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Review Article

Non-Invasive Methods of Assessing Stress in Cattle

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Abstract

Important components of farm animal welfare are the assessment and alleviation of pain and stress during and following routine husbandry procedures used on farms (e.g., disbudding and castration of calves). There is now a higher level of public awareness and concern for animal welfare on-farm, and in recent years consumer demand has placed pressure on agricultural industries to provide 'welfare friendly' products that also meet high standards of food quality and safety. The assessment and management of stress in farm animals have been reviewed previously. A major issue for animal welfare research is that currently most of the techniques and methods we use to measure stress or pain are invasive (e.g., blood sampling) and there is a lack of non-invasive, reliable tools. This article provides a concise review of new technologies and tools to evaluate the welfare impact of different husbandry practices in order to improve animal welfare.

Keywords: Animal Welfare, Assessment, Cattle, Non-invasive, Stress

Introduction

Dairy animals particularly cattle hold first position in terms of their population in India. Cattle also holds a strong position for fulfilling daily needs of many marginal farmers. However due to farmers' view of getting more production from animals, they also suffer a lot. They are subjected to stress in terms of either behavioural or physiological means. Stress has been defined as "a state that occurs when an animal makes extreme or abnormal adjustments in its behaviour or physiology in order to cope with adverse aspects of its environment and management" (Fraser *et al*., 1975). Due to the shift toward automation and the declining number of experienced stock people entering the industry, along with increasing herd sizes, automated remote monitoring of health and welfare on-farm is needed. Animals have an extensive range of requirements which can be explained as deficiency in an animal that can be fulfilled by obtaining a particular resource or responding to a particular environmental or physical stimulus (Broom, 1991). Currently most of the methods we use to measure stress or pain are invasive (e.g., blood sampling) and there is a lack of reliable, non-invasive tools (Stewart *et al*., 2005).

Explained below are some of such non-invasive methods which can be used for assessing stress in farm animals mainly cattle.

Animal Behaviour as Stress Indicator

Behaviour is classified as the most reliable animal-based indicator as its expression comes from the animal itself and indicates a measure in which it is adapted to the environment (Ostojić *et al*., 2019). Also, man-animal relationships during handling can have major impact on both production and welfare of animals (Munksgaard *et al*., 2005). Several methods of assessing animal welfare have been developed and used on farm, such as the Animal Needs Index in Germany and Austria, the Bristol Welfare Assurance Programme in the UK, and the current gold-standard in Europe. However, such assessments were formulated and designed to evaluate housed dairy cows and thus do not mark the unique challenges of pasture-based systems (Crossley, 2018). Temperament, flight zones, visual fields and vocalization scoring are the commonly used behavioural measurement methods which can be used to measure stress (Biswal and Somagond, 2020). Morbidity and mortality in beef cattle are important events with relevant economic and welfare implications; however, behavioural changes could be useful in the early prediction of diseases in beef cattle (Belaid *et al*., 2019). Assemblage of these measurements is effortless and economical such that marginal and resource-poor farmers if trained appropriately can generate accurate on farm welfare audits stressing on animal behaviour.

The Hair Cortisol Concentration (HCC)

It is assumed to be a retrospective marker of integrated cortisol secretion and stress over longer periods of time. Its quantification is increasingly used in psycho-neuroendocrinological studies in animal stress and welfare research. As an indicator for chronic stress or long-term activity of the hypothalamic-pituitary-adrenal axis, the measurement of HCCs from tail of Holstein cows (Cerri *et al.*, 2012) for the assessment of stress offers many considerable benefits for use, especially due to the easy and minimally invasive sampling procedure. There exists a wide array of adverse pathological conditions and stressors that alters the cortisol concentrations in hair and that HCC thereby provides an accurate, reliable and valid reflection of long-term cortisol secretion in many species (Heimbürge *et al*., 2019)

Measurement of Respiration Rate (RR) as Stress Indicator

RR can be affected by stress and heat exposure, pain (Stewart *et al*., 2013), and disease (Gershwin *et al*., 2015); therefore, changes in RR can be a useful indicator of these physiological states. One method for measuring RR in dairy cows is to record the number of flank movements as the animal inhales and exhales. Although to assess heat stress in cows, this method has been used reliably (Schütz *et al*., 2014), but the disadvantages lie in that it is very labor-intensive, and flank movements can be difficult to observe with shallow breathing (e.g., during disease onset) or in colder climates. Alternative methods for RR measurement include the use of thermistors (Milan *et al.*, 2016), infrared lasers, strain gauge or pressure transducers (Strutzke *et al.*, 2019), thoracic belts and spirometry masks (Maia *et al.*, 2014). Thermistors and spirometry masks need to be fitted to individual morphologies. However, these methods are limited by battery life when battery powered. Typically, sensors are used in remote devices that measure chest-wall movements (e.g., strain gauges or pressure transducers). A RR monitoring system has also been developed for cattle in which a thoracic belt was used to keep the transducer in place (Eigenberg *et al*., 2000).

This approach is impractical and susceptible to displacement and damage. The animal itself or other animals may try to move the belt resulting in sensor damage or signal interference (Milan *et al.,* 2016). Also, there are changes in normal behavioural patterns because of having such bulky equipment fitted to the animal that could increase stress, affecting the accuracy of the results.

In addition, a laser distance sensor was developed by Pastell et al. (2007) to measure RR during milking. The sensor measured the movement at the side of the abdominis lateralis region of the cow; however, the drawback is that device is not mobile and continuous measurement is impossible along with interference in RR measurement due to stress during milking. Infrared lasers, a non-invasive method for measuring RR were also used but the laser was absorbed by the animal's dark hair. This imposes a major limitation in that it could not be used on black cows (Pastel *et al.*, 2006).

Infrared Thermography (IRT)

Infrared thermography (IRT) is another non-invasive method that has been corroborated for the measurement of RR in adult dairy cattle (Stewart *et al.,* 2017). Infrared thermography (IRT) is the computation 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 shortest wavelengths are seen in photons with the highest energy. In the electromagnetic spectrum, broad range infrared radiation wavelengths (3-12 micrometers) are longer than visible light and in animals, 40-60% of heat loss is within this range. Small changes in temperature may result in substantial amounts of emitted photons (or radiated energy) that can be detected very sensitively using IRT.

When an animal becomes stressed, the HPA axis is activated. As a result of increases in catecholamines and cortisol levels as well as blood flow responses, will generate changes in heat production and loss from the animal (Schaefer *et al.,* 2002). This can be detected using a specialized infrared camera to collect real-time pictorial images at a distance from the subject, 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. This technology can detect thermal changes before clinical signs occur in the onset of many diseases like bovine viral diarrhea, bovine respiratory disease, mastitis, stress, pain and neonatal calf diarrhea in cattle. The diseases mentioned above are all associated with localized inflammatory response (e.g., respiratory tract, intestines), and during their onset, animals use other mechanisms for heat loss (e.g., radiated heat) to maintain a normal core body temperature.

Limitations in using Infrared Thermography (IRT)

Images must be collected out of wind drafts and direct sunlight, 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. The effects of weather conditions, circadian and ultradian rhythms, time following feeding, milking, lying and ruminating are also factors that need to be considered and require further investigation as part of validating IRT as a stress measurement tool.

However, by combining RR monitoring with IRT, it could be possible to detect other conditions that may not cause an inflammatory response, such as metabolic diseases (e.g., ketosis, rumen acidosis, bloat) or stress and discomfort.

Accelerometers

These small devices are attached to the hind leg or neck collar and are likely to be less invasive for the animal and less susceptible to damage or displacement than other devices, such as thoracic belts. If other valuable animal information indicative of pain or discomfort due to lameness, mastitis, or other disease onset could be collected from these devices, it would be beneficial for the farmer (Stewart *et al.,* 2017).

Heart Rate Variability as Non-Invasive Measures of Autonomic Nervous System Activity

Measurement of heart rate variability (HRV) allows more detailed interpretation of cardiac activity in terms of the autonomic nervous system. It has therefore been suggested that HRV is a more accurate measure of stress in animals

(Porges, 1995).

Measurement of Glucocorticoid Excretion in Faeces, Urine, Milk and Saliva

Indicators of HPA activity can be measured in faeces, urine, milk and saliva thus avoiding blood sampling procedures. These indicators require minimal contact with the animal, the ideal being collection of faeces in a paddock or housing system where no restraint or sampling is required and there is no interference with the stress levels of subsequent samples. There is a significant increase in faecal glucocorticoid excretion in dairy cows following administration of adrenocorticotropic hormone (ACTH), exposure to a novel environment or transport, suggesting that glucocorticoid metabolites measured in faeces can reliably indicate acute adrenal activity in dairy cattle (Morrow *et al*., 2002). Delay times of faecal excretion need to be taken into account in the interpretation of stress responses that occurred at a certain time before the sampling period. This delay (approximately 10-12 hours 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.

In ruminants, both faecal glucocorticoids (Morrow *et al.,* 2002; Möstl *et al.,* 2002; Saco *et al.,* 2008) and salivary cortisol (Cook and Jacobson, 1995; Cooper *et al.,* 1989; Fell and Shutt, 1988) have been proven to accurately reflect adrenocortical responses to stressors. However, the predictive and explanatory value of these techniques with regard to stress assessment is limited to short periods of time, and final result may be interfered by some factors, such as the circadian rhythm of peripheral cortisol (Lefcourt *et al.,* 1993) or the stress induced by animal handling to collect the sample (Moberg and Mench, 2000).

Conclusion

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, there has been development of non-invasive or minimally invasive systems for measuring stress, but these 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.

Conflict of Interests

There is no conflict of interest.

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