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► **To cite this version:**

Armelle Prunier, Luc Mounier, Pierre Le Neindre, Christine Leterrier, Pierre Mormède, et al.. Identifying and monitoring pain in farm animals: a review. *Animal*, 2013, 7 (6), pp.998-1010. 10.1017/S1751731112002406 . hal-01129733

HAL Id: hal-01129733

<https://hal.science/hal-01129733v1>

Submitted on 29 May 2020

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Identifying and monitoring pain in farm animals: a review

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(Received 6 July 2012; Accepted 2 November 2012; First published online 20 December 2012)

One important objective for animal welfare is to maintain animals free from pain, injury or disease. Therefore, detecting and evaluating the intensity of animal pain is crucial. As animals cannot directly communicate their feelings, it is necessary to identify sensitive and specific indicators that can be easily used. The aim of the present paper is to review relevant indicators to assess pain in several farm species. The term pain is used for mammals, birds and fish, even though the abilities of the various species to experience the emotional component of pain may be different. Numerous behavioural changes are associated with pain and many of them could be used on farms to assess the degree of pain being experienced by an animal. Pain, as a stressor, is associated with variations in the hypothalamic–pituitary–adrenal axis as well as in the sympathetic and immune systems that can be used to identify the presence of pain rapidly after it started. However, most of these measures need sophisticated equipment for their assessment. Therefore, they are mainly adapted to experimental situations. Injuries and other lesional indicators give information on the sources of pain and are convenient to use in all types of situations. Histopathological analyses can identify sources of pain in experimental studies. When pronounced and/or long lasting, the pain-induced behavioural and physiological changes can decrease production performance. Some indicators are very specific and sensitive to pain, whereas others are more generally related to stressful situations. The latter can be used to indicate that animals are suffering from something, which may be pain. Overall, this literature review shows that several indicators exist to assess pain in mammals, a few in birds and very few in fish. Even if in some cases, a single indicator, usually a behavioural indicator, may be sufficient to detect pain, combining various types of indicators increases sensitivity and specificity of pain assessment. Research is needed to build and validate new indicators and to develop systems of pain assessment adapted to each type of situation and each species.

Keywords: pain, behaviour, hormone, lesion, performance

Implications

Assessing pain is crucial for pain prevention and alleviation. The aim of the present paper is to review relevant indicators in several farm species, including fish. Four types of indicators, related to physiology, behaviour, lesions and performance, are discussed. Some indicators are very specific and sensitive to pain, whereas others are more general indicators of stressful situations, but can be used to alert persons in charge of the animals that these animals are suffering from something, which could be pain. A single behavioural indicator may be sufficient to detect pain, but combining indicators improves efficiency of pain assessment.

Introduction

Welfare of animals includes both physical and mental aspects and implies that animals should be free from pain, injury or disease (Farm Animal Welfare Council, 1992). Therefore, it is crucial to assess, that is, to detect and, if possible, to evaluate the intensity of pain, in order to prevent pain or to alleviate it as rapidly as possible (Guatteo *et al.*, 2012). Molony and Kent (1997) defined pain in animals as 'an aversive sensory and emotional experience [...], it changes the animal's physiology and behaviour to reduce or avoid damage, to reduce the likelihood of recurrence and to promote recovery'. According to this definition, pain has an emotional component, and one important question is whether animals, especially fish, have the conscious emotional states needed to experience pain. It is not the purpose of the

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present review to debate about this question. Therefore, we will use the term pain for all species included in the present review, even though the degree of conscious emotional states may vary greatly between species (Le Neindre *et al.*, 2009).

In humans, the best evaluation of pain is self-report, on the basis of oral or written communication (Herr *et al.*, 2006). In animals, it is necessary to use indicators that can be detected by external observers. Consequently, pain assessment in animals is difficult. The numerous reviews of literature or guidelines that have been written focussed mainly on mammalian species (e.g. see: Morton and Griffiths, 1985; Molony and Kent, 1997; Holton *et al.*, 2001) and more recently on birds (Gentle, 2011). Indicators used for animals are often similar to those described for humans. Most of them are based on physiological or behavioural reactions aiming at stopping the cause or reducing the consequences of the noxious stimuli (Molony and Kent, 1997; Mellor *et al.*, 2000). Indicators of injuries and lesions may be used additionally as they often cause pain. Finally, pain can lead to a decrease in production performance, such as growth rate (bulls: Earley and Crowe, 2002) or milk production (Fourichon *et al.*, 1999), which may also be used as a pain indicator.

The aim of the present paper is to describe the four main types of indicators (behavioural, physiological, lesional and production indicators) of pain in mammals (essentially in sheep, cattle and pigs), birds (mainly in poultry) and fish, on the basis of examples from the literature without attempting to be exhaustive. Strengths and weaknesses of each type of indicator will be evaluated. This review is based on a comprehensive report of the French National Institute for Agricultural Research (INRA) on pain in food-producing farm animals, with the aims to propose methods for assessment and solutions to prevent or alleviate pain (Le Neindre *et al.*, 2009). A review on the means to prevent and alleviate pain was also published (Guatteo *et al.*, 2012).

Situations of pain evaluation

Pain indicators in farm animals have been described for various situations using (a) experimental models of pain, (b) application of painful procedures that are commonly performed in commercial farms for production purposes (e.g. tail docking in mammals or beak trimming in birds) or (c) animals that are suffering from pain due to diseases or lesions (e.g. arthritis). Experimental models of pain often mimicked situations of 'natural' pain like intra-articular pain induced by urate injection (Hocking *et al.*, 1997) or feather pecking mimicked by feather removal (Gentle and Hunder, 1991). Some models were more artificial, such as subcutaneous injection of acetic acid in the lips of fish (Sneddon, 2003).

In some experiments, authors compared various combinations of experimental treatments including a painful procedure, a sham procedure, an analgesic treatment (local anaesthetic treatment with lidocaine in most cases and/or a non-steroidal anti-inflammatory drug (NSAID)), a sham treatment and/or

non-handled animals to validate potential pain indicators. Animals used in experimental models of pain as well as animals suffering from 'natural' pain, receiving or not analgesic treatments, were also used in validation studies.

Postural and behavioural indicators

In pigs and ruminants

Numerous postural and behavioural indicators of pain have been described in mammals (Table 1). They can be distinguished in five main categories. Four of these aim directly or indirectly to avoid or alleviate the painful stimulus: (1) avoidance and defensive behaviours, (2) vocalisations, (3) behaviours directed towards the painful areas and (4) postures and behaviours aiming to reduce stimulation of the painful area. The fifth category is related to general changes in activity, being motionless or agitated, feeding, drinking, social and grooming behaviours. These categories will be discussed in more detail below.

The nociceptive withdrawal reflex is the simplest form of avoidance and defensive behaviours. The withdrawal movement is involuntary and rapid, mediated by a reflex arc synapsing in the spinal cord. The brain receives the sensory input but does not intervene in the reflex action. The withdrawal reflex protects the animal from potentially damaging stimuli. The reflex is frequently used to measure the response to a controlled painful stimulus in order to test the efficiency of analgesic drugs or the influence of the physiological state of the animal. An example is the latency to the occurrence of a withdrawal or kicking movement when a limited area of the leg, shoulder or rump is subjected to a painful stimulus, for instance, by heating the skin locally with a laser beam (pigs: Jarvis *et al.*, 1997; calves: Veissier *et al.*, 2000; cows: Herskin *et al.*, 2003). Avoidance and defensive behaviours, including leg and body movements as if animals were trying to avoid or escape the painful stimuli, were observed during castration (Marx *et al.*, 2003), teeth resection and tail docking in young piglets (Noonan *et al.*, 1994; Torrey *et al.*, 2009). Similarly, dairy cows or growing calves jumped or kicked when subjected to hot-iron or liquid-nitrogen branding (Lay *et al.*, 1992a; Schwartzkopf-Genswein *et al.*, 1998). These behaviours may also be observed during palpation of a painful area. Reactions to palpation of the scrotum or of the neck of the scrotum in lambs that were castrated with constriction rings, ranged from no reaction or a slight tension of the hind limbs to bucking (Thornton and Waterman-Pearson, 1999).

Vocalisations are often used to identify pain in pigs, sheep and cattle. Many studies found that the number and features of these vocalisations were modified in case of painful situations (reviewed by Watts and Stookey, 2000; Manteuffel *et al.*, 2004). For example, during painful interventions, the number or duration of vocalisations increased in lambs (Molony *et al.*, 1997), cattle (Schwartzkopf-Genswein *et al.*, 1997) and pigs (Weary *et al.*, 1998). Detailed studies on pigs found that high-frequency screams (>1000 Hz) were more frequent, lasted longer and were more powerful when

Table 1 List of physiological and behavioural changes that may indicate the existence of pain in mammals

Physiological indicators	Behavioural indicators
Hormonal concentrations in blood, saliva or urine	Vocalizations
Adrenal axis: ACTH, cortisol	Number and duration
Sympathetic axis: adrenaline, noradrenaline	Intensity
Blood energetic metabolites	Spectral characteristics
Glucose, lactate	Postures
Free fatty acids	Abnormal lying (legs tucked under the body, etc.)
Blood concentrations of inflammatory markers ¹	Abnormal standing (not on all legs, rigid, etc.)
Haptoglobin, fibrinogen, IL-1, etc.	Behaviours
Activity of the autonomous nervous system	Frequent licking, scratching, rubbing
Heart rate	Avoidance and escape
Respiratory rate	Tonic immobility
Arterial blood pressure	Lack or excessive locomotion
Internal, cutaneous or eye temperature	Aggressiveness
Pupil diameter	Agitation or lack of activity
Sweating, skin electric conductivity	Prostration
Muscle tremor	Isolation
Brain activity	Loss of appetite, etc.
EEG	

ACTH = adrenocorticotrophic hormone; IL = interleukin; EEG = electroencephalogram.

Adapted from Mellor *et al.* (2000), Prunier *et al.* (2002) and Hay *et al.* (2003).

¹Inflammatory markers indicate the existence of an inflammatory state that may generate pain.

piglets were castrated than when they were just handled to simulate castration, and the high-frequency screams were much reduced when piglets received a local anaesthetic before castration (White *et al.*, 1995; Marx *et al.*, 2003). In addition, Puppe *et al.* (2005) showed that the entropy of the high-frequency vocalisation was lower during castration compared with the pre-surgical period. Similarly, Watts and Stookey (1999) observed that, compared with controls, calves subjected to hot-iron branding showed a greater frequency range in the fundamental or lowest harmonic of the audiospectrogram of their vocalisations, a higher maximum frequency and a higher peak sound level. However, many animals also vocalise during non-painful handling. Consequently, sometimes, no differences are found between the control and painful situation (Lay *et al.*, 1992a; Schwartzkopf-Genswein *et al.*, 1998). It was further shown in ruminants that after the acute response to a painful intervention, monitoring of vocalisations was of little efficacy to detect pain (Molony *et al.*, 2002; Grant, 2004).

Behaviours directed towards the painful areas are relatively easy to observe. Licking or scratching are probably performed to relieve the pain, as simultaneous activation of non-nociceptive sensory receptors of the skin inhibits the transmission of nociceptive signals (Melzack and Wall, 1965). Thus, calves licked the end of their tail after tail docking (Eicher *et al.*, 2000) or the scrotal area for 48 days following castration (Molony *et al.*, 1995). When licking is not possible for anatomical reasons, animals may scratch the painful area. For example, calves scratched their head with the hind foot after heat cauterisation of the horn-producing area (i.e. disbudding; Morisse *et al.*, 1995). Similarly, the days following surgical castration, pigs displayed scratching

of the scrotum against the floor (Hay *et al.*, 2003; Llamas Moya *et al.*, 2008; Figure 1) or dog-sitting postures (Llamas Moya *et al.*, 2008). Other specific movements directed to the painful area may involve head movements towards the painful area after castration and/or tail docking in lambs (Molony *et al.*, 2002), teeth champing (opening and closing of the mouth not associated to feeding) after teeth clipping in pigs (Noonan *et al.*, 1994) and head shaking after disbudding in calves (Morisse *et al.*, 1995).

Postures and behaviours to reduce stimulation of the painful area can also indicate the presence of pain. The most common example is lameness. Foot lesions frequently stop the animal putting weight on the affected leg (O'Callaghan *et al.*, 2003; Flower and Weary, 2006). Administration of an analgesic treatment reduced the degree of lameness in cattle, demonstrating the implication of pain in this behaviour (Rushen *et al.*, 2007). Pigs (Hay *et al.*, 2003), lambs (Molony *et al.*, 1993) and calves (Robertson *et al.*, 1994) were more often lying on their sides with their legs extended after castration than before. Abnormal ventral lying also occurred in lambs after castration combined or not with tail docking (Molony *et al.*, 1993 and 2002). Animals that suffer from pain may lie with legs tucked under the body (=huddle up; lambs: Molony *et al.*, 1993; pigs: Hay *et al.*, 2003; Llamas Moya *et al.*, 2008). All these postures supposedly reduce stretching of tissues, and hence painful stimulation of the injured area. The reduction in locomotion is observed not only in lame animals (cows: O'Callaghan *et al.*, 2003) but also after castration (pigs: Llamas Moya *et al.*, 2008), probably because it also reduces stimulation of the painful area.

Being motionless or agitated may both occur after a painful procedure and can be a pain indicator. For example,

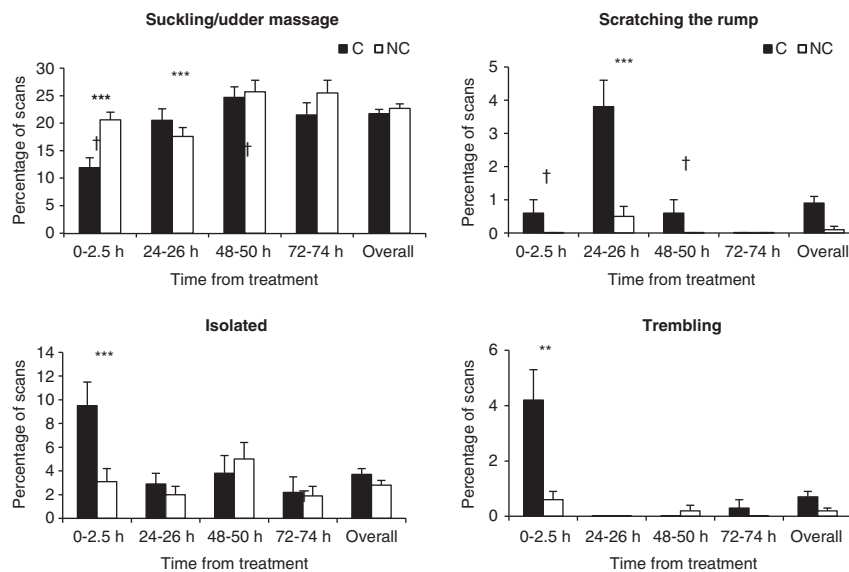


Figure 1 Comparison of some behavioural indicators of pain in piglets castrated at 5 days of age (C) or not (NC) at various periods from treatment (adapted from Hay *et al.*, 2003). Symbols indicate statistical differences between treatments within periods at $^{\dagger}P < 0.1$, $^{**}P < 0.01$ and $^{***}P < 0.001$.

'statue standing' (standing still for more than 10 s) and being awake without any activity were more frequent after rubber-ring castration in lambs (Molony *et al.*, 2002) and after surgical castration in pigs (Hay *et al.*, 2003), respectively, compared with non-castrated controls. Jumping, foot stamping and kicking, rolling from one side to the other side and restlessness measured by the frequency of alternating standing and lying postures were also more frequent after rubber-ring castration and/or tail docking (Molony *et al.*, 1995; Grant, 2004). Injection of a local anaesthetic into the two sides of the neck of the scrotum of lambs subjected to rubber-ring castration reduced these behaviours (Molony *et al.*, 1995 and 2002). Tail wagging can be interpreted as a sign of agitation and/or as a signal masking nociceptive signals from the rear part of the body and was observed after castration in piglets (Hay *et al.*, 2003), lambs (Molony *et al.*, 2002) or calves (Robertson *et al.*, 1994).

Pain may influence other behaviours such as those related to feeding, drinking, social and grooming. For example, less suckling or feeding behaviour, social isolation, behavioural desynchronisation with littermates and/or less social interactions with the dam were observed in pigs after surgical castration (McGlone and Hellman, 1988; Hay *et al.*, 2003; Llamas Moya *et al.*, 2008) or tail docking (Torrey *et al.*, 2009). Similarly in calves, reduction in feed intake was observed after castration (Fisher *et al.*, 1996).

In poultry

Like mammals, poultry show withdrawal reactions when an area is painfully stimulated. For example, automatic withdrawal of the foot occurred when the foot of a Japanese quail was plunged in hot water (Evrard and Balthazart, 2002). Defensive or flight behaviours during painful interventions involve attempts to escape (jumps, wing flapping) observed in chickens submitted to feather

removal (Gentle and Hunder, 1991). In a situation where birds could not escape from painful stimulations, they stopped to perform defensive and flight behaviours and crouched immobile (Gentle and Hunder, 1991).

Vocalisations can also be an expression of pain. Several studies show that hens vocalise during repeated removal of feathers from different anatomical regions (leg, cape, etc.) and during pecking (Collias, 1987; Gentle and Hunder, 1991). Collias (1987) described the vocalisations of hens during pecking. They were characterised by a short duration (0.1 to 0.2 s) and a high frequency (>8 kHz). They were moderately loud and of lower intensity than distress calls emitted during capture. In flocks where pecking was present, laying hens emitted more vocalisations, particularly more squawks (Bright, 2008).

Similar to mammals, birds can adopt postures and/or change their behaviour to reduce stimulation of the painful area. For example, after beak trimming, hens and turkeys gave fewer pecks (Gentle and Hunder, 1991). To avoid musculoskeletal pain, birds can limp or take relieving postures, ranging from a slight change in the positioning of the leg to a complete loss of support by the painful member (Kestin *et al.*, 1992; Leterrier and Nys, 1992). Lame chickens spent more time lying and walked less (Weeks *et al.*, 1997 and 2000), and turkeys with musculoskeletal disorders were less generally active than controls (Duncan *et al.*, 1991). Administration of analgesic treatments (local anaesthesia or NSAID) reduced dose dependently the time spent lying or standing on a single foot (Hocking *et al.*, 1997 and 2001).

Chickens with intra-articular pain induced by an urate injection reduced their grooming, feeding and drinking activities (Hocking *et al.*, 1997 and 2001), and chickens with musculoskeletal disorders dust-bathed less than healthy chickens (Vestergaard and Sanotra, 1999).

In fish

It has long been known that fish also demonstrate avoidance/escape behaviours when they are submitted to nociceptive stimuli such as electric shocks, and that they are able to learn to avoid these stimuli (e.g. Bintz, 1971; Ehrensing *et al.*, 1982 with goldfish). Learning did not occur if fish received an analgesic treatment with morphine (Ehrensing *et al.*, 1982), showing the implication of pain in the behaviour. Two types of escape behaviours were described both in goldfish and trout: a high speed 'panic' swimming response ceasing immediately after leaving the electrical zone and a more calm response ('tail-flip'), allowing the fish to cruise from the zone (Dunlop *et al.*, 2006).

Administration of a noxious stimulus, such as a subcutaneous injection of acetic acid in the lips, may cause reduction in frequency of swimming, cessation of food intake or induce abnormal behaviours such as rocking from side to side or rubbing the lips against the tank (Sneddon, 2003; Reilly *et al.*, 2008). One or more of these behavioural changes occurred in the three studied species: zebrafish, trout and carp (Reilly *et al.*, 2008). Experiments in rainbow trout showed that morphine or local anaesthesia with lidocaine reduced these behavioural changes in acid-treated animals (Sneddon, 2003; Mettam *et al.*, 2011), indicating that they were dependant on pain.

Drawbacks and advantages of using postural and behavioural indicators

A frequent criticism of behavioural assessment of pain is its supposed subjectivity, because of differences in perception and interpretation by observers. In order to avoid biases, it is necessary to ensure the validity and reproducibility of the behavioural measures.

First, it should be checked that behavioural indicators are specifically influenced by pain and not by handling or stress resulting from the procedure. For many indicators, this was verified by comparing the behaviour of animals submitted or not to painful/nociceptive stimuli with and without a pharmacological pain-relieving treatment (see above). However, some behavioural changes may also be observed during illness and situations of stress or discomfort without a nociceptive component. Apathy, social isolation and loss of appetite are examples. These behaviours cannot specifically identify pain but they can contribute to its overall evaluation if they are associated with more specific indicators.

Reproducibility of pain detecting measures was rarely evaluated except for lameness for which reliability between two observers was assessed (for example in sheep: Welsh *et al.*, 1993 and in cattle: Flower and Weary, 2006; Leach *et al.*, 2009). Lack of reliability may come from the observers and from the animal variability in the expression of pain. Regarding observers, many potential sources of biases identified for ratings (Meagher, 2009) may be relevant for behavioural and postural observations: insufficient precision of the definitions of the behavioural categories, insufficient experience and training of the observers, as well as a lack of

impartiality. Solutions include improvement of definitions, better training of observers, the assurance of their independency and, if relevant, the use of blind protocols. Regarding animals, the expression of pain may vary over time and across animals. For example, they can be modulated by endogenous analgesic processes. In some contexts, highly motivated behaviours, such as laying in hens, or feeding, may be expressed despite pain, and may reduce or even cancel pain expression (Gentle and Corr, 1995; Wylie and Gentle, 1998).

Although caution should be taken, postural and behavioural indicators have many advantages. First, they are not invasive, do not need any intervention on the animals and can be carried out by direct observation. Therefore, they can be used under field conditions, by veterinarians, technicians or farmers. Second, postural and behavioural indicators are sensitive and appear immediately during acute pain. Third, numerous pain-related behaviours are specific and help identify the location of the pain, thus facilitating its treatment.

Methods to record and analyse behavioural indicators can be very sophisticated and time-consuming but necessary for research purposes (e.g. Molony *et al.*, 1993 in lambs; Hay *et al.*, 2003 in pigs). However, at the farm level, rapid and simple systems of observation should be developed, for example, systems of scoring or systems of automatic recording of the behavioural activity on the basis of localisation of the animals by telemetry.

Physiological indicators

Physiological variations related to pain are mainly because of two interrelated mechanisms. First, pain is a powerful stressor stimulating directly the release of hormones from the hypothalamic–pituitary–adrenal (HPA) and sympathetic axes in mammals and birds and their equivalents in fish. Second, tissue damage activates the immune system and the release of numerous inflammatory mediators, which, in turn, may also activate the adrenal axis (reviewed by Turnbull and Rivier, 1999; Lamont *et al.*, 2000). For example, the pro-inflammatory cytokine, interleukin-1, released by immune cells after a tissue lesion, stimulates the release of adrenocorticotrophic hormone (ACTH) and cortisol and plays an active role in transmitting nociceptive stimuli to the brain (Turnbull and Rivier, 1999; Ren and Torres, 2009). Third, some substances (haptoglobin, fibrinogen, etc.) that are not involved in the control of pain but are released during inflammation can be used as indirect indicators of pain, as inflammatory states are highly susceptible to generate pain. Therefore, physiological indicators of pain involve hormones from the adrenal and sympathetic axes, their metabolic and physiological consequences, plasma markers of an inflammatory state and mediators involved in the physiological mechanisms of pain.

In pigs and ruminants

Various physiological indicators of pain have been used in mammals (Table 1). Many studies have examined cortisol

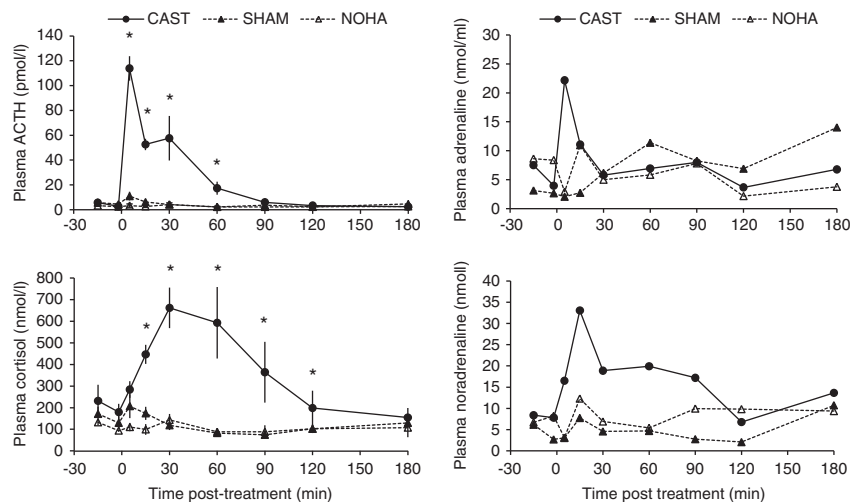


Figure 2 Comparison of plasma concentrations of adrenocorticotrophic hormone (ACTH), cortisol, adrenaline and noradrenaline in 5- to 6-day-old piglets that were submitted to surgical castration (CAST) or sham castration (SHAM) at time 0 or were not handled (NOHA) (adapted from Prunier *et al.*, 2005 and 2006). *Within experimental groups, concentration at a given time differs at $P < 0.05$ from concentration at time 0.

concentrations in blood or saliva samples collected at regular intervals before and after painful interventions in pigs (castration: Prunier *et al.*, 2005; Carroll *et al.*, 2006; Sutherland *et al.*, 2010, snaring (=squeezing the upper jaw with a nose sling) or tattooing: Merlot *et al.*, 2011; Figure 2), calves (castration: Cohen *et al.*, 1990; dehorning: Morisse *et al.*, 1995; Fisher *et al.*, 1996; Sylvester *et al.*, 1998) and lambs (castration: Lester *et al.*, 1991 and 1996). Handling had no significant effect on plasma cortisol in pigs (Prunier *et al.*, 2005), lambs (Mellor and Murray, 1989) or calves (Lay *et al.*, 1992a, 1992b), whereas the painful intervention induced a significant increase in the hours and sometimes days following its application in all cases except after tail docking and tooth resection in very young piglets. If local anaesthesia was used, the amplitude and duration of the cortisol increase were lower following castration (lambs: Graham *et al.*, 1997; Molony *et al.*, 2002; pigs: Prunier, 2002; Courboulay *et al.*, 2010; calves: Stafford *et al.*, 2002; Ting *et al.*, 2003), tail docking (lambs: Graham *et al.*, 1997) or dehorning (calves: reviewed by Stafford and Mellor, 2005). Similarly, post-surgery increases in plasma cortisol were reduced in animals treated with caudal epidural anaesthesia or NSAID in calves submitted to castration (Earley and Crowe, 2002; Stafford *et al.*, 2002; Ting *et al.*, 2003) or in lambs submitted to tail docking (Graham *et al.*, 1997).

Variations in plasma ACTH, the pituitary hormone that stimulates cortisol release, were more marked than variations in plasma cortisol after a painful procedure such as castration and other common procedures applied to pigs (Prunier *et al.*, 2005; Merlot *et al.*, 2011; Figure 2). The increase occurred earlier and amplitude of the peaks was higher. Thus, ACTH is a more sensitive pain indicator than cortisol. In parallel to ACTH, endogenous β -endorphin may be released in the general circulation in response to pain. An increase in plasma concentration of β -endorphin was observed after snaring or painful orthopaedic surgery in

horses (McCarthy *et al.*, 1993; Roozen *et al.*, 1995), tooth resection by grinding in piglets (Marchant-Forde *et al.*, 2009) and after surgical mulesing (removal of strips of skin from the rump area) in sheep (Shutt *et al.*, 1987). In contrast, horses with painful chronic lameness had plasma concentrations of β -endorphin similar to those of normal horses (McCarthy *et al.*, 1993). Therefore, the relevance of β -endorphin as a chronic pain indicator may be limited contrarily to its usefulness in detecting acute pain.

Plasma adrenaline increased rapidly but briefly after painful procedures such as castration in pigs (Prunier *et al.*, 2006), dehorning in calves, castration and tail docking in lambs (Mellor *et al.*, 2002). In the same experiments, plasma noradrenaline also increased, but the increase occurred later and lasted longer compared with adrenaline. In pigs, painful procedures resulted further in an increase in plasma lactate, probably because of the catabolism of muscular glycogen (Prunier *et al.*, 2005; Merlot *et al.*, 2011) triggered, at least in part, by the adrenaline release (Fernandez *et al.*, 1995). Similarly, increases in free fatty acids or glucose due to release of catecholamines and/or cortisol are expected. The increased muscular activity associated with the defence movements during acute pain may also contribute to higher plasma lactate in response to anaerobic muscular glycogen mobilisation.

The activity of the sympathetic axis can also be evaluated by other physiological variables. For example, arterial blood pressure and heart rate increased during castration in piglets (White *et al.*, 1995; Haga and Ranheim, 2005), and respiratory and heart rates increased during freeze or hot-iron branding in cattle (Lay *et al.*, 1992b and 1992c; Stewart *et al.*, 2008). These increases were less pronounced when a local anaesthesia was applied before surgery (pigs: White *et al.*, 1995; Haga and Ranheim, 2005). Heart rate variability (HRV) is under the control of both the sympathetic and parasympathetic systems and could thus be influenced by

chronic pain (Von Borell *et al.*, 2007a). However, HRV as a pain indicator has been poorly investigated. To our knowledge, there is only one study showing that treatment of horses, suffering from laminitis, with NSAID resulted in changes in HRV with an increase of high-frequency components and a decrease of low-frequency components together with less weight shifting behaviours (Rietmann *et al.*, 2004). Trembling resulting probably from activation of the sympathetic nervous system was observed during the first hours following castration in piglets (Hay *et al.*, 2003; Llamas Moya *et al.*, 2008) and in lambs (Molony *et al.*, 2002). The activity of the sympathetic axis may further be measured by maximal eye temperature, using an infrared camera recording, at regular intervals. The area measured includes the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle, the distance and angle of the beam being standardised. Calves with the head blocked in a restraint system and submitted to various treatments showed a transient decrease in eye temperature after disbudding without local anaesthesia followed by a prolonged increase, whereas only the prolonged increase was observed after disbudding with anaesthesia (Stewart *et al.*, 2008). Similarly, a prolonged increase was observed in calves after surgical castration (Stewart *et al.*, 2010).

Acute phase proteins may be used as markers of an inflammatory state susceptible to generate pain. An increase in plasma haptoglobin in the days following surgery occurred after surgical castration in lambs (Paull *et al.*, 2009) and calves (Earley and Crowe, 2002; Ting *et al.*, 2003) and after tail docking in heifers (Eicher *et al.*, 2000).

Electrical activity of the brain was modified by nociceptive stimuli as shown by variations in the electroencephalographic signals (EEG) in piglets undergoing surgical castration under isoflurane anaesthesia (Haga and Ranheim, 2005). These variations, especially a decrease in the absolute theta power, were less marked in piglets receiving local anaesthesia with lidocaine before surgery (Haga and Ranheim, 2005). Similarly, EEG was modified by scoop dehorning of calves maintained under general anaesthesia by halothane. These variations were reversed by local anaesthesia with lidocaine (Gibson *et al.*, 2007).

Variations in substances from the nervous system that are directly involved in the detection, perception or control of pain were also investigated. For instance, in piglets, after surgical castration, the expression of the protein *c-fos* in neurons from the spinal cord was lower when, before castration, local anaesthesia was administered (Nyborg *et al.*, 2000).

In poultry

The responses of the adrenal and sympathetic axes to painful stimuli have been poorly investigated in birds. Results from a study by Davis *et al.* (2004) indicated that corticosterone (the main glucocorticoid in birds) increased after beak trimming. An increase in the mean blood pressure (systole and diastole pressures) was observed after feather removal and this increase was followed by a gradual return to pre-stimulus levels, indicating a temporal activation of the sympathetic

nervous system (Gentle and Hunder, 1991). Heart rate increased in chickens submitted to beak trimming as well as in sham-handled controls, but trimmed chickens took longer to return to basal heart rates (Glatz, 1987; Glatz and Lunam, 1994). However, pain may induce opposite effects on heart rate. For example, after feather removal, heart rate decreased in 20% of the chickens, whereas it increased in 62% of them (Gentle and Hunder, 1991). To our knowledge, there are no available data regarding the use of HRV as an indicator of pain in poultry. Cardiovascular changes can also be detected by changes in the colour of the crest that turns pale in case of peripheral vasoconstriction. Changes in the colour of the crest were observed after feather removal but with a high variability between animals and even within animals when observations were repeated over time (Gentle and Hunder, 1991).

EEG patterns were also evaluated in birds. Electrodes were implanted at the surface of the telencephalon several weeks before feather removal (Gentle, 1974; Gentle and Hunder, 1991). During the period of immobility following feather removal, the EEG showed a characteristic high amplitude and low-frequency pattern similar to that observed during states of tonic immobility (Gentle *et al.*, 1989) that are indicative of fear.

In fish

Submitting teleost fish to a stressor activates the brain–sympathetic–chromaffin cell axis (equivalent of the sympathetic axis in mammals and birds) and the hypothalamic–pituitary–interrenal axis (equivalent of the HPA axis in mammals and birds), which influence the respiratory and cardiovascular systems, the hydromineral balance and the energetic metabolism (reviewed by Bonga, 1997). Only a few studies have examined the influence of nociceptive stressors on these axes. The injection of acetic acid in the lips of rainbow trout did not influence cortisol levels (Mettam *et al.*, 2011), but increased the opercular beat rate indicating an increase in the ventilation rate during the first hours following the administration (Sneddon, 2003; Mettam *et al.*, 2011). This increase was reversed when trouts were treated with morphine, indicating the nociceptive implication of the changes (Sneddon, 2003).

Drawbacks and advantages of using physiological indicators

As most of these indicators can also be related to stress or to illness without a pain component, their interpretation should be made with caution. Handling or restraining an animal to sample blood, urine or saliva, to measure heart rate, blood pressure, eye temperature or EEG may induce stress reactions (Moberg, 2000). For example, fattening pigs that are not fitted with permanent catheters are generally blood sampled from the anterior vena cava under restraint with a nose sling (snaring), and this procedure is sufficient to increase plasma cortisol and ACTH within a few minutes (Merlot *et al.*, 2011). Variables related to the sympathetic axis are even more sensitive and may change within seconds, reaching high levels with a large range of stimuli (Matteri *et al.*, 2000).

For example, in chickens, physical restraint alone or physical restraint combined with beak trimming caused similar increases in heart rate (Glatz and Lunam, 1994). In order to avoid the effects of handling and restraint, remote data acquisition techniques, such as telemetric measures in freely moving animals, should be used as much as possible. However, even in this situation, data should be interpreted with care as spontaneous activities such as feeding, sleeping or moving may also influence the sympathetic and parasympathetic activities, their balance and all related variables (Von Borell *et al.*, 2007a).

The release of indicators of inflammation is not closely related to the occurrence of pain. For example, in lambs, haptoglobin levels increased after surgical but not after rubber-ring castration, while rubber-ring castration resulted in more pain-related behaviour (Paull *et al.*, 2009).

Physiological measurements may require sophisticated equipment that can be fitted on the animals or specialised laboratory techniques to analyse biological markers. Some methods may require surgery and maintenance of the animals in especially adapted buildings. Owing to these specific requirements and the need to avoid other disturbances causing stress, physiological methods are difficult to use in commercial farms. However, they are very useful in experimental situations to identify sources of pain, compare painful procedures and test the efficacy of pharmacological treatments to relieve pain.

Injuries and other lesional indicators

The physiological mechanisms underlying pain perception are very similar in humans and other mammals. There are also many similarities with birds and fish. By analogy, it can be expected that lesions susceptible to cause pain in humans do so in animals. The most common are: fractures, cutaneous lesions, tissue trauma after amputation, abscesses and neuromas (random proliferations of axons and glial support cells). Therefore, clinical examination of live animals or carcasses as well as histopathological analyses can reveal possible sources of pain even though a lesion is not necessarily accompanied by pain.

In pigs and ruminants

Macroscopic lesions, including wounds and abscesses, have been used to assess the effect of resection of teeth of piglets on the sows' teats or on the other piglets (Brown *et al.*, 1996; Gallois *et al.*, 2005). Many studies evaluated pain-causing lesions including lesions on the tail, ears and feet of pigs (Moultotou *et al.*, 1999; Widowski *et al.*, 2003; Valros *et al.*, 2004; Gillman *et al.*, 2009) and on the feet of heifers and cows (Logue *et al.*, 1994; Capion *et al.*, 2009).

Histopathological analysis allowed demonstrating, for example, neuromas in histological sections of tail stumps of pigs following tail docking (Simonsen *et al.*, 1991; Done *et al.*, 2003). Such neuromas could be responsible for neuropathic pain that is chronic but often intermittent as shown in human amputees (Lewin-Kowalik *et al.*, 2006; Wolff *et al.*, 2011).

Histological analysis of longitudinal sections of teeth at different ages demonstrated anomalies in teeth clipped the day after birth, including dentin fractures, pulpitis and abscesses (Hutter *et al.*, 1994; Hay *et al.*, 2004). Such anomalies are very likely sources of pain.

In poultry

In laying hens, plumage condition and wounds resulting from pecking may be used to assess possible sources of pain. Similarly, scores used to measure pododermatitis in chickens indicated whether it involves inflammation only or ulceration arising from secondary infection (Allain *et al.*, 2009). High scores of pododermatitis were associated with withdrawal reactions of chickens when touched, indicative of pain.

A few histopathological studies were carried out after beak trimming. In contrast to early beak trimming, late beak trimming in chickens resulted in the development of painful neuromas (Beward and Gentle, 1985). However, this effect of age on beak trimming was not found in a later study on turkeys (Gentle *et al.*, 1995).

In fish

Lesions of various origins (bacterial or viral infections, UV light, dietary deficiency, attacks by conspecifics, etc.) have been described in farmed fish (Abbott and Dill, 1985; Turnbull *et al.*, 1998), particularly fin or skin erosion and eye lesions. They have a detrimental influence on health and welfare of the animals (Turnbull *et al.*, 1998), but no study was conducted to investigate the nociceptive character of such lesions.

Drawbacks and advantages of clinical measures

One important problem of using lesions as indicators of pain is that they are not necessarily sources of pain. Other difficulties are related to the constraints in conditions of observation: animals should be clean, light and space should be sufficient to examine animals or carcasses.

Applied to live or even to slaughtered animals, clinical measures are part of the diagnosis to determine a treatment to alleviate pain in commercial or in experimental situations. Histopathological methods are additional measurements often, but not exclusively, reserved to experimentation. They can help identifying sources of pain only detectable at a microscopic level.

Indicators related to production performance

Sustained behavioural and physiological changes due to pain can induce decreases in performance of the animals. Pain may reduce feeding behaviour (see: postural and behavioural indicators) or induce stress and immune reactions known to influence nutrient fluxes (see: physiological indicators) and utilisation (Elsasser *et al.*, 2000) or to inhibit physiological axes such as the gonadotropic axis (reviewed by Tilbrook *et al.*, 2000; Von Borell *et al.*, 2007b) or the somatotropic axis (Carroll, 2008; Borghetti *et al.*, 2009), thus influencing performance.

In pigs and ruminants

Studies in pigs on the effects of painful procedures such as tooth resection, tail docking or surgical castration often include data on growth rate. Most of them did not show significant effects on growth (tooth resection: Brown *et al.*, 1996; Gallois *et al.*, 2005; castration: Hay *et al.*, 2003; Carroll *et al.*, 2006; Marchant-Forde *et al.*, 2009; tail docking: Torrey *et al.*, 2009). However, some studies investigating the influence of castration in very young piglets (McGlone *et al.*, 1993; Kielly *et al.*, 1999) or the influence of clipping teeth of the heaviest piglets of the litter (Fraser and Thompson, 1991; Robert *et al.*, 1995) showed a negative impact on growth, possibly because the pain due to the treatment is a disadvantage when competing for teats before the teat order is established.

In cattle, lameness has a negative influence on reproductive performance of both males and females. For example, 88% of the bulls culled for infertility had lesions of at least one joint (Persson *et al.*, 2007). Similarly, in lactating cows, lameness was associated with a higher frequency of ovarian cysts in early lactation, a prolonged interval between calving and subsequent conception and higher rates of culling and mortality (Melendez *et al.*, 2003; Bicalho *et al.*, 2007). Many studies found that lameness is associated with lower milk production (reviewed by Fourichon *et al.*, 1999). In bull calves, pain had a negative influence on growth rate. Indeed, when an analgesic treatment was applied to castrated animals, pain was alleviated and the negative impact of surgical castration on growth was less marked (Earley and Crowe, 2002).

In poultry

Cannibalism, feather and vent pecking in laying hens increased mortality rate and decreased egg production (reviewed by Glatz, 2000; Cheng, 2006). The decrease in egg production may be explained, at least in part, by less feeding behaviour consecutive to pain in the victims. Lameness may reduce growth rate, at least partly because of reduced feeding and feed conversion as reviewed in broilers (Bizeray *et al.*, 2004). In adult male turkeys, hip disorders were associated with lower spontaneous activity and slower movements in sexual activity tests (Duncan *et al.*, 1991). The effects were reduced by anti-inflammatory corticosteroid administration. In poultry, performances are generally registered at the flock level, which makes it difficult to identify problems at the individual level.

In fish

As in other vertebrates, exposure of aquacultured fish to stressful situations has negative consequences on growth, reproduction, immunity and their ability to cope with their environment and therefore may reduce performance (reviewed by Barton and Iwama, 1991). Pain is a stressor and consequently may reduce performance; however, to our knowledge, there is no study available on this subject.

Drawbacks and advantages of measurements of performance

Performance measurements as pain indicators have many disadvantages that are related to their lack of sensitivity and

specificity, and to the fact that they are often measured at the flock level. Indeed, reductions in performance will occur only if changes in behaviour and physiology are sufficiently marked and prolonged. Performance indicators are often retrospective and of little value for treatment of individuals suffering from pain. In addition, changes in performance may have many other causes than pain, such as environmental conditions, disease or stressors. Moreover, good production performance does not preclude the existence of pain.

The advantage of performance measurements is their facility of use under field conditions and the relevance for the producers who are interested and register performance for economic reasons.

General conclusion

It is not possible to allocate a 'pain score' on the basis of a simple biochemical or electrophysiological test in farm animals, but many indicators allow monitoring of pain in farm animals. The amount of documentation on indicators of pain varies between species. In mammals, numerous indicators have been described, less in birds and even less in fish. Research is still necessary to describe or validate new indicators, especially in birds and fish. In the context of commercial farms, only indicators that can be measured directly and easily are useful for assessing pain. They are mainly related to behaviour, clinical examination and performance. Behavioural indicators have the best potential for an early detection in the process of pain and hence for an efficient treatment to alleviate pain. In some cases, one indicator, usually a behavioural indicator, is sufficient to detect pain, but combining various types of indicators increases the chance of detecting and evaluating the intensity of pain.

Acknowledgements

The authors thank the INRA-DEPE team for their help during the preparation of the Collective Scientific Survey Report on pain in farm animals that formed the basis of the present literature review. They are especially grateful to C. Sabbagh, H. Chiapello and D. Fournier for their contributions.

References

- Abbott JC and Dill LM 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo Gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 42, 1702–1706.
- Allain V, Mirabito L, Arnould C, Colas M, LeBouquin S, Lupo C and Michel V 2009. Skin lesions in broiler chickens measured at the slaughterhouse: relationships between lesions and between their prevalence and rearing factors. *British Poultry Science* 50, 407–417.
- Barton BA and Iwama GK 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* 1, 3–26.
- Bicalho RC, Vokey F, Erb HN and Guard CL 2007. Visual locomotion scoring in the first seventy days in milk: impact on pregnancy and survival. *Journal of Dairy Science* 90, 4586–4591.
- Bintz J 1971. Between- and within-subject effect of shock intensity on avoidance in goldfish (*Carassius auratus*). *Journal of Comparative and Physiological Psychology* 75, 92–97.

- Bizeray D, Faure JM and Leterrier C 2004. Making broilers walk: what for and how. *Productions Animales* 17, 45–57.
- Bonga SEW 1997. The stress response in fish. *Physiological Reviews* 77, 591–625.
- Borghetti P, Saleri R, Mocchegiani E, Corradi A and Martelli P 2009. Infection, immunity and the neuroendocrine response. *Veterinary Immunology and Immunopathology* 130, 141–162.
- Breward J and Gentle MJ 1985. Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry. *Experientia* 41, 1132–1134.
- Bright A 2008. Vocalisations and acoustic parameters of flock noise from feather pecking and non-feather pecking laying flocks. *British Poultry Science* 49, 241–249.
- Brown JME, Edwards SA, Smith WJ, Thompson E and Duncan J 1996. Welfare and production implications of teeth clipping and iron injection of piglets in outdoor systems in Scotland. *Preventive Veterinary Medicine* 27, 95–105.
- Capion N, Thamsborg SM and Enevoldsen C 2009. Prevalence and severity of foot lesions in Danish Holstein heifers through first lactation. *Veterinary Journal* 182, 50–58.
- Carroll JA 2008. Bidirectional communication: growth and immunity in domestic livestock. *Journal of Animal Science* 86, E126–E137.
- Carroll JA, Berg EL, Strauch TA, Roberts MP and Kattesh HG 2006. Hormonal profiles, behavioral responses, and short-term growth performance after castration of pigs at three, six, nine, or twelve days of age. *Journal of Animal Science* 84, 1271–1278.
- Cheng H 2006. Morphopathological changes and pain in beak trimmed laying hens. *Worlds Poultry Science Journal* 62, 41–52.
- Cohen RDH, King BD, Thomas LR and Janzen ED 1990. Efficacy and stress of chemical versus surgical castration of cattle. *Canadian Journal of Animal Science* 70, 1063–1072.
- Collias NE 1987. The vocal repertoire of the Red Junglefowl: a spectrographic classification and the code of communication. *The Condor* 89, 510–524.
- Courboulay V, Hémonic A, Gadonna M and Prunier A 2010. Castration avec anesthésie locale ou traitement anti-inflammatoire: quel impact sur la douleur des porcelets et quelles conséquences sur le travail en élevage?. In 42. Journées de la Recherche Porcine, Paris (FRA), pp. 27–33.
- Davis GS, Anderson KE and Jones DR 2004. The effects of different beak trimming techniques on plasma corticosterone and performance criteria in single comb White Leghorn hens. *Poultry Science* 83, 1624–1628.
- Done SH, Guise J and Chennells D 2003. Tail biting and tail docking in pigs. *Proceedings of the Pig Veterinary Society Meeting, Burleigh Court, Loughborough, UK, 14–15 November 2002*, pp. 136–154.
- Duncan IJ, Beatty ER, Hocking PM and Duff SR 1991. Assessment of pain associated with degenerative hip disorders in adult male turkeys. *Research in Veterinary Science* 50, 200–203.
- Dunlop R, Millsopp S and Laming P 2006. Avoidance learning in goldfish (*Carassius auratus*) and trout (*Oncorhynchus mykiss*) and implications for pain perception. *Applied Animal Behaviour Science* 97, 255–271.
- Earley B and Crowe MA 2002. Effects of ketoprofen alone or in combination with local anesthesia during the castration of bull calves on plasma cortisol, immunological, and inflammatory responses. *Journal of Animal Science* 80, 1044–1052.
- Ehrensing RH, Michell GF and Kastin AJ 1982. Similar antagonism of morphine analgesia by MIF-1 and naloxone in *Carassius auratus*. *Pharmacology Biochemistry and Behavior* 17, 757–761.
- Eicher SD, Morrow-Tesch JL, Albright JL, Dailey JW, Young CR and Stanker LH 2000. Tail-docking influences on behavioral, immunological, and endocrine responses in dairy heifers. *Journal of Dairy Science* 83, 1456–1462.
- Elsasser TH, Klasing KC, Filipov N and Thompson F 2000. The metabolic consequences of stress: targets for stress and priorities of nutrient use. In *The biology of animal stress. Basic principles and implications for animal welfare* (ed. GP Moberg and JA Mench), pp. 77–110. CAB International, Wallingford, UK.
- Evrard HC and Balthazard J 2002. The assessment of nociceptive and non-nociceptive skin sensitivity in the Japanese quail (*Coturnix japonica*). *Journal of Neuroscience Methods* 116, 135–146.
- Farm Animal Welfare Council (FAWC) 1992. Updates the five freedoms. *Veterinary records* 17.
- Fernandez X, Levasseur P, Ecolan P and Wittmann W 1995. Effect of epinephrine administration on glycogen-metabolism in red and white muscle of anesthetized pigs (*Sus Scrofa domestica*). *Journal of the Science of Food and Agriculture* 68, 231–239.
- Fisher AD, Crowe MA, delaVarga MEA and Enright WJ 1996. Effect of castration method and the provision of local anesthesia on plasma cortisol, scrotal circumference, growth, and feed intake of bull calves. *Journal of Animal Science* 74, 2336–2343.
- Flower FC and Weary DM 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. *Journal of Dairy Science* 89, 139–146.
- Fourichon C, Seegers H, Bareille N and Beaudeau F 1999. Effects of disease on milk production in the dairy cow: a review. *Preventive Veterinary Medicine* 41, 1–35.
- Fraser D and Thompson BK 1991. Armed sibling rivalry among suckling piglets. *Behavioural Ecology and Sociobiology* 29, 9–15.
- Gallois M, Cozler YL and Prunier A 2005. Influence of tooth resection in piglets on welfare and performance. *Preventive Veterinary Medicine* 69, 13–23.
- Gentle MJ 1974. Changes in habituation of the EEG to water deprivation and crop loading in *Gallus domesticus*. *Physiology & Behavior* 13, 15–19.
- Gentle MJ 2011. Pain issues in poultry. *Applied Animal Behaviour Science* 135, 252–258.
- Gentle MJ and Hunder LN 1991. Physiological and behavioural responses associated with feather removal in *Gallus gallus var domesticus*. *Research in Veterinary Science* 50, 95–101.
- Gentle MJ and Corr SA 1995. Endogenous analgesia in the chicken. *Neuroscience Letters* 201, 211–214.
- Gentle MJ, Jones RB and Woolley SC 1989. Physiological changes during tonic immobility in *Gallus gallus var domesticus*. *Physiology & Behavior* 46, 843–847.
- Gentle MJ, Thorp BH and Hughes BO 1995. Anatomical consequences of partial beak amputation (beak trimming) in turkeys. *Research in Veterinary Science* 58, 158–162.
- Gibson TJ, Johnson CB, Stafford KJ, Mitchinson SL and Mellor DJ 2007. Validation of the acute electroencephalographic responses of calves to noxious stimulus with scoop dehorning. *New Zealand Veterinary Journal* 55, 152–157.
- Gillman CE, Kilbride AL, Ossent P and Green LE 2009. A cross-sectional study of the prevalence of foot lesions in post-weaning pigs and risks associated with floor type on commercial farms in England. *Preventive Veterinary Medicine* 91, 146–152.
- Glatz PC 1987. Effects of beak trimming and restraint on heart rate, food intake, body weight and egg production in hens. *British Poultry Science* 28, 601–611.
- Glatz PC 2000. Beak trimming methods – review. *Asian-Australasian Journal of Animal Sciences* 13, 1619–1637.
- Glatz PC and Lunam CA 1994. Production and heart-rate responses of chickens beak-trimmed at hatch or at 10 or 42 days of age. *Australian Journal of Experimental Agriculture* 34, 443–447.
- Graham MJ, Kent JE and Molony V 1997. Effects of four analgesic treatments on the behavioural and cortisol responses of 3-week-old lambs to tail docking. *Veterinary Journal* 153, 87–97.
- Grant C 2004. Behavioural responses of lambs to common painful husbandry procedures. *Applied Animal Behaviour Science* 87, 255–273.
- Guatteo R, Levionnois O, Fournier D, Guémené D, Latouche K, Leterrier C, Mormède P, Prunier A, Servière J, Terlouw C and Le Neindre P 2012. Minimising pain in farm animals: the 3S approach – ‘suppress, substitute, soothe’. *Animal* 6, 1261–1274.
- Haga HA and Ranheim B 2005. Castration of piglets: the analgesic effects of intratesticular and intrafunicular lidocaine injection. *Veterinary Anaesthesia and Analgesia* 32, 1–9.
- Hay M, Vulin A, Génin S, Sales P and Prunier A 2003. Assessment of pain induced by castration in piglets: behavioral and physiological responses over the subsequent 5 days. *Applied Animal Behaviour Science* 82, 201–218.
- Hay M, Rue J, Sansac C, Brunel G and Prunier A 2004. Long-term detrimental effects of tooth clipping or grinding in piglets: a histological approach. *Animal Welfare* 13, 23–32.
- Herr K, Bjoro K and Decker S 2006. Tools for assessment of pain in nonverbal older adults with dementia: a state-of-the-science review. *Journal of Pain and Symptom Management* 31, 170–192.

- Herskin MS, Muller R, Schrader L and Ladewig J 2003. A laser-based method to measure thermal nociception in dairy cows: short-term repeatability and effects of power output and skin condition. *Journal of Animal Science* 81, 945–954.
- Hocking PM, Robertson GW and Gentle MJ 2001. Effects of anti-inflammatory steroid drugs on pain coping behaviours in a model of articular pain in the domestic fowl. *Research in Veterinary Science* 71, 161–166.
- Hocking PM, Gentle MJ, Bernard R and Dunn LN 1997. Evaluation of a protocol for determining the effectiveness of pretreatment with local analgesics for reducing experimentally induced articular pain in domestic fowl. *Research in Veterinary Science* 63, 263–267.
- Holton L, Reid J, Scott EM, Pawson P and Nolan A 2001. Development of a behaviour-based scale to measure acute pain in dogs. *Veterinary Record* 148, 525–531.
- Hutter S, Heinritz K, Reich E and Ehret W 1994. Efficacité de différentes méthodes de résection des dents chez le porcelet non sevré. *Revue de Médecine Vétérinaire* 145, 205–213.
- Jarvis S, McLean KA, Chirmside J, Deans LA, Calvert SK, Molony V and Lawrence AB 1997. Opioid-mediated changes in nociceptive threshold during pregnancy and parturition in the sow. *Pain* 72, 153–159.
- Kestin SC, Knowles TG, Tinch AE and Gregory NG 1992. Prevalence of leg weakness in broiler chickens and its relationship with genotype. *Veterinary Record* 131, 190–194.
- Kielly J, Dewey CE and Cochran M 1999. Castration at 3 days of age temporarily slows growth of pigs. *Swine Health and Production* 7, 151–153.
- Lamont LA, Tranquilli WJ and Grimm KA 2000. Physiology of pain. *Veterinary Clinics of North America, Small Animal Practice* 30, 703–728.
- Lay DC, Friend TH, Bowers CL, Grissom KK and Jenkins OC 1992a. A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *Journal of Animal Science* 70, 1121–1125.
- Lay DC, Friend TH, Randel RD, Bowers CL, Grissom KK and Jenkins OC 1992b. Behavioral and physiological effects of freeze or hot-iron branding on crossbred cattle. *Journal of Animal Science* 70, 330–336.
- Lay D, Friend T, Grissom K, Bowers C and Mal M 1992c. Effects of freeze or hot-iron branding of Angus calves on some physiological and behavioral indicators of stress. *Applied Animal Behaviour Science* 33, 137–147.
- Le Neindre P, Guattéo R, Guéméné D, Guichet JL, Latouche K, Leterrier C, Levionnois O, Mormède P, Prunier A, Serrie A and Serviere J 2009. Animal pain identifying, understanding and minimising pain in farm animals. Multi-disciplinary scientific assessment. Summary of the Expert Report, INRA, France, 98pp. Retrieved October 18, 2012, from http://www.international.inra.fr/the_institute/scientific_expertise/expert_reports/pain_in_farm_animals
- Leach KA, Dippel S, Huber J, March S, Winckler C and Whay HR 2009. Assessing lameness in cows kept in tie-stalls. *Journal of Dairy Science* 92, 1567–1574.
- Lester SJ, Mellor DJ, Ward RN and Holmes RJ 1991. Cortisol responses of young lambs to castration and tailing using different methods. *New Zealand Veterinary Journal* 39, 134–138.
- Lester SJ, Mellor DJ, Holmes RJ, Ward RN and Stafford KJ 1996. Behavioural and cortisol responses of lambs to castration and tailing using different methods. *New Zealand Veterinary Journal* 44, 45–54.
- Leterrier C and Nys Y 1992. Clinical and anatomical differences in varus and valgus deformities of chick limbs suggest different etiopathogenesis. *Avian Pathology* 21, 429–442.
- Lewin-Kowalik J, Marcol W, Kotulska K, Mander M and Klimczak A 2006. Prevention and management of painful neuroma. *Neurologia Medico-Chirurgica* 46, 62–67.
- Llamas Moya SL, Boyle LA, Lynch PB and Arkins S 2008. Effect of surgical castration on the behavioural and acute phase responses of 5-day-old piglets. *Applied Animal Behaviour Science* 111, 133–145.
- Logue DN, Offer JE and Hyslop JJ 1994. Relationship of diet, hoof type and locomotion score with lesions of the sole and white line in dairy-cattle. *Animal Production* 59, 173–181.
- Manteuffel G, Puppe B and Schon PC 2004. Vocalization of farm animals as a measure of welfare. *Applied Animal Behaviour Science* 88, 163–182.
- Marchant-Forde JN, Lay DC Jr, McMunn KA, Cheng HW, Pajor EA and Marchant-Forde RM 2009. Postnatal piglet husbandry practices and well-being: the effects of alternative techniques delivered separately. *Journal of Animal Science* 87, 1479–1492.
- Marx G, Horn T, Thielebein J, Knubel B and Von Borell E 2003. Analysis of pain-related vocalization in young pigs. *Journal of Sound and Vibration* 266, 687–698.
- Matteri RL, Crarroll JA and Dyer CJ 2000. Neuroendocrine responses to stress. In *The biology of animal stress: basic principles and implications for animal welfare* (ed. GP Moberg and JA Mench), pp. 1–21. CABI Publisher, Wallingford, UK.
- McCarthy RN, Jeffcott LB and Clarke IJ 1993. Preliminary studies on the use of plasma beta-endorphin in horses as an indicator of stress and pain. *Journal of Equine Veterinary Science* 13, 216–219.
- McGlone JJ and Hellman JM 1988. Local and general anesthetic effects on behavior and performance of two- and seven-week-old castrated and uncastrated piglets. *Journal of Animal Science* 66, 3049–3058.
- McGlone JJ, Nicholson RI, Hellman JM and Herzog DN 1993. The development of pain in young pigs associated with castration and attempts to prevent castration-induced behavioral changes. *Journal of Animal Science* 71, 1441–1446.
- Meagher RK 2009. Observer ratings: validity and value as a tool for animal welfare research. *Applied Animal Behaviour Science* 119, 1–14.
- Melendez P, Bartolome J, Archbald LF and Donovan A 2003. The association between lameness, ovarian cysts and fertility in lactating dairy cows. *Theriogenology* 59, 927–937.
- Mellor DJ and Murray L 1989. Changes in the cortisol responses of lambs to tail docking, castration and ACTH injection during the first seven days after birth. *Research in Veterinary Science* 46, 392–395.
- Mellor DJ, Cook CJ and Stafford KJ 2000. Quantifying some responses to pain as a stressor. In *The biology of animal stress: basic principles and implications for welfare* (ed. GP Moberg and JA Mench), pp. 171–198. CAB International, Wallingford.
- Mellor DJ, Stafford KJ, Todd SE, Lowe TE, Gregory NG, Bruce RA and Ward RN 2002. A comparison of catecholamine and cortisol responses of young lambs and calves to painful husbandry procedures. *Australian Veterinary Journal* 80, 228–233.
- Melzack R and Wall PD 1965. Pain mechanisms – a new theory. *Science* 150, 971–971.
- Merlot E, Mounier AM and Prunier A 2011. Endocrine response of gilts to various common stressors: a comparison of indicators and methods of analysis. *Physiology & Behavior* 102, 259–265.
- Mettam JJ, Oulton LJ, McCrohan CR and Sneddon LU 2011. The efficacy of three types of analgesic drugs in reducing pain in the rainbow trout, *Oncorhynchus mykiss*. *Applied Animal Behaviour Science* 133, 265–274.
- Moberg GP 2000. Biological response to stress: implications for animal welfare. In *The biology of animal stress: basic principles and implications for animal welfare* (ed. GP Moberg and JA Mench), pp. 1–21. CABI Publisher, Wallingford, UK.
- Molony V and Kent JE 1997. Assessment of acute pain in farm animals using behavioral and physiological measurements. *Journal of Animal Science* 75, 266–272.
- Molony V, Kent JE and Robertson IS 1993. Behavioural responses of lambs of three ages in the first three hours after three methods of castration and tail docking. *Research in Veterinary Science* 55, 236–245.
- Molony V, Kent JE and Robertson IS 1995. Assessment of acute and chronic pain after different methods of castration of calves. *Applied Animal Behaviour Science* 46, 33–48.
- Molony V, Kent JE and McKendrick IJ 2002. Validation of a method for assessment of an acute pain in lambs. *Applied Animal Behaviour Science* 76, 215–238.
- Molony V, Kent JE, Hosie BD and Graham MJ 1997. Reduction in pain suffered by lambs at castration. *Veterinary Record* 139, 205–213.
- Morisse JP, Cotte JP and Huonnic D 1995. Effect of dehorning on behaviour and plasma cortisol responses in young calves. *Applied Animal Behaviour Science* 43, 239–247.
- Morton D and Griffiths P 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* 116, 431–436.
- Mouttoutu N, Hatchell FM and Green LE 1999. Foot lesions in finishing pigs and their associations with the type of floor. *Veterinary Record* 144, 629–632.

- Noonan GJ, Rand JS, Priest J, Ainscow J and Blackshaw JK 1994. Behavioural observations of piglets undergoing tail docking, teeth clipping and ear notching. *Applied Animal Behaviour Science* 39, 203–213.
- Nyborg PY, Srig A, Lykkegaard K and Svendsen O 2000. Nociception after castration of juvenile pigs determined by quantitative estimation of c-Fos expressing neurons in the spinal cord dorsal horn. *Dansk Veterinærtidsskrift* 83, 16–17.
- O'Callaghan KA, Cripps PJ, Downham DY and Murray RD 2003. Subjective and objective assessment of pain and discomfort due to lameness in dairy cattle. *Animal Welfare* 12, 605–610.
- Paull DR, Lee C, Colditz IG and Fisher AD 2009. Effects of a topical anaesthetic formulation and systemic carprofen, given singly or in combination, on the cortisol and behavioural responses of Merino lambs to castration. *Australian Veterinary Journal* 87, 230–237.
- Persson Y, Soderquist L and Ekman S 2007. Joint disorder: a contributory cause to reproductive failure in beef bulls? *Acta Veterinaria Scandinavica* 49, 31.
- Prunier A 2002. Evaluation et prévention de la douleur induite par les interventions de convenue chez le porcelet. In *Journée d'étude bien-être des porcs et des volailles*, Gembloux (BEL), 2002/10/16.
- Prunier A, Hay M and Servière J 2002. Evaluation et prévention de la douleur induite par les interventions de convenue chez le porcelet. *Journées de la Recherche Porcine* 34, 257–268.
- Prunier A, Mounier AM and Hay M 2005. Effects of castration, tooth resection, or tail docking on plasma metabolites and stress hormones in young pigs. *Journal of Animal Science* 83, 216–222.
- Prunier A, Bonnaeu M, Von Borell EH, Cinotti S, Gunn M, Fredriksen B, Giersing M, Morton DB, Tuytens FAM and Velarde A 2006. A review of the welfare consequences of surgical castration in piglets and the evaluation of non-surgical methods. *Animal Welfare* 15, 277–289.
- Puppe B, Schon PC, Tuchscherer A and Manteuffel G 2005. Castration-induced vocalisation in domestic piglets, *Sus scrofa*: complex and specific alterations of the vocal quality. *Applied Animal Behaviour Science* 95, 67–78.
- Reilly SC, Quinn JP, Cossins AR and Sneddon LU 2008. Behavioural analysis of a nociceptive event in fish: comparisons between three species demonstrate specific responses. *Applied Animal Behaviour Science* 114, 248–259.
- Rietmann TR, Stauffacher M, Bernasconi P, Auer JA and Weishaupt MA 2004. The association between heart rate, heart rate variability, endocrine and behavioural pain measures in horses suffering from laminitis. *Journal of Veterinary Medicine Series A – Physiology Pathology Clinical Medicine* 51, 218–225.
- Ren K and Torres R 2009. Role of interleukin-1 β during pain and inflammation. *Brain Research Reviews* 60, 57–64.
- Robert S, Thompson BK and Fraser D 1995. Selective tooth clipping in the management of low-birth weight piglets. *Canadian Journal of Animal Science* 75, 285–289.
- Robertson IS, Kent JE and Molony V 1994. Effect of different methods of castration on behavior and plasma cortisol in calves of three ages. *Research in Veterinary Science* 56, 8–17.
- Roozen Awn, Tsuma VT and Magnusson U 1995. Effects of short-term restraint on plasma-concentrations of catecholamines, beta-endorphin, and cortisol in gilts. *American Journal of Veterinary Research* 56, 1225–1227.
- Rushen J, Pombourcq E and Passillé AMD 2007. Validation of two measures of lameness in dairy cows. *Applied Animal Behaviour Science* 106, 173–177.
- Schwartzkopf-Genswein KS, Stookey JM and Welford R 1997. Behavior of cattle during hot-iron and freeze branding and the effects of subsequent handling ease. *Journal of Animal Science* 75, 2064–2072.
- Schwartzkopf-Genswein KS, Stookey JM, Crowe TG and Genswein BM 1998. Comparison of image analysis, exertion force, and behavior measurements for use in the assessment of beef cattle responses to hot-iron and freeze branding. *Journal of Animal Science* 76, 972–979.
- Shutt DA, Fell LR, Connell R, Bell AK, Wallace CA and Smith AI 1987. Stress-induced changes in plasma concentrations of immunoreactive beta-endorphin and cortisol in response to routine surgical procedures in lambs. *Australian Journal of Biological Sciences* 40, 97–103.
- Simonsen HB, Klinken L and Bindseil E 1991. Histopathology of intact and docked pigtailed. *British Veterinary Journal* 147, 407–412.
- Sneddon LU 2003. The evidence for pain in fish: the use of morphine as an analgesic. *Applied Animal Behaviour Science* 83, 153–162.
- Stafford KJ and Mellor DJ 2005. Dehorning and disbudding distress and its alleviation in calves. *The Veterinary Journal* 169, 337–349.
- Stafford KJ, Mellor DJ, Todd SE, Bruce RA and Ward RN 2002. Effects of local anaesthesia or local anaesthesia plus a non-steroidal anti-inflammatory drug on the acute cortisol response of calves to five different methods of castration. *Research in Veterinary Science* 73, 61–70.
- Stewart M, Stafford KJ, Dowling SK, Schaefer AL and Webster JR 2008. Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. *Physiology & Behavior* 93, 789–797.
- Stewart M, Verkerk GA, Stafford KJ, Schaefer AL and Webster JR 2010. Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *Journal of Dairy Science* 93, 3602–3609.
- Sutherland MA, Davis BL, Brooks TA and McGlone JJ 2010. Physiology and behavior of pigs before and after castration: effects of two topical anesthetics. *Animal* 4, 2071–2079.
- Sylvester SP, Mellor DJ, Stafford KJ, Bruce RA and Ward RN 1998. Acute cortisol responses of calves to scoop dehorning using local anaesthesia and/or cautery of the wound. *Australian Veterinary Journal* 76, 118–122.
- Thornton PD and Waterman-Pearson AE 1999. Quantification of the pain and distress responses to castration in young lambs. *Research in Veterinary Science* 66, 107–118.
- Tilbrook AJ, Turner AI and Clarke IJ 2000. Effects of stress on reproduction in non-rodent mammals: the role of glucocorticoids and sex differences. *Reviews of Reproduction* 5, 105–113.
- Ting STL, Earley B, Hughes JML and Crowe MA 2003. Effect of ketoprofen, lidocaine local anesthesia, and combined xylazine and lidocaine caudal epidural anesthesia during castration of beef cattle on stress responses, immunity, growth, and behavior. *Journal of Animal Science* 81, 1281–1293.
- Torrey S, Devillers N, Lessard M, Farmer C and Widowski T 2009. Effect of age on the behavioral and physiological responses of piglets to tail docking and ear notching. *Journal of Animal Science* 87, 1778–1786.
- Turnbull AV and Rivier CL 1999. Regulation of the hypothalamic–pituitary–adrenal axis by cytokines: actions and mechanisms of action. *Physiological Reviews* 79, 1–71.
- Turnbull JF, Adams CE, Richards RH and Robertson DA 1998. Attack site and resultant damage during aggressive encounters in Atlantic salmon (*Salmo salar* L.) parr. *Aquaculture* 159, 345–353.
- Valros A, Ahlstrom S, Rintala H, Hakkinen T and Saloniemi H 2004. The prevalence of tail damage in slaughter pigs in Finland and associations to carcass condemnations. *Acta Agriculturae Scandinavica Section A – Animal Science* 54, 213–219.
- Veissier I, Rushen J, Colwell D and De Passille AM 2000. A laser-based method for measuring the pain sensitivity of cattle. *Applied Animal Behaviour Science* 66, 289–304.
- Vestergaard KS and Sanotra GS 1999. Relationships between leg disorders and changes in the behaviour of broiler chickens. *Veterinary Record* 144, 205–209.
- Von Borell E, Langbein J, Despres G, Hansen S, Leterrier C, Marchant-Forde J, Marchant-Forde R, Minero M, Mohr E, Prunier A, Valance D and Veissier I 2007a. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals – a review. *Physiology & Behavior* 92, 293–316.
- Von Borell E, Dobson H and Prunier A 2007b. Stress, behaviour and reproductive performance in female cattle and pigs. *Hormones and Behavior* 52, 130–138.
- Watts JM and Stookey JM 1999. Effects of restraint and branding on rates and acoustic parameters of vocalization in beef cattle. *Applied Animal Behaviour Science* 62, 125–135.
- Watts JM and Stookey JM 2000. Vocal behaviour in cattle: the animal's commentary on its biological processes and welfare. *Applied Animal Behaviour Science* 67, 15–33.
- Weary DM, Braithwaite LA and Fraser D 1998. Vocal response to pain in piglets. *Applied Animal Behaviour Science* 56, 161–172.
- Weeks CA, Davies HC and Hunt P 1997. Effect of leg weakness on feeding behaviour of broilers. *BSAS Occasional Publication* 20, 124–125.
- Weeks CA, Danbury TD, Davies HC, Hunt P and Kestin SC 2000. The behaviour of chickens and its modification by lameness. *Applied Animal Behaviour Science* 67, 111–125.

Prunier, Mounier, Le Neindre, Leterrier, Mormède, Paulmier, Prunet, Terlouw and Guatteo

Welsh EM, Gettinby G and Nolan AM 1993. Comparison of a visual analogue scale and a numerical rating scale for assessment of lameness, using sheep as a model. *American Journal of Veterinary Research* 54, 976–983.

White RG, Deshazer JA, Tressler CJ, Borcher GM, Davey S, Waning A, Parkhurst AM, Milanuk MJ and Clemens ET 1995. Vocalization and physiological response of pigs during castration with or without a local anesthetic. *Journal of Animal Science* 73, 381–386.

Widowski TM, Cottrell T, Dewey CE and Friendship RM 2003. Observations of piglet-directed behavior patterns and skin lesions in eleven commercial swine herds. *Journal of Swine Health and Production* 11, 181–185.

Wolff A, Vanduyhoven E, van Kleef M, Huygen F, Pope JE and Mekhail N 2011. Phantom pain. *Pain Practice* 11, 403–413.

Wylie LM and Gentle MJ 1998. Feeding-induced tonic pain suppression in the chicken: reversal by naloxone. *Physiology and Behavior* 64, 27–30.