



# Infrared thermography as a tool for the measurement of negative emotions in dairy cows

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## Abstract

In commercial dairy cows, the conditions in which they are kept may lead to negative emotional states associated with the development of chronic physiological and behavioural abnormalities that may compromise their health, welfare and productivity. Such states include fear, stress or anxiety. Behavioural rather than physiological tests are more likely to be used to indicate these states but can be limited by their subjectivity, need for specialised infrastructure and training (of the operator and sometimes the animal) and the time-consuming nature of data collection. Popularly used physiological measures such as blood cortisol may be more appropriate for acute rather than chronic assessments but are easily confounded, for example by a response to the act of measurement *per se*. More sophisticated physiological measures such as functional magnetic resonance imaging (fMRI) and electroencephalography (EEG) may be impractical due to cost and time and, like blood cortisol, have the confounding associated with the act of measurement. By contrast, infrared thermography of external body surfaces is remote, non-invasive, easily repeated and follows an objective methodology, allowing longitudinal data acquisition for the inference of changes in chronic emotional state over time. The objective of this review was to investigate the potential of infrared thermography to measure cow emotions. In lactating dairy cows, maximum IRT of the eyes and coronary band of the limbs seem to be most representative of thermoregulatory changes, which are repeatable and correlate with behavioural and physiological indicators of emotional state. IRT methodologies have the potential to become a fundamental tool for the objective assessment of welfare state in dairy cows.

**Keywords** Emotion · Infrared thermography · Surface temperature · Non-invasive · Stress · Cow welfare

## Introduction

Emotions are internal psychological states derived from short but intensive affective responses to circumstances (Cabanac 2002; Mota-Rojas et al. 2021a). There are two

fundamental dimensions in emotions—valence and arousal (Mendl et al. 2010; Zebunke et al. 2011). Valence is the intrinsic attractiveness (positive valence) or averseness (negative valence) of a circumstance, and arousal is graduated from a low to a high level of emotion at a given valence (Tamioso et al. 2017). In cattle, regular feelings of negative emotions associated with unavoidable events encountered daily, such as but not limited to an unfamiliar object, person, environment, feeding, housing, regrouping or contact with a more dominant herd mate have been linked to abnormal behavioural and physiological responses, such as vigilance, a left eye view of the threat, connected to right brain hemisphere processing, decreased feed intake and increased plasma cortisol and catecholamine concentrations (Bristow and Holmes 2007; Phillips et al. 2015; Du et al. 2018; Goma et al. 2018). Such abnormalities may compromise the health, welfare and productivity of dairy cows.

It is important to identify cows in negative emotional states in order to prevent stressful situation or conspecific

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confrontations and for the additional husbandry requirements that they need to improve their wellbeing (Dawkins 2012; Proctor 2012). Cows suffering from negative emotions eat less, pass more time in a vigilant state and show frequent fight or flight responses, and thus, they have a lower productive and reproductive performance (Breuer et al. 2000; Kilgour et al. 2006; Gibbons et al. 2011; Hulbert and Moisé 2016; Lee et al. 2018). It further increases their susceptibility to diseases (Fell et al. 1999; Goff et al. 2002). Emotional responses are hard to determine in animals as the opportunities for self-report is limited. Instead, behavioural and physiological measures are relied on. However, behavioural measures of emotions such as flight speed or forced lateralisation tests are time-consuming and require the active involvement of animals and infrastructure to carry out the test. Behavioural responses to emotions could also vary between animals due to genotype, individual factors of the animals such as sex and age, as well as evaluator-related factors such as their experience and knowledge of the natural repertoire of the species (Clark et al. 1997; Veenema et al. 2003). Similarly, problematic are physiological measures such as cortisol and or catecholamine concentration in plasma, faeces, urine, milk, saliva and hair, as they do not always represent the sympathetic and cognitive responses to emotions, their severity or duration (Stewart et al. 2007). Other sophisticated physiological measures of emotions used in humans, such as of functional magnetic resonance imaging (fMRI) and electroencephalography (EEG), are difficult to apply to cattle due to their relative novelty and/or technological limitations. Non-invasive and/or remote devices have been used to evaluate the heart (Lefcourt et al. 1999) and respiratory (Eigenberg et al. 2000) rates, as well as foraging (Rutter et al. 1997) and lying, standing and walking (Champion et al. 1997) behaviours. These have the disadvantages of being relatively expensive, still requiring handling of the cattle, and a need for cattle to carry these devices, all of which can alter emotion state (Stewart et al. 2005). New options for the non-invasive measurement of emotions in cattle are therefore required.

Core body or peripheral surface temperatures have been used to monitor emotional states in a range of species of animals and humans (Baker et al. 1976; Lowe et al. 2005; Vianna and Carrive 2005; Stewart et al. 2008a; Ludwig et al. 2010; Vinkers et al. 2013; Lee et al. 2018). In cattle, an increase in rectal or vaginal temperature has been proposed as an indicator of negative emotions (Smith and Risco 2005), but these techniques may be confounded as they also require the restraint of the cattle (Stewart et al. 2008a). The use of thermal data loggers could avoid the frequent handling of animals, although the maintenance of uninterrupted data delivery is a major limitation, including the need to rehandle the animal for device maintenance and further invasive procedures (McCafferty et al. 2015).

Compared to thermal data loggers or hand-held thermometers, infrared thermography is a tool that uses a specialized camera to evaluate emitted radiation from objects, and as a result should be even less invasive for the estimation of body surface temperature and therefore negative emotions (Mota-Rojas et al. 2021b). Infrared temperature (IRT) of external body surfaces have been used to estimate changes in the internal body temperature of animals in association with oestrus, respiration, disease onset and behavioural responses indicative of change in emotions (Stewart et al. 2017; Lowe et al. 2019; Marquez et al. 2019; Uddin et al. 2019). Infrared thermography measures the radiated heat of external body surfaces, such as the eyes and limbs, from a distance by an infrared sensor (Jenkins et al. 2009). It allows the visualisation of changes in the radiated heat as a radiometric image of pixels varying in colours or shades according to different infrared temperature, which is non-invasive, thus allowing long-term monitoring of animals and has a greater potential for automation than the measurement of rectal or vaginal temperature (Tamioso et al. 2017; Unruh et al. 2017; Macmillan et al. 2019). This review presents the current state of knowledge, limitations and future recommendation of research related to negative emotions and infrared thermography, for the improvement of the health, welfare and productivity of dairy cows.

## Emotions of non-human animals

An emotion is an internal psychological state derived from a short, intense conscious or unconscious affective response to circumstances which are associated with (a) physiological; how the body responds, (b) behavioural; what one shows to others, (c) motivational; behaving in a particular way, (d) motor; facial expression and movement, (e) subjective; influenced by individual judgement and (f) cognitive; making judgements of circumstances based on information perceived and processed in the brain components (Arnold 1960; Cabanac 2002; Désiré et al. 2006). Processing of emotion encompasses different components of the brains such as the limbic system, hypothalamus, amygdala and cerebral cortex (Lezama-García et al. 2019). Valence is an intrinsic attractiveness (positive valence) or averseness (negative valence) of an event, object or situation, and arousal indicates graduation from a low to a high level of emotion at a given valence (Tamioso et al. 2017).

In cattle, positive valence could be associated with feeding, playing, stroking, grooming, brushing, and other tactile contacts (Schmied et al. 2008), facilitating animal curiosity for social bonding through the activation of the parasympathetic nervous system (Fredrickson 2001). Contrary to this, negative valence is associated with aversive handling that involves shouting, quick or unpredictable movements, use of

flags, sticks and prods to hit animals. Other causes of negative valence that have been investigated includes exposure to an unfamiliar person, object, or arena or more dominant herd mates, climatic extremes, sudden changes in diet, transition from lactation to dry-off, transportation and disruption to social groupings (Hemsworth et al. 1996; Boissy and Le Neindre 1997; Munksgaard et al. 2001; Phillips et al. 2015; Franchi et al. 2021). Negative emotions activate the sympathetic nervous system which has physiological, endocrine, metabolic and behavioural consequences when overstimulated for the possible actions to avoid threatening situations or to cope with the threat (Tooby and Cosmides 1990), while positive emotions activate the parasympathetic nervous system to stimulate curiosity and facilitate social bonding (Fredrickson 2001).

Besides, the external stimulus of emotion, the genetic potential and idiosyncratic events in the life of an animal play a vital role in the development of negative emotions (Lang et al. 2000). Genes such as that encoding for Troponin receptor kinase C (TrkC) have been reported to modulate the susceptibility of cattle to negative emotions such as fear, stress or anxiety due to restraint in a cattle crush (Veenema et al. 2003). However, the genetic component is complex, as several genes may be involved in the initiation of negative emotions and the behavioural responses that result (Clement and Chapouthier 1998).

## Impact of emotional valence on cattle productivity

Emotional valences have an impact on the health, welfare and productivity of cattle. Cattle suffering from negative emotions showed more vigilance, right side laterality, left eye view of the threat (Phillips et al. 2015; Du et al. 2018; Goma et al. 2018), enhanced plasma cortisol and catecholamine concentrations (Bristow and Holmes 2007), may eat less (Osei-Amponsah et al. 2020), thus reducing the productive and reproductive performance (West 2003; Bernabucci et al. 2010), display abnormal behaviours (Lee et al. 2018) and have a weakened immune system (Fell et al. 1999), making them susceptible to mastitis and other infectious and or metabolic diseases (Goff et al. 2002). Thus, identification of cows suffering from anxiety is of importance to allow targeted interventions to maintain sound health, welfare and productivity.

Measuring blood cortisol has been indicating valences, where a high cortisol level indicates negative emotions and or stressed animals and a low cortisol level indicates positive emotions and or non-stressed animals. Bristow and Holmes (2007) differentiated behavioural responses of cattle having high and low blood cortisol. Cattle with high blood cortisol had shown less time spent in rumination while rest, more

vocalisation, and hesitation to enter a stressful situation (a holding corral). Goma et al. (2018) evaluated the milk fat percentage of behaviourally anxious (extreme right lateralised) and non-anxious (extreme left lateralised) cows and reported that **anxious/right lateralised cows produced milk with a lower fat percentage than that of non-anxious/ left lateralised cows**. Cattle with more excitable temperaments, as measured by faster flight speed, chute score, pen score and exit velocity, exhibit greater basal concentrations of glucocorticoids and catecholamines were considered to have a ‘poorer’ temperament (more anxious); conversely, cattle with calm temperament measured by such as but limited to slower flight speed were considered to have a ‘good’ temperament (Burrow and Dillon 1997; Petherick et al. 2002). Cattle with good temperaments have been recommended for breeding programmes in Australia for their improved productivity (Burrow and Corbet 2000).

## The limitations of current measures of negative emotions

Compared to human research, behavioural and physiological measurements are more commonly used to infer emotional state of non-human animals than more sophisticated EEG and fMRI techniques. Physiological measures in cattle have tended to focus on an elevation in blood cortisol and catecholamine concentrations as an indication of the extent of suffering from negative emotion (Breuer et al. 2000). A major limitation of blood cortisol is that the process of collecting blood requires restraint and handling of cattle and that itself might affect the emotional state, in addition to the stressor (Alam and Dobson 1986; Stewart et al. 2005). To overcome this limitation but not always successfully, cortisol has also been measured in faeces (Morrow et al. 2002), urine (Pol et al. 2002), milk (Verkerk et al. 1998), saliva (Negrao et al. 2004) and hair (Sharma et al. 2019). However, even with better measurement, cortisol does not represent the entire sympathetic and cognitive responses to negative emotion (Stewart et al. 2007). Additionally, cortisol does not necessarily vary according to the severity of emotional arousal, may not persist for the full period of emotional arousal, and is highly sensitive to a range of other stimuli that may not be related to the negative emotions such as excitement during sex or the anticipation of a reward (Mormède et al. 2007). Central nervous system (CNS) activities of negative emotional responses of cattle have also been measured by quantification of adrenaline and noradrenaline concentrations (neurotransmitter) in the blood (e.g., Sinclair et al. 2016). The logic is that the level of adrenaline and noradrenaline is linked to brain function and negative emotions. That in itself requires further validation, but assaying

CNS activities in commercial herds may be impractical due to the shortlived changes.

To further discount the utility of physiological measures, Jensen and Toates (1997) have claimed that emotional responses are basically a behavioural and or psychological phenomenon. Since behavioural measures of emotions are thought to cover the entire sympathetic and cognitive responses, they could provide a good indication of the duration and severity of emotional responses (Mellor et al. 2000; Kilgour et al. 2006; Stewart et al. 2007; Gibbons et al. 2011; Phillips et al. 2015). Consequently, different behavioural measures of emotional response have been developed (Kilgour et al. 2006; Gibbons et al. 2011; Phillips et al. 2015; Hulbert and Moisé 2016; Lee et al. 2018). These behavioural measures range from subjective to objective in nature; some have been validated as repeatable but other have limited research available on them. Subjective measures depend on people's judgement and interpretation of results, while objective measures rely on timing or distance to a situation. Repeatable measures indicate their efficiency to measure emotional responses of animals consistently over time despite the possible influences of habituation to that measure. Commonly used behavioural measures of emotional responses are flight speed, crush score, the human approach test and the forced lateralisation test (Burrow 1991; Burrow and Dillon 1997; Petherick et al. 2002; Müller and von Keyserlingk 2006; Winckler et al. 2007; Windschnurer et al. 2008; Gibbons et al. 2011; Phillips et al. 2015).

In a flight speed test, the time taken to traverse a fixed distance (m) by the cattle while leaving the crush is recorded in s, and flight speed is calculated as m/s. Burrow (1991) and Burrow and Dillon (1997) reported that faster flight speed indicates poor temperament in cattle, and slower flight speed indicates good temperament. Flight speed is a repeatable and objective measure of temperament in cattle (Petherick et al. 2002; Müller and von Keyserlingk 2006).

The Human approach test measures the fearfulness of cattle in their home environment without removing them from their social group. It is a more subjective measure, where an unfamiliar person approaches the cattle with quick unpredictable shouting or movements or by confronting cattle with flags, sticks or electric prods on the cattle and estimate the flight distance with eye estimation as avoidance or approach behaviour (Winckler et al. 2007; Windschnurer et al. 2008; Gibbons et al. 2011). The greater the flight distance, the greater the fearfulness of the cows. The human approach test is moderately repeatable in dairy cows (Gibbons et al. 2009).

In the crush score test, cattle are moved into the crush in a calm manner by an unfamiliar person and the behavioural responses are scored as shown by cattle around the crush. Cattle with a higher crush score are more likely to be affected by restraint in the crush. Crush score is another

subjective measure of emotion in mature cattle and but may not be repeatable (Gibbons et al. 2011).

As described earlier in this review, the forced lateralisation test is a psychological or cognitive test. It tests a cow's preference to pass either the right or left side (from the cow's perspective) of an unfamiliar person in a familiar laneway (measured on a laterality index) whilst the cow is exiting the milking parlour after milking as they walk towards their feeding area (Phillips et al. 2015; Goma et al. 2018). There is associated behaviour evidence that cows that more consistently pass the person on the right side of the lane are more anxious than those on the left side (less anxious) (Phillips et al. 2015; Goma et al. 2018). Laterality is a highly repeatable test in lactating dairy cows (Goma et al. 2018; Uddin et al. 2021), but its relationship to cognitive processes needs further research.

There are practical limitations of behavioural measures of emotions. It is not always possible to conduct such tests on-farm, or off-farm, due to a lack of required labour, time, infrastructure and personnel with the required expertise in cattle behaviour. The separation of experimental cattle from their social groups, deprivation of feed or water and restraining for experimental purposes also confounds the result of the test. Additionally, behavioural measures are mostly published from the perspective of temperament traits in beef rather than dairy cattle, with the exception of the forced lateralisation test. Beef cattle are normally more sensitive than dairy cattle, as dairy cattle are in more regular contact with humans and other environmental changes in the commercial dairy farm for a longer period of their lifespan than beef cattle. Behavioural exhibition of emotions may be confounded by other factors such as an animal's individual characteristics, species, breed, nature and level of emotional arousal (Clark et al. 1997). For example, one animal may act aggressively in defence, whereas another may withdraw in response to the same threat.

In order to overcome confounding of the common physiological and behavioural measures of emotions, non-invasive and/or remote sensing devices have been used to monitor heart rate (Lefcourt et al. 1999), respiratory rate (Eigenberg et al. 2000), foraging behaviour (Rutter et al. 1997) and lying, standing and walking behaviours (Champion et al. 1997). Using these devices still requires handling and restraint of animals, and animals further need to carry the device, which itself could cause aroused emotions to the animals (Stewart et al. 2005). Sometimes, animals with these devices are kept separate from their herd which might change their social behaviour. Moreover, animals may not behave normally in their herd even after removal of such devices. Thus, it is important to investigate new measures of emotions in dairy cows that can overcome the limitations of the above mentioned physiological and behavioural measures.



## Potential of body temperature as a measure of emotional response

Changes in the valence and arousal of emotions are associated with sudden changes in blood flow and temperature, as detected in humans, animals and birds (Franzini et al. 1981; McFarland 1985; Herborn et al. 2015; Jerem et al. 2019; Franchi et al. 2021). The sequence is that threat stimuli first disorganise the metabolic homeostasis (a stable internal environment) of the body (Taylor et al. 2008). Then, in threatening situations, an immediate attempt occurs to restore metabolic homeostasis through the activation of the hypothalamic pituitary adrenal (HPA) and CNS axis. Cross-linking between the HPA and CNS axes also occurs through the secretion of endorphins by the pituitary gland. The HPA axis is responsible for the secretion of cortisol that promotes gluconeogenesis to provide extra energy to respond to the threat by the means of fight or flight (Moberg and Mench 2000). The CNS is responsible for the secretion of adrenaline and noradrenaline which also prepares the animal for fight or flight. The fight or flight responses are linked to a range of biological changes, such as greater blood flow from lesser to more important parts of the body such as brain and skeletal muscles. This occurs through the dilation and constriction of blood vessels and stimulation of the sweat glands, changes which lead to an increase in the loss of heat from the external body surfaces (Worthley and Connolly 2000; Luzi et al. 2013; McManus et al. 2016; Godyń et al. 2019). This cascade of events in acute or repeated stress increases core body temperature and is known as stress-induced hyperthermia (Jerem et al. 2019). An increase in core body temperature can be accompanied by a reduced blood circulation and therefore reduced temperature, in certain peripheral areas. This is because the temperatures of peripheral areas, including the external surfaces of the body, are mainly determined by blood flow to the skin and the metabolic rate of adjacent tissues (Berry et al. 2003). The external body surfaces act as a buffer between the internal body and external ambient temperature and are quite variable, while core body temperature, in normal situations, is relatively stable (Sokabe and Tominaga 2009). Negative emotional responses have been associated with increased core body temperature in humans (Vinkers et al. 2013), mice (Borsini et al. 1989), cattle (Macaulay et al. 1995; Lee et al. 2018) and rats (Nakamori et al. 1993). Decreased body temperatures have been recorded in association with negative emotions on the surface of the tail and paws in rats (Vianna and Carrive 2005); the muzzle, nasal mucosa, ears, hands, feet and tail of pigs and monkeys (Baker et al. 1976); and the ear pinnae of sheep and rabbits (Lowe et al. 2005; Stewart et al. 2008a; Ludwig et al. 2010), as blood is directed from external to internal body parts. Therefore, measurement of body surface temperature could

indicate a range of negative emotional responses associated with autonomic nervous system activation.

## Limitations of common techniques of body temperature measurement

Rectal or vaginal temperature are considered “gold standard” measures of core body temperature in cattle. They are recorded by manually placing a thermometer into and against the wall of the rectum or vagina for approximately 30 s prior to reading. Recording of rectal or vaginal temperature requires displacement of cattle from their social group, ideally restraint in a cattle crush and physical intervention to penetrate the thermometer into the rectum or vagina. Measurement per se may enhance the emotional stimuli themselves and confound the estimated results (Stewart et al. 2008b, Burfeind et al. 2010 and Naylor et al. 2012). Uddin et al. (2021) reported that measurements of rectal temperature in lactating dairy cows were not repeatable across three time periods, each separated by a month.

More advanced and less invasive data loggers can be used to overcome the frequent handling of animals (Small et al. 2008; Gloster et al. 2011; Kou et al. 2017). But uninterrupted data delivery requires insertion or implantation of loggers into the body, which may cause pain and distress to cattle. Insertion or removal of data loggers has a risk of infection, too. The cost of loggers and skilled labour required make them unsuitable to use in many commercial herds (Stewart et al. 2005; McCafferty et al. 2015). Thus, it is desirable to find a technique for screening groups of cattle with minimal or no restraint and labour use to identify cattle with negative emotional responses, in order to allow targeted interventions to maintain sound health, good welfare and a high level of productivity (Head et al. 2001).

## Infrared thermography as a measure of body temperature

Infrared cameras are now readily available that interpret non-visual radiation of external skin surfaces of animals or objects into contrasting colours or shades, each indicating different infrared temperatures (IRT) to form an image called a radiometric image (Speakman and Ward 1998; Montanholi et al. 2010; Proctor and Carder 2015). IRT measurement has the potential for future automation and has recently been investigated as a less invasive, remote and innovative alternative to the use of conventional thermometers (Stewart et al. 2007; Proctor and Carder 2016; Tamioso et al. 2017; Unruh et al. 2017; Macmillan et al. 2019).

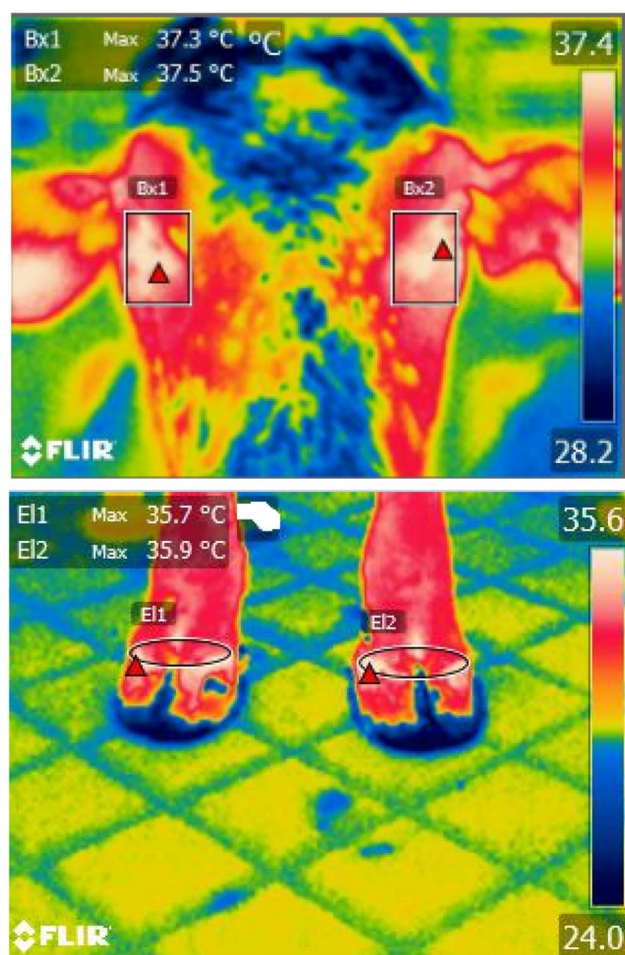
Uddin et al. (2019) investigated the key external body surface's IRT of lactating dairy cows in association with emotional responses and milk production, with the aim of capturing radiometric images without additional restraint during routine milking of a commercial herd. The radiometric image of cows' heads and forelimbs were reliably taken during milking in a herringbone milking parlour, using a handheld camera at a distance of one metre. Maximum IRT of external body surfaces was a more reliable measure of body temperature than minimum or average IRT or rectal temperature. In particular, the IRT measures appeared to be better predictors of heat stress than rectal temperature, perhaps because cows' metabolism is designed to maintain core body temperature or homeostasis. Other researchers have also found that maximum IRT of external body surfaces are better associated with emotional responses and productivity than minimum or average IRT (Cook et al. 2001; Metzner et al. 2014; Byrne et al. 2017, 2019). However, radiometric image analysing software calculates minimum and maximum IRT based on a single pixel, as the lowest and highest values, respectively, whilst average IRT is the mean value for all pixels within the defined zone of a radiometric image. Because ambient temperature is cooler than cows' body surface temperature, minimum and average IRT calculation are prone to variation due to positioning the frame of the radiometric image, as well as external factors such as water droplets from a sprinkler system or mud on lower forelimbs. Although using just one pixel is subject to be between pixel error, there may be high variance in average and minimum values because of variation in the proportion of the frame that includes hot body parts. This was supported by the statement of Byrne et al. (2019) and Uddin et al. (2020), who reported maximum IRT of external body surfaces had lower variances than that of minimum or average. Unlike average and minimum IRT, maximum temperature is not influenced by artifacts at ambient temperature that are within the frame, because they are at lower temperatures. Thus, it is recommended that maximum IRT of external body surfaces is more likely to be accurately measured than average or minimum temperatures and may be better associated with emotional responses.

Comparing body parts of cattle showed that the eyes' and coronary band of forelimbs' maximum IRT were most representative of body temperatures (rectal temperature) than muzzles' and ears' IRT (Uddin et al. 2019, 2021). Anatomically, the eye is closer to the brain hemispheres than the muzzle. Additionally, eyes have capillary beds in the small area around the medial posterior palpebral border of the lower eyelid and the lacrimal caruncle innervated by the sympathetic nervous system, which may actively respond to changes in the blood flow in the eye, by vessel from the infraorbital artery, and nervous control by the sympathetic system (facial nerve and auricular palpebral nerve) during

threatening situations (Stewart et al. 2007, 2009; McGreevy et al. 2012). Moreover, the eyes have retinal vessels similar to the important brain vessels, and choroid vessels and ciliary process which are similar to the small intestine and kidney. Similarly, the coronary band is a muscular structure actively involved in the movement of the animal and supporting the weight of the body and has profuse blood circulation to provide nutrition to the hooves (Cockcroft et al. 2000; Nikkhah et al. 2005; Gloster et al. 2011; Alsaad and Büscher 2012). Hence, it is not surprising that average eye and coronary band temperatures have been associated with right side laterality in a forced lateralisation test (Uddin et al. 2019, 2021), considered to be indicative of cows being more stressed by the presence of the person. The reviewed scientific literature suggests that muzzle and ear IRT have more limitations than those of eyes and coronary band of limbs (Eddy et al. 2001; Nikkhah et al. 2005; Bowers et al. 2009; Alsaad and Büscher 2012). For example, IRT of the muzzle region often has debris or excess moisture that could impede or exaggerate the reflectance spectra that the thermal camera detects. Similarly, frequent ear movements and the presence of ear tags meant that the targeted highly vascularised and hairless ear pinnae were easily missed. Moreover, in some designs of milking parlour cows' heads may be obscured by hardware in the dairy parlour while capturing radiometric images, obscuring the ears and muzzle (Uddin et al. 2019, 2021). The coronary band and eyes are more easily recorded in the five minutes or so available to the camera operator as the cow stood in the dairy to be milked. Also, the eye IRT is less confounded by hair than the muzzle and ear IRT (Schaefer et al. 2004). Nonetheless, the topographical region of the eyes is free from hair or fur, including a high density of near-surface blood vessels and arteriovenous anastomosis, which may control radiant energy through frequent changes in the volume of superficial venous blood: thermal window (Mota-Rojas et al. 2021b).

Direct sunlight may artificially elevate body surface temperature. Furthermore, heat stress has been observed to cause lateralised responses in maximum IRT of the eye (Church et al. 2014), with greater increases in the IRT of the left than right eye in response to direct sunlight on the measured eye. This might be expected because there is better connectivity of the left eye than the right eye with the right brain hemisphere, the predominant controller of the negative emotions (Robins and Phillips 2010; Phillips et al. 2015). Using data from both left and right sides appears advantageous; Uddin et al. (2019) found that maximum IRT of both eyes averaged and both limbs averaged were more closely associated with right laterality in a forced lateralisation test than IRT of left or right eye or limb alone (Fig. 1).

These best practice IRT measures—maximum IRT of both eyes and coronary band of forelimbs—are at least as



**Fig. 1** Front on view radiometric images of a cow's head (A) and lower forelimbs (B). Adapted from Uddin et al. (2021)

repeatable as the established measure of stress behaviour and flight speed (Uddin et al. 2021). By contrast, the most used measure of body temperature, rectal temperature, was not very repeatable, highlighting its weak reliability as a measure of stress responses. Scoley et al. (2018) reported that right eye IRT was more repeatable than the IRT of the anal area in calves. Recording of rectal temperature is influenced by depth of penetration of thermometer into the rectum, the presence of faeces and the length of time the thermometer is held against the rectal wall (Burfeind et al. 2010; Naylor et al. 2012).

### Sampling strategy for IRT

Uddin et al. (2020) reported a sampling strategy for the maximum IRT of both eyes; (left + right eye)/2 and both limbs; (left + right coronary band of forelimb)/2 of lactating dairy cows. This study removes confusion regarding the required number of radiometric images in a day or session,

the number of days and animals to estimate biologically relevant changes in the maximum IRT of the eyes and limbs. These are important to establish, if IRT is to be used widely in assessing the emotions of dairy cows. Uddin et al. (2020) suggest capturing two radiometric images of eyes and/or limbs for 14–16 cows in rapid succession, or three radiometric images on 10–12 cows, based on variance components of IRT measures (eyes and limbs) to calculate the least significant differences between two theoretical treatment groups under different sampling scenarios. Previously, researchers have evaluated an inconsistent number of radiometric images, experimental animals and days for the estimation of treatment effects, due to the lack of a precise sampling strategy. For example, Proctor and Carder investigated the effect of emotional change on the muzzle IRT of cows in two different years with 2 different sampling strategies. In 2015, they evaluated the radiometric images of the muzzle of 13 cows, captured in a day at 3 different time points (2 radiometric images at each time point). Then, in 2016, they evaluated the muzzle IRT of 22 cows by capturing radiometric images for 5 days. In another study, Gómez et al. (2018) evaluated the maximum eye IRT of 30 cows. They captured 3 radiometric images during and before claw trimming over 3 sessions. They found a 0.4 °C higher eye IRT of cows during claw trimming than at the start of claw trimming ( $P \leq 0.05$ ). By following the sampling strategy of Uddin et al. (2020), the same effect could have been determined by infrared thermography of approximately half the number of cows, or fewer sessions and replicates of the radiometric images in a session. This potentially reduces the stress to the cows and cost of the experiment.

### Association of IRT with emotional responses

The association of IRT of the eyes and the coronary band with emotional state has been tested in several studies with cattle. In one (Stewart et al. 2007), maximum IRT of the left eye was increased, from 37.8 °C 30 min before injection to 38.5 °C for up to an hour afterwards, following adrenocorticotrophic hormone (ACTH) administration, but not bovine corticotrophin-releasing hormone or social isolation. Their results indicate the possibility that not all types of exogenous HPA stimulation indicate or drive emotional responses similarly and that ACTH deserves further testing as an arbiter of emotional response. The relationship between IRT and lateralised movement in the forced lateralisation test was tested by Uddin et al. (2019, 2021). The earlier study found positive associations between right side laterality and both eye IRT and the IRT of the coronary band of the forelimbs; the right lateralised (R) cows had higher IRT at these sites than the left lateralised (L) cows. The later study did not observe such relationships, but the lactation stage was identified



as a potential confounder of responses. Alternatively, the much greater range of the cows' laterality in Uddin et al.'s 2019 study than their 2021 study may explain the absence of connections in the latter. Franchi et al. (2021) reported that eye IRT of Holstein–Frisian cows was increased when they were regrouped into a dry-off management paddock. The “dry-off” is the period between the regular lactation management period and the next calving which imposes stressors that include changes in diet, milking frequency, intramammary antimicrobial infusion, housing and herd mates (Zobel et al. 2015).

Uddin et al. (2021) detected positive associations between sniffing behaviour and the IRT of both eyes. Haley et al. (2000) has argued that an increase in sniffing behaviour in cows and heifers in tie-stall compared to loose housing is a negative emotional response to a lack of housing comfort or an inability to adapt. Kappel et al. (2017) suggested that sniffing is a hesitation behaviour in cattle, while Goma et al. (2018) suggested that it was related to anxiety. Further research is needed to correlate IRT responses to behaviour in cattle.

IRT responses could differ according to the type of emotional challenge. Contrary to expectations, Uddin et al. (2021) reported that the IRT of both eyes and rectal temperature were negatively associated with flight speed. During the acute stress induced by briefly holding a cow in a crush and then releasing it, blood flow might redirect from peripheral to internal body parts; thus, core body temperature increases, and external temperature decreased (Luzi et al. 2013; McManus et al. 2016; Godyń et al. 2019). In agreement with this, Stewart et al. (2008b) reported that eye IRT decreased after acute stress (disbudding of calves) for 5 min but increased thereafter. Thus, the evaluation of IRT in studies of emotional response should be accompanied by defined time points, to differentiate between acute and chronic responses.

Uddin et al. (2021) reported that eye IRT and THI was positively associated, whereas in this study, rectal temperature and THI were negatively associated. These findings are consistent with mechanisms of thermoregulation in mildly heat-stressed cows. At high ambient temperature and THI, cows release more heat through external body surfaces to maintain core body temperature within a stable range. This heat may be redirected due to altered blood circulation from the core body to external body surfaces, and it was identified as higher eye IRT but lower rectal temperature in that situation. These findings are consistent with those of Alsaaod and Büscher (2012) and Stokes et al. (2012), who reported that IRT measures were positively associated with ambient temperature. However, in more extreme heat stress conditions, positive correlations are observed between THI and rectal temperature, for example in lactating dairy cows by Kaufman et al. (2018). So, the

context of stress should be considered in the interpretation of the relationship between IRT measures and environmental stress responses.

In the studies of Uddin et al. (2019, 2021), negative associations between eye IRT and milk fat content were observed. An emotionally relaxed cow could produce milk of higher fat content, due to a more effective milk let-down, milk fat being retained in the alveoli of the udder, or it could reflect better forage digestion. Stressed cows are known to restrict their rumination activity, which helps to digest forages (Osei-Amponsah et al. 2020). Uddin et al.'s studies also detected that cows with high IRT had longer waiting times before milking. More relaxed cows wait longer before being milked (Varlyakov et al. 2011), allowing them to release more heat from their limbs or to be cooled by sprinklers. Limbs are an important source of heat loss in cattle (Van Den Heuvel et al. 2004). IRT could provide a practical and effective means of monitoring the extent of heat loss in response to cooling strategies.

## Confounders of IRT

Internal confounders for IRT use in cattle include emissivity of the skin, hair colour, unexpected movement and health status of animal, whilst external factors include ambient temperature, relative humidity, sunlight, wind speed, distance between camera lens and measuring objects (Idris et al. 2021). As identified by Uddin et al. (2021), stage of lactation, as indicated by the measure, days in milk (DIM), should also be accounted for in any future studies in cows. Others have similarly reported that cows with lower DIM had higher coronary band IRT (Nikkhah et al. 2005; Alsaaod and Büscher 2012). This could be explained by cows producing more metabolic heat in early lactation, being comparatively less habituated to the milking routine, resulting in a greater emotional sensitivity to their environment and potential threats, as well as being more likely to be in oestrus. Cows at a lower DIM would be expected to expend more energy grazing in order to consume more feed to fulfil the demand of high milk yield and a higher blood flow to accommodate that, therefore increasing metabolic rate and the need to lose metabolic heat. During movement, skeletal muscle metabolism increases, releasing heat (Taylor et al. 2014). More cows will be in oestrus in early compared to late lactation, which can increase physical activity and vaginal blood flow, LH surge and ovulation and the secretion of progesterone in the luteal phase, all of which contribute to the enhancement of body temperature (Lewis and Newman 1984; Redden et al. 1993; Talukder et al. 2014).



## Future perspectives of the use of infrared thermography for the dairy industry

The IRT shows potential relationships with emotional responses identified by their behaviours and milk fat percentage. A lower maximum IRT of the eyes and coronary band of forelimbs and a higher waiting time pre-milking show potential as predictors of the most relaxed cows and those most likely to produce the most milk fat (Uddin et al. 2019, 2021). These measures also have the potential to indicate the success of cooling strategies for a herd. Cows with higher waiting time (and a lower limb IRT) could have more oxytocin secretion and a more complete milk let-down than those cows who waited for less time (and had a higher limb IRT). Studies of cows' motivation are warranted as it is possible that cows passing rapidly through the parlour system are not only anxious but also more stressed by the close proximity of other cows in the collecting yard. Against that, potential confounders of the IRT response such as the amount of feed intake and its composition should also be accounted for as these would change the production of metabolic heat in the cow. Future studies should also focus on causal rather than associative methodologies. Discrepancies in the relationship between IRT measures and laterality of dairy cows between studies indicated further research was warranted on different subsets of cows: anxiety, stress extreme but oppositely lateralised, and randomly selected cows with comparisons made at similar THI, DIM and SCC are needed. It would be prudent to include facial expression and pupillometry responses of cows in any future studies. Finally, with the advancement of time the above emotional responses could be compared with electroencephalograms, a valuable measure of emotional state.

## Conclusions

Negative emotional responses in dairy cows are not only important to cow welfare but also productivity. Commonly used measures of emotions, be they behavioural or hormonal tests, are easily confounded due to the relative invasiveness of such tests. Contactless estimation of emotional responses in animals is required and IRT methodologies offer that opportunity due to the relationship between the IRT of selected external surfaces and physiological changes, that in turn drive changes in the distribution of blood and therefore temperatures between core and peripheral tissues, consistent with negative emotions. Key IRT measures show high repeatability. There is also scope for future improvements of IRT methodologies through the

development of software capable of the automatic extraction of IRT data from pictures and/or video clips that could also readily take into account potential confounders such as DIM. This review provides a proof of concept that strategic sampling of maximum IRT of a dairy cow's eyes and the coronary band of the forelimbs has the potential to predict and therefore better manage those most at risk of suffering from long-term negative emotions.

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**Data availability** None of the data was deposited in an official repository.

## Declarations

**Conflict of interest** The authors declare no conflicts of interest.

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