

INFRARED THERMOGRAPHY AS A METHOD FOR EVALUATING THE WELFARE OF ANIMALS SUBJECTED TO INVASIVE PROCEDURES – A REVIEW*

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Abstract

In recent years, there has been a growing interest in animal welfare. Consumers of animal products are paying more attention to maintaining good husbandry conditions on the farms, but also to some of the procedures entailing pain and suffering. The most invasive procedures are castration and dehorning (disbudding), which are often performed without anaesthesia. Pain associated with tissue damage causes behavioural and physiological changes. Observation of behaviour combined with measurements of autonomic nervous system activation, and hormones of the hypothalamic-pituitary-adrenal axis, is the main method for assessing the emotional state of an animal. Immobilization and blood collection may themselves be a source of stress for these animals. There is a strong prerequisite for the development of non-invasive methods of assessing the level of animal welfare. One of them is infrared thermography. The study of eye temperature changes in cattle, measured with an infrared thermography camera, confirmed the activation of the autonomic nervous system in response to pain.

Key words: welfare, infrared thermography, disbudding, castration

Consumers of animal products are paying ever increasing attention to the conditions in which farm animals are kept and to some of the procedures applied in these establishments that entail pain and suffering. A low level of animal welfare provision is considered a negative development, not only for ethical reasons, but also from the viewpoint of animal health status and the quality and safety of food products. Despite the increasing emphasis being placed on these concerns, the issue of animal discomfort or suffering still seems to be of secondary importance in many farms, with the high profitability of production being the main concern. Particular examples of this kind of attitude are the continuing use of painful procedures such as dehorning or castration without pain relief.

* This study was financed from statutory activity, project No. 08.3.11.9.

It seems that scientific research focused on feeling pain and stress by animals can throw new light on the issue of evaluating the level of animal welfare in farms.

Welfare and stress reaction

In recent years we have seen a change in awareness regarding the psychical life of animals, exemplified by, for instance, the physical pain experienced by animals but also their psychic suffering (Bakke, 2007). According to Dawkins (2006), the science of animal welfare includes a number of disciplines such as behavioural ecology, evolutionism, neurobiology, genetics, and the studies concerning the consciousness of animals.

Because of the ambiguity of the term, evaluating welfare is not easy. Welfare is defined as a good physical and mental feeling (i.e. the most comfortable conditions for a captive animal), and where the requirements regarding its existence in appropriate environmental conditions are being met (Broom, 1996).

As a result of efforts to unify the principles of welfare, the right of animals to 'five freedoms' has been established (FAWC, 1993). This term applies to the most essential needs of animals which should be met in any circumstances. They include freedom from the following:

- thirst and hunger,
- discomfort (by making sure that animals have the right environmental conditions and sufficient space),
- pain, injury and disease,
- fear and distress, and
- the freedom to behave naturally.

According to Fraser (2008), from the viewpoint of ethics and science, the rules of proper conduct in relation to animals may be summarised in four principles. The first principle is to ensure that animals are kept in a healthy condition. Fraser lists such factors as the provision of sufficient food, permanent access to water, vaccination against disease, the provision of proper living conditions (e.g. suitable air temperature), and injury prevention, as well as maintaining productivity at a high level. This author highlights the fact that health is an important factor, but not the only one guaranteeing a high level of welfare.

The second principle is to limit the pain and stress experienced by animals. Fraser notes the significance of applying anaesthetic drugs in invasive procedures, thereby preventing lameness, wounds and other bodily injuries, as well as discontinuing the existing stressful and brutal methods of handling animals. The second principle also includes preventing heat or cold stress.

The third principle pertains to the expression of natural behaviour and the emotional well being of animals. Examples include the provision of suitable litter to sows, or nests to laying hens. The last principle of good animal husbandry regards the provision of natural environmental elements. The animals should have access to pens and to adequate sunlight.

Good nutrition, access to water, proper microclimatic conditions as well as caring for the health of herds, are the principal pre-conditions for obtaining high pro-

ductivity. These factors are not disputable, and they are obvious to any good animal breeder or producer. High productivity levels and reasonable health status, however, do not always testify to a high level of welfare. The genetic potential of the present population or breeding lines of livestock animals can assure high productivity, even when the mental state of an animal is not good (Hewson, 2003). There is, therefore, a strong premise for directing more attention towards the emotional life of animals, including their experiencing of pain and fear. This task is not easy as highlighted by Stewart et al. (2005), as there is no universally accepted scientific definition regarding the stress and fear felt by an animal. Selye (1978) described stress as a state where an animal is exposed to the effects of some environmental factors operating as non-specific stimuli, whose force exceeds a certain threshold value and results in a creature having to adapt to the new environment. Fear is an early, emotional reaction to danger which motivates an animal to avoid the imminent threat (Rushen et al., 1999). The definition of pain, given by IASP (International Association for the Study of Pain) states that it is the feeling of an unpleasant sensation and emotion associated with real or potential damage to tissues (IASP, 1975).

According to Dawkins (2006), when the psychic state of an animal is evaluated, the most important thing is to observe the behaviour and to study some physiological indicators. The observation of behaviour should include both typical actions performed by the animal (also including its preferences for acquiring certain elements from the environment), as well as manifestations of aggression or stereotypical behaviour (Broom, 1996). The most important physiological indicator of welfare is the stress reaction mechanism.

The effective response of an organism to a threat is made possible by co-operation between the nervous, hormonal, and immunological systems. The stimulus provoking stress causes an excessive reaction, including neurohormonal changes, which aim to maintain or restore the homeostasis of the organism (Carrasco and Van de Karl, 2003).

When in a state of danger, there is activation – chiefly via the limbic system where individual impulses are analysed – of, *inter alia*, the hypothalamic-pituitary-adrenal axis as well as of the sympathetic-adrenal system (Zimecki and Artym, 2004). When an animal is exposed to stress or pain, the principal role in preparing a response is initially played by the sympathetic division of the autonomic nervous system. As a consequence, catecholamines, adrenaline and noradrenaline are secreted from the adrenal medulla. These hormones affect various systems and organs preparing the organism for a ‘fight or flight’ response. The action of catecholamines, for example, increases the heart rate and the blood pressure. The spleen releases red blood cells stored there, in order to supply additional oxygen to the muscles. Also released are the lipid and carbohydrate reserves accumulated in the liver. The ventilation of the lungs intensifies as a result of dilation of the bronchioles and the number of lymphocytes responsible for repairing damaged tissues increases. The pupils dilate in order to increase visual acuity and the blood flow is redistributed to the skeletal and heart muscles. The vasoconstriction of skin vessels protects against excessive loss of blood in the event of damage to external tissues and the blood flow in internal organs and muscles increases (Stewart et al., 2005). The vasoconstriction of the blood

vessels in external organs, results in the lowered thermal emissivity of these sites (Nakayama *et al.*, 2005).

The action of the other branch of the autonomic nervous system – the parasympathetic system – consists chiefly in helping the organism achieve a state of homeostasis as well as restore energy reserves. Then the heart rate slows down and the oxygen and nutrient demands of the muscles decrease. The blood flow in the skin vessels increases and the blood vessels in the internal organs contract (Stewart *et al.*, 2005). The effective response of the body is also made possible due to activation of the hypothalamus and the release of a neurohormone – corticoliberin (CRH). When transferred to the anterior lobe of the hypophysis, this hormone stimulates the release of the adrenocorticotrophic hormone (ACTH), which affects the cells of the adrenal cortex and stimulates the synthesis of glucocorticoids, including, among others, cortisol. The glucocorticoids influence the work of many organs, regulate the metabolism of proteins, carbohydrates and lipids and boost the effects of catecholamines (Longstaff, 2002).

Assessment of activation of the autonomic nervous system and the level of hormones released, during the activation of the hypothalamic-pituitary-adrenal axis, is a principal method for evaluating animal welfare (Mormede *et al.*, 2007). In practice, it is very difficult to obtain reliable results for measuring the levels of adrenaline and noradrenaline due to their low concentration in plasma and short half-life (Hjemdahl, 1993). The level of cortisol in the blood, maintained for a relatively long period, is the reason that this hormone is usually studied as an indicator of the response by an organism to stress (Matteri *et al.*, 2000).

The identifiers of the physiological response of an organism to threats or pain include, among others, dopamine, serotonin, opioids, immunological cells, or neuropeptides such as substance P (Zimecki and Artym, 2004).

Invasive procedures on farms

The most usual sources of stress among farm animals could be listed as follows: unsuitable climatic conditions prevailing in the buildings housing livestock, social relationships between individual animals, too high levels of stock, noise, and other environmental production factors which require extremely high adaptation expenditure on the part of the animal. However, the most invasive procedures to which the farm animals are subjected include castration, dehorning, and branding (Stafford and Mellor, 2005).

Castration of males is performed in order to improve the quality of meat, and reduce aggression and sexual activity in the herds (Currah *et al.*, 2009; Sutherland *et al.*, 2012). Generally, three methods for this procedure can be distinguished, namely physical, chemical, and hormonal. The surgical method is still the most common method used in pig herds (Fredriksen *et al.*, 2009). In cattle herds, the surgical removal of testes is applied although another common method is to apply special rubber rings which cut the blood flow within the testicles. These procedures are often performed on very young animals without using anaesthesia (Fredriksen *et al.*, 2009; Fajt *et al.*, 2011). The reports published by the PIGCAS organisation show that of the 125 million pigs slaughtered in Europe, 77% are castrated without pain-killers

(Fredriksen et al., 2009). In Poland, the law allows this procedure to be conducted without anaesthesia, in piglets up to 7 days old (Ordinance of the Minister of Agriculture and Rural Development of 15 February 2010 on the requirements and methods of handling and maintenance of livestock animal species, for which the protection standards were determined in the legal provisions of the European Union (Council Directive 2008/120/EC)).

The authors of the PIGCAS report state, however, that there are known cases of illegal castrations having been performed without anaesthesia in piglets older than 7 days. The procedure of castration performed without pain relief, even on very young animals is increasingly being criticised, particularly by animal rights groups but also by consumers themselves (Sutherland et al., 2012). Mechanical, thermal or chemical stimuli of scrotal skin nociceptors causes activation of various-order neurons, which transmit the information from the periphery to the brain, where they can be interpreted as pain (Lamont et al., 2000).

The pain associated with the procedure results in the following: a decrease in food intake, dejection, muscle tremor, increased vocalisation, diarrhoea, inflammations, and increased levels of ACTH, cortisol, substance P, or opioids (Thuer et al., 2007; Moya et al., 2008; Coetzee et al., 2008; Gonzalez et al., 2010; Sutherland et al., 2012). As indicated by the results of some studies, in younger animals the castration process is easier, and the cortisol level stays at a lower level, as does the stress vocalisation (Ting et al., 2005; Taylor et al., 2001). Other studies, made on piglets castrated at the age of 1, 5, 10, 15, and 20 days after birth showed, however, that the behavioural reactions to pain and stress associated with this procedure were similar, irrespective of the age of the animal (Mc Glone et al., 1993). Additionally, Marx et al. (2003) states that the vocalisation ability in piglets increases with age (development of the larynx). Therefore, the measurement of sounds emitted by young animals during the castration procedure, are ambiguous indicators of the suffering they experience. According to Jäggin et al., (2001), the number of nociceptors in the skin of a piglet's scrotum is similar to their number in the scrotum of other species. Moreover, studies in human and rodents show that neonates can suffer from pain or even feel exacerbated pain as the endogenous mechanisms of pain control do not function (Andrews and Fitzgerald, 1994).

In cattle herds, the practice of dehorning is widespread. Its main purpose is to avoid wounds being inflicted in the herd, as well as making the animals easier and safer to handle. According to the report by the ALCASDE organisation (2009), more than 80% of milk cattle in Europe are hornless. The method used most often is the removal of horn buds (disbudding) by burning them out using a hot iron. The procedure is conducted on calves up to two months old. Apart from this thermal method, the chemical method of disbudding is also used (chiefly in Spain) by applying a caustic paste. Surgical methods are also used. In the case of adult cattle, the removal of horns is most often carried out with the use of a wire saw. These methods cause tissue damage, sensitization of the nociceptors, and as a consequence, considerable pain (Anderson and Muir, 2005).

Surgical dehorning and disbudding can be more painful than other methods (Stafford and Mellor, 2005). Moreover, Stilwell et al. (2009) found higher cortisol re-

sponses in calves disbudded with a hot iron than in calves disbudded with caustic paste. However, according to the ALCASDE report (2009), hot iron disbudding is often performed on very young calves without the use of any anaesthesia. Faulkner and Weary (2000) observed the cortisol response lasting from 1–4 hours after hot iron disbudding and behavioural signs of pain such as head jerks and ear flicking. Graf and Senn (1999) found that the higher levels of this hormone continued up to one hour after the burning procedure, although the higher heart rate remained for more than three hours, compared with the calves in the control group and those that had hot iron disbudding performed under local anaesthesia

Marking the livestock animals by branding or freezing of marks, permitted in Poland until very recently, constitutes a source of long-lasting suffering for animals (Schwartzkopf-Genswein and Stookey, 1997). Tagging for identification purposes by fixing earrings seems to be a less painful procedure, although there is still a lack of any scientific studies pertaining to the discomfort felt by animals during such marking processes.

Infrared thermography – a non-invasive research method

Contrary to examination of the physiological reaction to stress, the study of behavioural reactions is conducted as a non-invasive procedure. Measuring the parameters of blood which prove the threat felt by animals, may themselves constitute a source of anxiety for an animal and, therefore, contribute to the secretion of stress hormones (Stewart *et al.*, 2005). The immobilizing of an animal provokes an excessive hormonal response in its organism (Zimecki and Artym, 2004). Thus there is a premise that non-invasive methods of investigation should be used, which do not result in additional discomfort to animals.

In recent years, the experiments on animals performed with the use of infrared cameras have become very popular. The principle of operating such equipment consists of the fact that the body, whose temperature is higher than absolute zero, emits thermal radiation within a 760 nm to 1 mm wavelength. The detector of an infrared thermovision camera enables the radiation energy to be converted into an electric signal. In individual modules, the signal is transformed as it undergoes amplification, and is converted into digital form and values representing temperatures of particular points on the image matrix. Colours from the palette (of colours) are then assigned to these points (pixels). In this way, a colour thermogram, which represents a map of temperature distribution in the object studied, is developed. Since the amount of energy released by bodies is a function of their temperature, the thermograms are quantitative representations of the surface temperature of the studied objects (Kulesza and Kaczorowski, 2004).

The newest generation infrared thermography cameras take measurements in real time. The equipment is light/portable and displays great sensitivity, which is significant in recording the temperature of an animal's skin. Additionally, the camera software permits analysis of temperature data in any area of the thermogram. The infrared thermography method has many applications, particularly in industry, medicine, and veterinary science. In studies of skin temperature, it has been proved that the intensity of infrared radiation emitted by the studied tissues is directly proportional

to the metabolic processes occurring in them and is associated with a simultaneous increase in blood supply to a given area (Fita et al., 2007). The blood flow in a given region of the body depends on the autonomic nervous system, and the local vessel-constriction and vessel-dilation mediators (Całkosiński et al., 2007).

Among the issues associated with farm animals, the infrared thermography method is the most widely used application in the thermal imaging of horse skin. The studies concerning this species primarily concern diagnoses of orthopaedic symptoms, inflammations, or the fitting of the saddle on the animal's back. At present, there is increased interest in applying infrared thermography for studies on other animal species. Using an infrared camera, Scolari et al. (2009) found the changes in skin temperature in sows during the oestrus. Rainwater-Lovett et al. (2009) analysed the thermograms of cow hooves infected with the foot-and-mouth disease virus. The infrared thermographic images show a marked increase in the temperature of hooves in animals suffering from this disease, before clinical symptoms appeared. Other studies using infrared thermography cameras have proved the existence of rhythmic changes in hoof temperature in sheep (D'Alterio et al., 2011). The authors suggest that thermographic method can effectively detect the disorders in peripheral blood circulation.

There are only few publications pertaining to the use of the aforementioned method concerning evaluation of the emotional state of animals. In human studies, infrared thermography has been used as a lie detector, where it was found that eye temperature increased in people who were lying (Pavlidis et al., 2002).

As suggested by studies carried out on horses by Cook et al. (2001), the eye temperature may be an indicator of the hypothalamic-pituitary-adrenal cortex axis activation. After exogenous ACTH challenge tests, an increase in eye temperature followed, that was found to correlate with an increase in the cortisol level in the blood and saliva of animals. Stewart et al. (2007), however, after applying similar stimulation in cows did not confirm the effect of the synthetic adrenocorticotrophic hormone on the increase in eye temperature. These researchers suggest that activation of the hypothalamic-pituitary-adrenal cortex axis is not the sole factor causing the eye temperature increase. However, infrared thermographic measurements in the eye can be indicative of activation of the sympathetic system-adrenal medulla axis. After an epinephrine challenge a rapid drop in eye temperature was noted. The authors detected changes in the temperature in a specific region of the eye near the caruncula lacrimalis. Probably, as they suggest, it was associated with the redirection of blood from the capillary vessels via the sympathetic nervous system mediated vasoconstriction. In the same study Stewart et al. (2007) also reached the conclusion that measuring eye temperature and monitoring the heart rate variability could be an effective and stress-free method for detecting the activation of the autonomic nervous system in response to pain. Using this method, the researchers studied calves undergoing the hot iron disbudding procedure, which was performed with and without local anaesthetic (two experimental groups). In the study there were also two control groups. One of them included calves subjected to simulated procedures of disbudding without analgesia, whereas in the second group the control animals had received a local analgesia during sham disbudding. The measurements

of temperature and heart rate variability were taken continuously. Both disbudding procedures performed with and without local analgesia, caused a rapid drop in eye temperature (statistically significant for the disbudding without analgesia), and after 5 minutes post treatment, the eye temperature in calves from both experimental groups increased and it was higher ($P < 0.001$) for both disbudded groups compared to the control groups. However, the increase was greater in calves subjected to disbudding without local anaesthesia. Heart rate increased during the 5 minutes after completion of the disbudding procedure in calves from two experimental groups, although it only remained elevated above the baseline in animals disbudded without local anaesthetic. There were no significant differences in eye temperature and heart rate variability in animals in these two control groups.

Stewart *et al.* (2010) determined the autonomic activity for evaluation of pain in surgically castrated bull calves. The authors took into account eye temperatures (measured with an infrared thermography camera), heart rate, heart rate variability, as well as the norepinephrine, epinephrine and cortisol level in blood samples. There were four groups, namely sham handling (the control group), surgical castration, local anaesthesia with simulated castration and a group of calves subjected to surgical castration with local anaesthesia. During the first 2 minutes following the procedures a decrease in eye temperature, followed by a rapid increase was noticed in a group of calves castrated with and without pain relief. However, the increase was greater in calves subjected to castration without local anaesthesia. The heart rate was elevated during the 2 minutes following both castration procedures, after which it decreased rapidly but lasted for more than 20 minutes after the treatment. Cortisol increased within 15 minutes after castration in both castrated groups.

The level of catecholamines in the blood increased within 2 minutes of the castration in the group without anaesthesia, compared to the control group. In the opinion of the authors (Stewart *et al.*, 2007, 2010) the invasive procedures caused initially the sympathetic nervous system to become activated. Later, an evident (particularly after surgical castration) increase in eye temperature, decrease in the heart rate variability and high level of cortisol in the blood maintained for a long time after the procedure, may be evidence of activation of the parasympathetic nervous system. The higher eye temperature may be caused by vasodilation of blood vessels, increased flow of peripheral blood and, therefore, higher thermal emissivity. Stewart *et al.* (2010) also found that applying local anaesthesia during castration did not minimize all the negative effects of the procedures. As stated by Stafford *et al.* (2002), only a combination of local anaesthesia and non-steroid anti-inflammatory drugs can prevent the high concentration of cortisol in blood serum after the castration procedure.

Summary

Humanitarian ways of handling animals fit well with the development of civilization. Increasing interest in the living conditions and the handling of animals is later matched by changes to the law. One such example is the amendment of the relevant Polish legislation concerning the protection of animals (Act of 16 September 2011 amending the law on protection of animals, and the law on cleanliness and order in municipalities), in force since 2012, which introduced, *inter alia*, increased penalties

for the ill-treatment of animals, demands for more humanitarian treatment of fish, and bans on keeping domestic animals tethered for longer than 12 hours.

Painful procedures performed in farms, although deemed necessary by some, are not in step with the accepted contemporary principles of animal welfare. The opinion that invasive procedures may be performed on younger animals without anaesthesia has stirred great controversy. Some countries, such as Norway and Switzerland, have introduced a requirement for surgical castration to be performed only under anaesthetic, irrespective of the age of piglets. Among the animal breeders in Europe, there are also pressure groups supporting a total ban on use of castration (the Brussels declaration – a voluntary initiative of the sector). Alternative methods are being sought, such as immunocastration which involves vaccinations with analogues of the gonadotropin-releasing hormone GnRH, combined with a protein carrier. This type of treatment blocks the function of the testes. However, this method, although seemingly humanitarian and convenient, has not been thoroughly studied yet. In addition it is not known whether it will be accepted by the consumers (Valeeva et al., 2009). In this situation, it seems that the best solution is to carry on using the procedures of surgical castration and dehorning (disbudding), but with the application of analgesic medicines. The local anaesthesia, non-steroid analgesic drugs or general anaesthesia are commonly applied in humans and domestic animals such as dogs and cats. The low usage of these methods on livestock animals can be explained by the following reasons: economic reasons, habitual attachment to routinely performed practices, or by lack of knowledge regarding the pain experienced by the animals.

There is a strong need to develop new, objective research methods that focus on the pain and stress felt by an animal. Infrared thermography, combined with monitoring of the heart rate, could be one of the most effective methods for evaluating the emotional state of animals. However, the described method still needs evaluation, especially in relation to researching other farm animal species. It is not clear if this non-invasive assessment will be useful in the case of smaller and more mobile animals, like piglets, whilst it is also not clear if only eye temperature changes during the invasive procedures performed in farms. This subject calls for more research, discussion and the establishment of principles.

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Accepted for printing 12 II 2013

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**Termografia jako metoda oceny dobrostanu zwierząt poddanych inwazyjnym zabiegom –
artykuł przeglądowy**

STRESZCZENIE

W ostatnich latach rośnie zainteresowanie dobrostanem zwierząt. Konsumentów produktów pochodzenia zwierzęcego skupiają uwagę nie tylko na warunkach utrzymania panujących na fermach, ale również na niektórych zabiegach powodujących ból i cierpienie zwierząt. Do najbardziej inwazyjnych zabiegów należą kastracja i dehornizacja. Zabiegi te wykonywane są często bez znieczulenia. Ból związany z uszkodzeniem tkanek powoduje zmiany behawioralne i fizjologiczne. Obserwacja zachowania oraz pomiary aktywacji autonomicznego układu nerwowego i hormonów osi podwzgórze-przysadka-nadnercza jest główną metodą oceny stanu emocjonalnego zwierzęcia. Unieruchomienie i pobieranie krwi mogą same stanowić źródło stresu dla zwierząt, dlatego istnieje silna potrzeba rozwijania bezinwazyjnych metod badawczych w ocenie poziomu dobrostanu. Jedną z nich jest termografia. Badanie zmian temperatury oka u bydła za pomocą kamery termograficznej potwierdziło aktywację autonomicznego systemu nerwowego w odpowiedzi na ból.