Animal Welfare 2005, 14: 319-325
ISSN 0962-7286

Infrared thermography as a non-invasive tool to study animal welfare

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Abstract

Growing public concern regarding animal welfare and consumer demand for humanely produced products have placed pressure on the meat, wool and dairy industries to improve and confirm the welfare status of their animals. This has increased the need for reliable methods of assessing animal welfare during commercial farm practices. The measurement of the stress caused by commercial farm practices is a major component of animal welfare assessment. However, a major issue for animal welfare science is that many of the techniques used to measure stress involve invasive procedures, such as blood sampling, which may themselves cause a stress response and therefore affect the measurement of interest. To reduce this problem, a number of non-invasive or minimally invasive methods and devices have been developed to measure stress. These include the measurement of cortisol concentrations in saliva and faeces, and remote devices for recording body temperature, heart rate and the collection of blood samples. This review describes the benefits and limitations of some of these methods for measuring stress. In particular, the review focuses on recent advances and current research in the use of infrared thermography (IRT) for measuring stress. Specific applications for IRT in the dairy and beef industries are also described including an automated, non-invasive system for early diagnosis of infection in cattle. It is essential that non-invasive measures of acute and chronic stress are developed for reliable assessment of animal welfare during standard farm management practices and IRT may be a useful tool for this purpose. IRT may offer advantages over many other non-invasive systems as it appears to be capable of measuring different components of the stress axis, including acute sympathetic and hypothalamic-pituitary-adrenocortical responses.

Keywords: *animal welfare, infrared thermography, non-invasive techniques, remote sampling, stress measurement*

Introduction

Stress has been defined as a state that occurs when an animal is required to make abnormal or extreme adjustments, in either its physiology or behaviour, in order to cope with adverse aspects of its environment and management (Fraser *et al* 1975); therefore, measuring stress is central to the assessment of animal welfare. In recent years there has been a dramatic rise in public awareness regarding animal welfare during routine farm animal management practices and concern about whether animals suffer when they are exposed to such situations. Some of these practices are coming under increasing public scrutiny, for example, live animal export trades, where high mortality rates occur, and intensive herd management of dairy cows, where the animals' normal behaviours may be compromised.

Poor welfare standards are not only undesirable on ethical grounds but also have a negative effect on animal health, meat quality and food safety. During transport and pre-slaughter handling, poor welfare can affect meat quality, by carcass damage from bruising, and can result in dark-cutting beef, reduced tenderness (Schaefer *et al* 1988) and susceptibility to bacterial spoilage (Barham *et al* 2002). Barham *et al* (2002) found that following transportation, cattle had increases in shedding of *Salmonella* spp, which leads to hide contamination.

Consumer demand has placed pressure on the dairy, meat and wool industries to provide 'welfare friendly' products that also meet high standards of food quality and safety. In order to improve animal welfare, safety and overall productivity, the development of new technologies and tools to evaluate the welfare impact of different management systems, particularly on-farm or pre-slaughter, are necessary.

Current measures of stress and their limitations

Both the behavioural and physiological systems are involved in the response to stress and are often used in combination to assess stress (Broom & Johnson 1993). Although many researchers have focussed on physiological measures, the importance of behavioural responses in this process has been highlighted by Jensen and Toates (1997), who suggested that stress is primarily a behavioural/psychological phenomenon. As a first line of defence, behavioural

https://doi.org/10.1017/S096272860002964X Published online by Cambridge University Press

responses to aversive stimuli often act to remove the animal from a potentially harmful situation (Clark *et al* 1997). Factors that may influence a behavioural response include the individual's characteristics, species and breed, previous experience, and the nature and severity of the stressor (Clark *et al* 1997). However, the measurement and interpretation of some behaviours can be subjective and the variation in responses can be misleading. For example, one animal may act aggressively in defence, whereas another animal may withdraw in response to the same stressor. In addition, behavioural observations can require a large number of resources, sometimes over a long period of time, such as during 24 h observations. Adequately trained personnel, who have a good knowledge of the animal's species-specific behaviour, are essential (eg in order to define behaviours and set objective criteria) and in some cases lighting, video and digital analysis equipment may also be required. Psychological factors, including an animal's prior experience, may also influence its response to a particular situation and are hard to control and determine. The more an animal can predict and/or control its situation, the lower its stress levels (Weiss 1972; Jensen & Toates 1997). For example, in addition to the physical stress of being restrained, an animal might also experience psychological stress attributable to the inability to avoid or escape from what it perceives to be a harmful or threatening situation.

Monitoring physiological responses, such as hypothalamic–pituitary–adrenocortical (HPA) activity and changes in plasma cortisol concentrations in particular, are frequently used to study the stress responses in farm animals (Broom & Johnson 1993). However, depending on the type of stressor being investigated, there may be some limitations to the usefulness of hormones as a measure of stress: these include difficulties in obtaining true baseline levels, inadequate sampling frequency that may miss rapid response times, and circadian and ultradian rhythms in hormone levels. One major problem is that methods such as blood sampling, which are used to measure some physiological indicators of stress, require the animal to be restrained, and handling procedures can themselves cause a stress response that may lead to confounding results. Collection of a single blood sample via jugular venipuncture or jugular vein catheterisation can increase cortisol concentrations in dairy cows for up to 60 min and 130 min, respectively (Alam & Dobson 1986). Hopster *et al* (1999) found that successive blood samples collected via jugular venipuncture from loose-housed dairy cows caused an increase in cortisol concentrations; however, the increase was less for cows that were accustomed to handling and restraint. Therefore, by habituating cows to sampling procedures, increased plasma cortisol concentrations attributable to handling associated stress can be avoided (Hopster *et al* 1999). However, this may not be true in all situations; De Silva *et al* (1986) found that the stress response of sheep to blood sampling was not reduced by previous handling, and cortisol and prolactin concentrations were lower when a remote blood sampling procedure was used.

Ultradian and circadian rhythms also need to be considered when analysing physiological responses to stress as they can alter baseline hormone concentrations. Strong ultradian rhythms of cortisol concentrations, with a duration of approximately 120 min, have been observed in lactating cows (Lefcourt *et al* 1993). Cortisol concentrations may also be affected by other factors, such as social status (Mulleder *et al* 2003), reproductive state (Bell *et al* 1991), and activity levels (Fisher *et al* 2002).

Other physiological indicators of stress that have been used are changes in immune function (Kelley 1985) and sympathetic responses, such as increased heart rate, respiratory rate, body temperature and the secretion of catecholamines (eg epinephrine) (Broom & Johnson 1993). Problems arise when using heart rate as a measure of stress, because changes attributable to metabolic activity are difficult to distinguish from those attributable to emotional responses (Broom & Johnson 1993). Mean heart rate can only be interpreted as the net effect of interactions between both divisions of the nervous system, whereas measurement of heart rate variability (HRV) allows more detailed interpretation of cardiac activity in terms of the autonomic nervous system (Marchant-Forde *et al* 2004). Therefore it has been suggested that HRV is a more accurate measure of stress in animals (Mohr *et al* 2002; Marchant-Forde *et al* 2004). One of the limitations of using HRV is the lack of remote equipment that is capable of storing large amounts of data required for HRV analysis; consequently, few studies have investigated HRV in farm animals. However, technologies for storage and data analysis are improving (Marchant-Forde *et al* 2004). In conclusion, there is no simple definitive method by which stress can be measured. Most researchers agree that a combination of physiological and behavioural measures may provide the best assessment of animal welfare (Clark *et al* 1997) and that there is a need for more reliable, non-invasive ways to measure stress.

Developments in non-invasive measures of stress and remote sampling

Remote devices have been developed to record physiological responses that may be useful for measuring stress, such as heart rate (Lefcourt *et al* 1999), respiratory rate (Eigenberg *et al* 2000), blood parameters (Ingram *et al* 1997), and ear pinna temperature (Ingram *et al* 2002; Beausoleil *et al* 2004; Lowe *et al* 2005). Core body temperature has also been measured using surgically implanted radio-transmitters (Lefcourt & Adams 1998) and intravaginal data-loggers (Bluett *et al* 2000). Furthermore, systems have been developed to measure behaviour remotely, such as foraging behaviour (Rutter *et al* 1997), lying, standing and walking (Champion *et al* 1997). However, most of these remote systems still require handling the animal and in some cases minor surgery, catheterisation and other manipulations, such as the insertion of a data-logger into the ear canal or vagina. Some systems require animals to carry bulky equipment, which itself could cause changes in physiology or behaviour. In addition, it may not always be possible to release animals carrying such devices

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back into their normal herd, which may disrupt normal behaviour patterns (eg social behaviour). Therefore, remote systems may be useful in fundamental studies investigating acute stress responses to management practices, such as transport or de-horning, but they may be less practical for assessing on-farm animal welfare. In commercial farming situations, animals are often exposed to multiple stressors or chronic stress, and although it is important to have accurate, non-invasive tools to measure levels of acute stress, it is also vital to have tools capable of measuring chronic stress and recovery over longer periods of time on-farm.

Indicators of HPA activity can be measured in faeces (Palme *et al* 2000; Morrow *et al* 2002), urine (Pol *et al* 2002), milk (Verkerk *et al* 1996) and saliva (Cook *et al* 1996; Negrao *et al* 2003); therefore, avoiding blood sampling procedures. Furthermore, these indicators require minimal contact with the animal, the ideal being the collection of faeces from a paddock or housing system where no restraint or sampling is required and there is no interference with the stress levels of subsequent samples. Morrow *et al* (2002) found a significant increase in faecal glucocorticoid excretion in dairy cows following administration of an adrenocorticotropic hormone (ACTH) challenge, exposure to a novel environment and following transportation, suggesting that glucocorticoid metabolites measured in faeces can reliably indicate acute adrenal activity in dairy cattle. However, the delay time of faecal excretion needs to be taken into account during the interpretation of stress responses that occurred during a specific time prior to the sampling period. This delay, which is approximately 10–12 h in cattle, is related to the digesta transit time between the bile duct and the rectum and is affected by season, feed intake and pasture digestibility (Morrow *et al* 2002).

Salivary 'free' cortisol may be a more reliable indicator of stress than 'total' cortisol measured in blood because cortisol concentrations in saliva represent an ultrafiltrate of the free steroid fraction in blood and are biologically active (Cook *et al* 1996). Cook *et al* (1996) found that handling and transportation stimulated significant increases in salivary cortisol in pigs, and serum and salivary cortisol were significantly correlated. In addition, urinary cortisol concentrations have been shown to increase significantly in response to an ACTH challenge in pigs (Pol *et al* 2002). Pol *et al* (2002) found that increases in stereotypic behaviour were related to higher urinary cortisol concentrations and suggested that this may be a useful tool for measuring chronic stress. For dairy cows, collection of milk samples for the analysis of cortisol is a convenient sampling method that, in modern facilities, requires minimal or no animal handling, and is a procedure to which cows become well habituated. Verkerk *et al* (1996) found that milk and plasma cortisol concentrations were highly correlated in dairy cows. However, short-duration increases in plasma cortisol were not well represented in milk cortisol concentrations at the following milking, so samples need to be obtained during the period of elevated cortisol (Verkerk *et al* 1996). In summary, current non-invasive measures of stress have limitations and there is still a demand for more non-invasive ways to measure stress and study animal welfare.

Infrared thermography: application for measuring stress and assessing animal welfare

Infrared thermography (IRT) is the measurement of radiated electromagnetic energy. Electromagnetic radiation can be described as a stream of photons, which are particles that have no mass, each travelling in a wave-like pattern and moving at the speed of light. The photons with the highest energy correspond to the shortest wavelengths. In the electromagnetic spectrum, broad range infrared radiation wavelengths (3–12 μm) are longer than visible light and in animals 40–60% of heat loss is within this range (Kleiber 1975). Small changes in temperature may result in substantial amounts of emitted photons (or radiated energy) that can be detected very accurately using IRT.

IRT has been used for many years in human and veterinary medicine. In 1980, Purohit and McCoy (1980) reported on the use of IRT in equine medicine to detect leg or hoof problems in race horses and in 1992, Yang and Yang (1992) reviewed the applications of IRT in various fields of medicine, including pharmacy and dentistry.

When an animal becomes stressed, the HPA axis is activated and heat production, as a result of increases in catecholamines and cortisol concentrations, in addition to blood flow responses, will produce changes in heat production and heat loss from the animal (Schaefer *et al* 2002). This can be detected using a specialised infrared camera to collect real-time pictorial images at a distance from the animal, usually with no need for contact or restraint. Minimal restraint may be necessary in some cases to simplify image collection, depending on the animal's flight distance and handling experience, the area of the body that is of interest and how close the operator needs to be to the animal.

There are some limitations and factors that need to be considered when using IRT. Images must be collected out of direct sunlight and wind drafts, and hair coats should be free of dirt, moisture or foreign material. Dirt on the animal alters the emissivity and conductivity, and excess moisture increases local heat loss to the environment or dryer areas of the coat (Palmer 1981). The effects of weather conditions, circadian and ultradian rhythms, time following feeding, milking, lying and rumination are also factors that need to be considered and require further investigation as part of validating IRT as a stress measurement tool.

IRT has been used successfully to detect cattle that are predisposed to producing 'dark–firm–dry' beef (Tong *et al* 1995), 'pale–soft–exudative' pork in swine (Schaefer *et al* 1989), and to monitor transportation stress in cattle (Schaefer *et al* 1988). Other studies have used IRT to assess inflammation attributable to hot iron branding in cattle (Schwartzkopf & Stookey 1997) and scrotal surface temperature as a measure of fertility in bulls (Kastelic *et al* 1996).

There are a number of body sites, alone or in combination, which can be monitored using IRT to indicate the impact of a wide range of potential adverse events. For example,

Figure 1

Infrared thermographic image (grey scale) of the udder and hind quarters of a dairy cow.

Cook and Schaefer (2002) found a significant increase in radiated heat from the dorsal surface of wapiti in response to removal of velvet antler. Other studies have shown increases in eye temperature in response to velvet antler removal in elk and reindeer (Cook *et al* 2005). In addition, eye temperature, measured using IRT, was more effective at detecting bovine viral diarrhoea (BVD) because changes were detected as early as day one of infection, compared with days 5–6 for other areas, such as the nose, ear, body and hooves (Schaefer *et al* 2003).

Infrared thermography in the dairy industry

So far, the uses of IRT in the dairy industry include early detection of oestrus (Hurnik *et al* 1985), mastitis (Scott *et al* 2000; Berry *et al* 2003) and lameness (Nikkhah *et al* 2005). Recent studies have focused on the use of IRT to detect mastitis much earlier than was previously possible (Scott *et al* 2000; Berry *et al* 2003). This is of considerable benefit because mastitis is a major welfare and economic concern for the dairy industry (Gill *et al* 1990). Currently, the industry's standard practice for detecting mastitis in milking cows is the use of somatic cell counts (SCC). However, changes in SCC are often detected late in the timescale of an udder infection and do not identify all classes of infection, subclinical infections, or those that take some time to display clinical signs. Furthermore, general clinical symptoms, such as changes in core or rectal temperature, also develop late in the course of an infection. However, one reliable sign of an inflammatory response is often the increase in temperature of the infected area itself (Marieb 1989); therefore, an alternative method for the early identification of mastitis would be to measure the radiated infrared temperature of the mammary gland, because this is the actual site of infection.

Using an endotoxin-induced mastitis model, Scott *et al* (2000) found that inflammation could be detected from temperature differences using IRT earlier than with either SCC or bovine serum albumin (BSA). BSA concentration peaked at 6 h post-induction, whereas IRT temperature increases were evident within 1 h post-induction. In Figure 1, an infrared thermographic image, the background and legs of the cow are cooler and therefore darker than the udder, which is warmer and therefore lighter. Skin temperature is affected by the flow of heat from the body core to the surface of the body, and by the flow of heat from the skin surface to the atmosphere; consequently, such images can reveal very early signs of udder infections in lactating cows.

Berry *et al* (2003) used IRT to study the effects of environmental factors on the daily variation in udder temperature. They found a distinct circadian rhythm in udder temperature and a significant increase in udder temperature caused by exercise. However, the daily variation in udder temperature was smaller than the rise in temperature resulting from an induced mastitis response. Therefore, they concluded that IRT has potential as an early detection tool for mastitis if it is combined with detailed monitoring of environmental temperature.

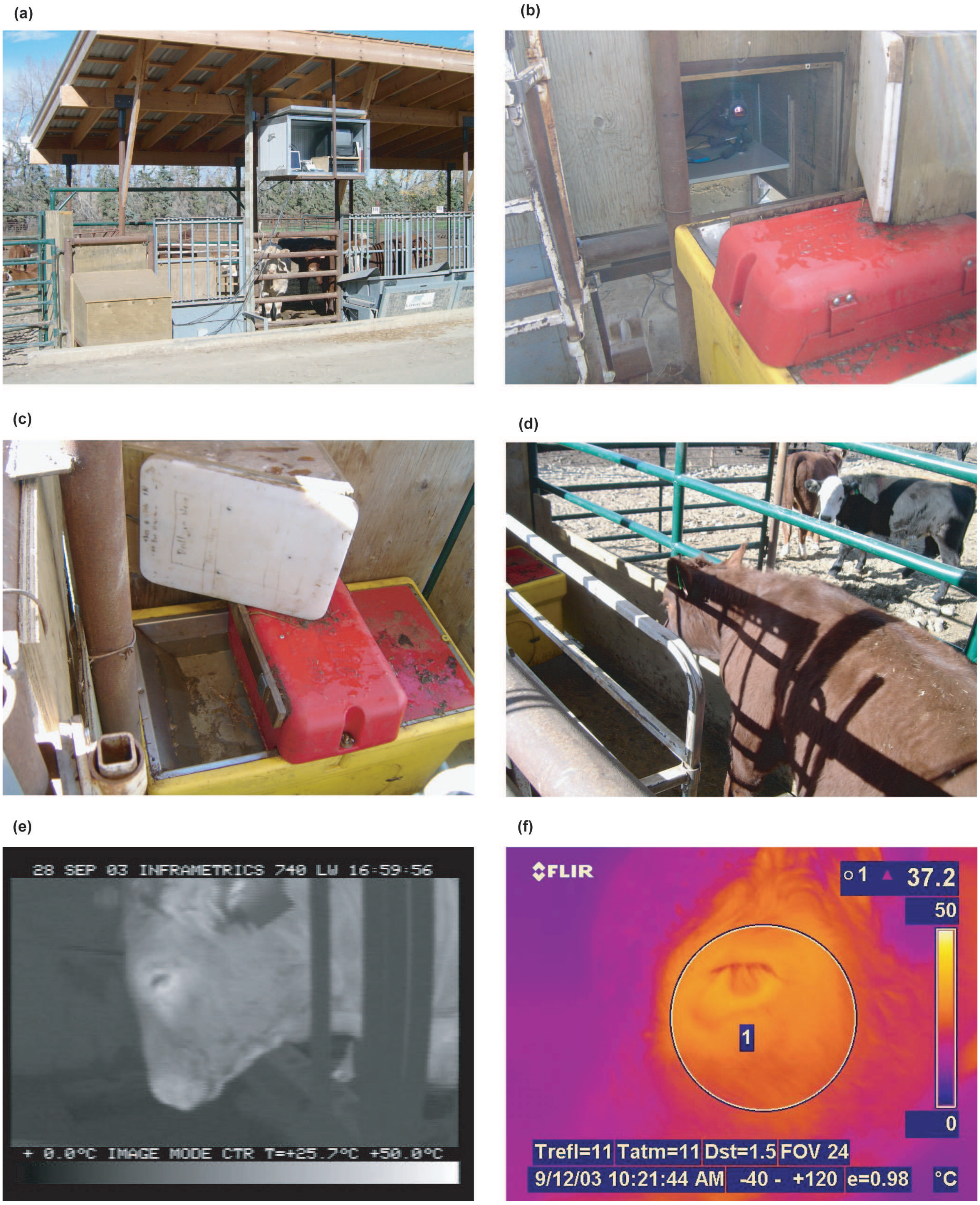
Infrared thermography in the beef industry

On a global scale, most beef calves are exposed to several transportation and handling experiences within their lifetimes, typically involving co-mingling and some form of auction. These stressors often predispose calves to an increased incidence of diseases, such as bovine respiratory disease (BRD) (Cusack *et al* 2003) and BVD (Houe 1999). These diseases have significant animal welfare and economic consequences for the beef industry, particularly in North America. The industry's standard practice to identify calves with disease is the observation of clinical symptoms by an experienced cattle handler, at which point intervention measures are taken. Unfortunately, the appearance of clinical symptoms is usually several days or more into the course of the disease and by then the animal often requires considerable medical attention, including the use of antibiotics, in order to recover. In a BVD virus induction model, Schaefer *et al* (2003) used IRT as a non-invasive, early detection method for identifying calves with BVD. They found that increases in eye temperatures, measured using IRT, were more consistent than other anatomical areas, as described earlier. There were also significant changes in eye temperatures several days to one week before other clinical signs of infection.

In such situations, the use of IRT for the non-invasive detection of early stages of disease in livestock would be possible. The authors of this paper have tested one such strategy, the incorporation of an infrared scanning station into a water trough, because the animals visit this site on a regular basis (Figure 2). The scanning station was coupled to an electronic ID system, to automatically identify which animal was present at the water station and to collect an infrared image of the eye region of that animal. From prediction indexes, such a system could then advise a feedlot operator or farm manager if an animal is showing early signs of disease and therefore requires medical attention.

https://doi.org/10.1017/S096272860002964X Published online by Cambridge University Press

Figure 2



Automated infrared image collection for detection of early infection in cattle. The photographs show: **(a)** the collection site, **(b)** an infrared camera aimed at a trough, **(c)** an electronic identification (EID) detection plate and water trough, **(d)** an animal entering for a drink, **(e)** a grey scale infrared image of an animal drinking, and **(f)** a close-up colour infrared image of the eye region.

https://doi.org/10.1017/S096272860002964X Published online by Cambridge University Press

Current research investigating infrared thermography as a tool to measure stress

The ability of IRT to detect and measure animal responses to stress has been the focus of recent research activity. Relationships between IRT and HPA activity were initially investigated by Cook *et al* (2001), who used IRT and cortisol concentrations to measure adrenocortical and metabolic activity in horses. Matched blood and saliva samples, and IRT eye images were collected at set intervals before and after an ACTH challenge. The results revealed a significant correlation between maximum eye temperature and both salivary and plasma cortisol concentrations suggesting that changes in eye temperature may be associated with activation of the HPA axis.

The study by Cook *et al* (2001) led to a need for further investigation into the relationship between the different components of HPA axis activity, IRT responses and changes in heat production and loss. In order to do this, Stewart *et al* (2005) conducted a study to validate IRT eye temperature as a measure of stress, using dairy cattle as a model. Cows were given an ACTH challenge and subjected to psychological stress (social isolation). Increases in both cortisol and ACTH concentrations confirmed that the stress axis had been stimulated. IRT eye temperatures not only increased following both ACTH and saline (control) treatments, but also prior to the treatments, possibly because of the effects of prior activity or handling stress. An interesting finding was that following social isolation, IRT eye temperature tended to fall rapidly and then increase again, which may reflect an acute sympathetic response attributable to the psychological stress. During this trial, cortisol concentration and IRT eye temperature were also measured in these cows following two catheterisation procedures. IRT eye temperatures and cortisol concentrations did not increase significantly following the first procedure; however, both were significantly higher ($P < 0.001$ for IRT; $P < 0.05$ for cortisol concentration) post-catheterisation one-week later, when the procedure was repeated. The increased response the second time the animals were catheterised suggests that the cows may have anticipated the procedure and that this caused the higher stress response. This supports the suggestion that some type of psychological component may be involved in the IRT eye response. Therefore, IRT may be capable of detecting acute sympathetic responses as well as HPA activity. However, research is currently being undertaken to investigate this possibility further.

Conclusions and animal welfare implications

Studies of animal welfare have commonly measured HPA axis activity, activation of the sympathetic system and behavioural responses to stress. However, a major problem for animal welfare researchers is that many of the methods used to measure these responses involve restraint or handling procedures, which may alter the stress response itself. Recently, non-invasive or minimally invasive systems for measuring stress have been developed, but these systems have limitations and no single measure of stress is perfect. Reliable, non-invasive tools that can be used to measure acute and chronic stress during commercial practices and pre-slaughter are required. IRT fits this criteria and has great potential as a way to assess animal welfare. The use of IRT in the dairy and beef industries has been developed and automated systems to monitor infection are currently being tested. Although IRT equipment is portable, simple to use and animal restraint is minimal or unnecessary, there are some variables, such as weather conditions and circadian rhythms, which need further investigation before IRT can be used reliably during commercial practices on-farm. However, IRT may have certain advantages over other non-invasive methods by offering insight into the metabolic consequences of stress and enabling measurement of short-term acute and long-term chronic responses to stress.

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