P = 0.0808

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.
Corresponding means with different superscripts (a or b) are significantly (P < 0.05) different.

Table 13: calculated cardiopulmonary parameters (mean ± SD) measured for 195 min following IM injection of xylazine (Xyl-LR; n= 6) or placebo (Plac-LR; n = 6) and cows were positioned in lateral recumbency (LR; grey underlay) 15 min after drug administration for 30 min and xylazine (Xyl-St; n= 6) but cows remained standing.

	BL		LR			Time [min]				
	-15	15	30	45	60	75	105	135	195	Effects
P_AO_2 [mmHg]										
Plac-LR	103 ± 3.02	103 ± 3.13[a]	106 ± 4.67[a]	105 ± 3.49	105 ± 4.07	103 ± 3.91	103 ± 2.57	102 ± 2.89*	102 ± 3.81	T : P = 0.0753
Xyl-LR	104 ± 3.20	100 ± 3.35*[a,b]	99.4 ± 3.57*[b]	99.9 ± 4.92*	100 ± 3.47*	98.8 ± 5.06	99.3 ± 3.17*	101 ± 3.26*	101 ± 3.43*	G : P = 0.1138
Xyl-St	105 ± 3.33	98.8 ± 2.65*[b]	98.3 ± 2.32*[b]	99.4 ± 3.29*	95.0 ± 11.1	100 ± 3.88*	97.5 ± 10.8	100 ± 4.4*	102 ± 5.18	TxG:P = 0.0272
C_cO_2 [ml dl^{-1}]										
Plac-LR	13.4 ± 0.73	13.1 ± 0.81*	14.9 ± 1.67*[a]	14.8 ± 1.45*[a]	14.7 ± 1.61*[a]	13.8 ± 1.25	12.9 ± 1.13	12.8 ± 0.8*	12.6 ± 0.76*	T : P < 0.0001
Xyl-LR	13.3 ± 1.00	12.0 ± 0.85*	12.3 ± 0.91*[b]	12.8 ± 0.70[b]	13.0 ± 0.87[a,b]	12.6 ± 0.67*	12.1 ± 0.44*	12.4 ± 0.53*	12.4 ± 0.44*	G : P = 0.0459
Xyl-St	13.8 ± 0.70	12.5 ± 0.75*	12.2 ± 0.68*[b]	12.0 ± 0.6*[b]	12.0 ± 0.66*[b]	12.5 ± 0.05*	12.6 ± 0.48*	12.8 ± 0.68	13.3 ± 0.86	TxG:P < 0.0001

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.

Corresponding means with different superscripts (a or b) are significantly (P < 0.05) different.

Table 14: Haematological parameters (mean ± SD) measured for 195 min following IM injection of xylazine (Xyl-LR; n= 6) or placebo (Plac-LR; n= 6) and cows were positioned in lateral recumbency (LR; grey underlay) 15 min after drug administration for 30 min and xylazine (Xyl-St; n= 6) but cows remained standing.

	Time [min]									
	-15	**15**	**30**	**45**	**60**	**75**	**105**	**135**	**195**	**Effects**
WBC [10^3*µl^{-1}]										
Plac-LR	7.40 ± 1.88	8.45 ± 1.69*	8.35 ± 1.99*	8.26 ± 1.92	8.26 ± 1.92	7.80 ± 2.14	8.35 ± 2.14*	10.2 ± 2.68*	11.1 ± 2.28*	T: P < 0.0001
Xyl-LR	7.83 ± 2.22	7.32 ± 2.63	7.17 ± 2.64	7.46 ± 2.71	7.85 ± 2.59	8.01 ± 2.57	8.21 ± 2.14	8.91 ± 2.25	9.43 ± 1.94	G: P = 0.5372
Xyl-St	7.75 ± 2.61	6.15 ± 1.84*	6.45 ± 1.84*	6.58 ± 1.79*	6.88 ± 1.77	7.10 ± 1.74	7.66 ± 1.89	7.85 ± 1.84	9.18 ± 3.58	TxG:P = 0.0476
RBCs [10^3*µl^{-1}]										
Plac-LR	5.94 ± 0.65	5.68 ± 0.43	5.30 ± 0.49	5.49 ± 0.53^{a}	5.55 ± 0.50^{a}	5.98 ± 0.73	5.56 ± 0.64	5.49 ± 0.56	5.43 ± 0.54*	T: P < 0.0001
Xyl-LR	5.71 ± 0.57	5.13 ± 0.43*	5.30 ± 0.49*	5.49 ± 0.53^{ab}	5.55 ± 0.50^{ab}	5.40 ± 0.43*	5.20 ± 0.35*	5.33 ± 0.38*	5.31 ± 0.31*	G: P = 0.2601
Xyl-St	6.05 ± 0.64	5.51 ± 0.55*	5.35 ± 0.58*	5.28 ± 0.59*^{b}	5.29 ± 0.58*^{b}	5.42 ± 0.61*	5.45 ± 0.52*	5.54 ± 0.63*	5.75 ± 0.75	TxG:P < 0.0001
Hb [g dl^{-1}]										
Plac-LR	9.63 ± 0.53	9.38 ± 0.59*	10.7 ± 1.23*^{a}	10.6 ± 1.06*^{a}	10.6 ± 1.18^{a}	9.90 ± 0.91*	9.28 ± 0.82	9.17 ± 0.58*	9.05 ± 0.55*	T: P < 0.0001
Xyl-LR	9.55 ± 0.73	8.58 ± 0.62*	8.83 ± 0.67*^{b}	9.17 ± 0.51^{b}	9.32 ± 0.64^{ab}	9.07 ± 0.50*	8.70 ± 0.33*	8.87 ± 0.39*	8.90 ± 0.32*	G: P = 0.0469
Xyl-St	9.93 ± 0.51	8.98 ± 0.55*	8.77 ± 0.50*^{b}	8.63 ± 0.45*^{b}	8.63 ± 0.48*^{b}	8.93 ± 0.36*	9.02 ± 0.33*	9.17 ± 0.49	9.52 ± 0.63	TxG:P < 0.0001
PCV [%]										
Plac-LR	26.1 ± 1.25	25.7 ± 1.29^{a}	28.9 ± 2.95*^{a}	28.6 ± 2.46*^{a}	28.6 ± 2.74*^{a}	27.0 ± 2.31^{a}	25.3 ± 1.79	25.0 ± 1.30*	24.7 ± 1.36*	T: P < 0.0001
Xyl-LR	25.9 ± 1.74	23.3 ± 1.47*^{b}	24.0 ± 1.52*^{b}	24.8 ± 1.00^{b}	25.0 ± 1.21^{b}	24.4 ± 1.19*^{b}	23.5 ± 0.77*	24.2 ± 0.96*	24.1 ± 0.74*	G: P = 0.0241
Xyl-St	27.3 ± 1.04	25.0 ± 1.06*^{ab}	24.3 ± 0.78*^{b}	24.0 ± 1.00*^{b}	24.0 ± 1.00*^{b}	24.6 ± 1.17*^{ab}	24.6 ± 1.13*	25.0 ± 1.39*	25.9 ± 1.58*	TxG:P < 0.0001
PLT [10^3*µl^{-1}]										
Plac-LR	336.6 ± 27.3	336.0 ± 25.8^{ab}	338.6 ± 27.5^{ab}	343.8 ± 227.6^{a}	340.3 ± 34.6^{ab}	322.1 ± 34.5^{ab}	317.6 ± 31.8*^{ab}	328.6 ± 35.10	317.5 ± 33.08*	T: P = 0.0275
Xyl-LR	385.3 ± 98.6	339.6 ± 67.7*^{a}	358.3 ± 79.5*^{a}	371.5 ± 864.9^{a}	378.3 ± 88.1^{a}	367.3 ± 75.5^{a}	357.6 ± 95.9*^{a}	381.8 ± 88.38	363.6 ± 85.89*	G: P = 0.0211
Xyl-St	294.6 ± 10.8	273.0 ± 15.1^{b}	270.5 ± 16.9*^{b}	257.0 ± 117.4*^{b}	272.8 ± 20.8*^{b}	251.6 ± 63.4^{b}	275.0 ± 18.8*^{b}	281.0 ± 14.87	284.6 ± 22.03	TxG:P = 0.0460
Total Protein [g dl^{-1}]										
Plac-LR	75.3 ± 4.93	73.8 ± 4.79	75.3 ± 5.13	75.3 ± 5.09	75.0 ± 4.20	75.0 ± 5.90	73.7 ± 5.75	74.3 ± 5.24	73.3 ± 6.80	T: P = 0.0023
Xyl-LR	75.7 ± 5.47	73.2 ± 6.37*	73.8 ± 6.74	73.8 ± 6.40	74.5 ± 5.89	73.2 ± 5.38*	72.0 ± 5.18*	74.0 ± 7.29	75.0 ± 6.23	G: P = 0.8545
Xyl-St	76.4 ± 3.98	73.0 ± 5.10*	71.3 ± 4.13*	71.3 ± 5.32*	71.5 ± 6.16*	71.5 ± 4.72*	71.8 ± 5.98*	73.0 ± 5.14*	76.0 ± 5.66	TxG:P = 0.0195

Means with an asterisk (*) differ significantly (P < 0.05) from baseline.

Corresponding means with different superscripts (a or b) are significantly (P < 0.05) different.

9.2 *Second study*

Table 15: Plasma cortisol, non-esterified fatty acid (NEFA) and lactate concentrations (Mean± SD) of placebo (controls; C; n= 12) and xylazine (X; n= 12) treated cows recorded for 6 hours following claw treatment in lateral recumbency (LR) on a surgical tipping table.

		LR			Post-op			
		Baseline	***+15min***	***LR end***	***+1h***	***+3h***	***+6h***	***Effects***
Cortisol	**C**	18.7±12.8	**38.9±15.5[a]**	**37.8±25.1**	**9.04±6.16**	**6.55±4.95**	**8.63±7.03**	T: P < 0.0001
[ng ml^{-1}]	**X**	14.6±11.1	16.4±12.8[b]	**39.1±25.5**	9.54±6.89	**7.23±5.82**	9.31±7.79	T: P = 0.4270
								TxG:P = 0.1408
NEFA	**C**	589±425	**856±462[a]**	**858±368**	493±296	554±374[a]	589±327[a]	T: P < 0.0001
[µmol L^{-1}]	**X**	493±217	498±361[b]	**781±435**	**293±153**	**243±144[b]**	**331±178[b]**	T: P = 0.0578
								TxG:P = 0.1600
L(+)Lactate	**C**	1.56±0.88	1.83±1.01[a]	1.57±0.91	**0.81±0.24**	**0.65±0.15**	**0.55±0.19**	T: P < 0.0001
[mmol L^{-1}]	**X**	0.98±0.39	0.85±0.26[b]	1.24±1.04	0.84±0.61	**0.63±0.40**	**0.45±0.24**	T: P = 0.1139
								TxG:P = 0.1321

Bold means differs significantly (P < 0.05) from base line within groups

Corresponding means with different superscripts (a or b) differ significantly (P < 0.05) among groups.

Table 16: Heart rate(HR), respiratory rate (RR) and body temperature (BT; Mean± SD) of placebo (controls; C; n= 12) and xylazine (X; n= 12) treated cows recorded for 6 hours following claw treatment in lateral recumbency (LR) on a surgical tipping table.

			LR		Post-op			
		Baseline	***+15min***	***LR end***	***+1h***	***+3h***	***+6h***	***Effects***
HR	**C**	82.2±10.8	85.7±16.1[a]	84.0±12.7[a]	80.3±10.8	82.2±16.7	82.0±16.1	T: P =0.0039
[beatsmin^{-1}]	**X**	81.8±7.90	**61.5±7.22[b]**	**67.5±9.02[b]**	77.5±9.59	82.7±12.9	80.9±9.51	T: P = 0.0577 TxG: P < 0.0001
RR	**C**	45.5±13.5	48.3±10.7[a]	51.3±14.1[a]	43.0±13.8	50.0±17.6[a]	47.5±17.5	T: P =0.0797
[breathsmin^{-1}]	**X**	35.6±10.1	**20.8±11.4[b]**	31.0±15.8[b]	32.8±11.4	36.2±12.7[b]	36.3±12.5	T: P = 0.0016 TxG: P = 0.0354
BT	**C**	38.9±0.31	**39.1±0.35**	38.9±0.24	38.8±0.17	38.9±0.14	38.9±0.24	T: P = 0.0030
[C°]	**X**	38.8±0.21	**39.1±0.28**	**39.1±0.30**	38.9±0.46	38.8±0.35	38.9±0.26	T: P = 0.7335 TxG: P =0.1321

Bold means differs significantly (P < 0.05) from base line within groups

Corresponding means with different superscripts (a or b) differ significantly (P < 0.05) among groups.

Table 17: Types of claw affections causing lameness during the entire study on cows treated with placebo (n= 12; controls) and cows treated with xylazine (n= 12).

Nr	***Affected claw***	***Claw affections***	***Type of treatment***
Controls (n= 12)			
1	FL / med.	Pod.sol.circum.superfacialis et sol.circum.prof.	Claw trimming, wooden block
2	HR / lat.	Septic arthritis of the coffin joint	Claw amputation
3	HL / med.	Lat.Pod.par.abax. et sol.prof. interdigital necrobacillosis	claw trimming, wooden block
4	HL / lat.	Pod. Sol. Circum.prof. pur. et necr with arthritis of coffien joint	Resection of coffin joint
5	HL / lat.	Pod. Solaris circum lat prof necrotic purulanta	Excision of the lesion
6	HR / med.	Pod. Solaris superficialis	Claw trimming
7	HL / med.	Seous arthritis of the fetlock joint	Joint lavage
8	HR / lat.	Prof. Podo circum paralateralis	Claw trimming, wooden block
9	HL / med.	Foot root, deep sole ulcer	Lesion excision
10	HL / med.	Arthritis of coffien joint with osteomylitis	Joint resection
11	HL / med.	Interdigital dermatitis	Paring and thining
12	HR / lat.	Pod. Profou abax. Circum perfo. With osteomylitis, arthritis	Claw amputation
Xylazine (n= 12)			
13	HR / lat	Toe ulcer	Toe resection
14	FR / lat	Pod septic diffu. profound	Trimming, wooden block
15	FR / med	Deep non-perforating sole ulcer, mild degree of osteomylitis of os-pedis	Trimming + wooden block
16	HR /lat, med	*Med:*Pod. Solaris profou., osteomylitis of pedal bone; *lat:* deep perforating sole ulcer	Trimming , blocking of uninjured claw
17	HL / lat	Superficial sole ulcer	claw trimming
18	HR / lat, med	Superficial sole ulcer	Claw trimming
19	FL / med	Superficial sole ulcer	Claw trimming
20	FR / med	Deep perforating sole ulcer with osteomylitis of os-pedis and sesamoid bone	Resection of coffin joint, excision of sesamoid bone
21	HL / med	Superficial sole ulcer	Claw trimming
22	HR /med HL / lat	Pod. Circ. Prof. solearis et pariet.abax	Trimming, blocking
23	HR /lat, HL/lat	Pod.sol.circum.prof; pod.sol.circu.sup.	Trimming and blocking
24	HR /lat, HL/lat	Pod.sol.circum.prof with arthritis purul.; pod.sol.circum.profou.	Resection of coffin joint, blocking

Fig.14: Caudal auricular artery catheter implantation in a cow (Seldinger technique)

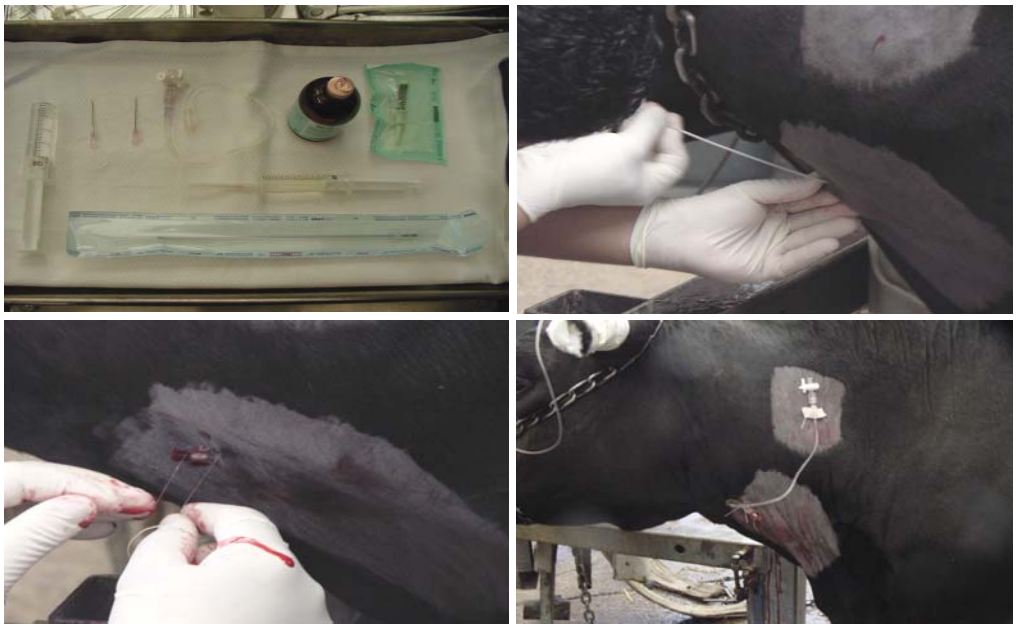

Fig.15: External jugular vein catheter implantation in a cow

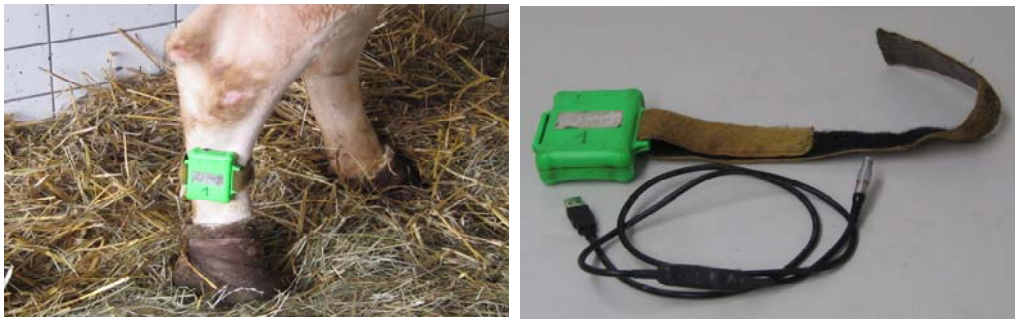

Fig.16: Pedometer

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12 ACKNOWLEDGEMENTS

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