

*Review Article***Assessment of Welfare through Behavioural, Physiological and Biochemical Measures in Dairy Animals: A Review****Prachurya Biswal and Arun Somagond***Livestock Production and Management Section, Indian Veterinary Research Institute, Bareilly,
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Abstract

Welfare is the adjustment of individuals to the surroundings and it is the state of wellbeing of an animal in the given environment. Its failure leads to sufferings, pain and poor welfare. Generally, the indicators of poor welfare in farm animals are lessened growth, impaired reproductive function, body injury, sickness, immunocompromisation, adrenal activity, behavioural anomalies and self-narcotization which leads to economic losses to the farm, farmer and country. Welfare can be measured in many ways and corrective measures can be taken to improve the poor welfare conditions and eliminate the sufferings of animals to provide them a better and healthy life. The commonly used behavioural measurement methods include temperament, flight zones, visual fields, vocalization scoring, analysing biochemical parameters, physiological parameters etc. If farmers are properly trained it is easy and cheap to compile these measurements so that resource-poor farmers can produce accurate audits on farm welfare. For achieving better production and economics, welfare of animals should be assessed in order to maintain their wellbeing. More research should be done for assessing welfare by modern methods so that animal rights will be protected as well as welfare will be maintained.

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The term “welfare” can be referred as the circumstances of an individual in relation to its surroundings which can be measured. Failure to manage with the surroundings and intricacy in adjusting are indicators of poor welfare. Sufferings and poor welfare often take place together, but welfare can be poor without suffering. Diminished life expectancy, lessened growth, impaired reproductive function, body injury, sickness, immunocompromisation, adrenal activity, behaviour anomalies, and self-narcotization are considered as the indicators of poor welfare. Usually welfare represents the mental and physical health of

an animal in relation to its environment (Smith and Pearson, 2005). Animal welfare also requires applying prudent and responsive animal husbandry methods to the farm's livestock. Good animal welfare has a positive effect on production (Ndou *et al.*, 2011) as well as good practices of animal welfare are underpinned by the framework provided in the five familiar freedoms that illustrate an animal's elementary requirements (Bech *et al.*, 2008; Vessier *et al.*, 2008). Animal management practices should aim at keeping animals free from thirst, hunger and malnutrition, discomfort, pain, injury and disease, fear and distress, and should also be able to engage in the normal pattern of animal behaviour (Bech *et al.*, 2008). The major constraints experienced by the farmers to follow proper calf welfare practices were lack of money, lack of knowledge and veterinary doctors in vicinity (Singh *et al.*, 2017). Non-fulfilment of these needs may expose the animal to stressors with detrimental effects on animal welfare. Prolonged exposure to stressors disrupts energy mobilization and reactions involved in stress response, thus affecting the normal body functions, immunity, growth, reproduction and expression of normal behaviour (West *et al.*, 2003). Hence, welfare needs to be measured for providing better living conditions, health, wellbeing to the animals and keeping these things in view this article emphasizes on measuring animal welfare by behavioural, physiological and last not the least by biochemical measures.

Animal Welfare: A Prospect to Peep in

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). However, there is a distinction between animal welfare and animal rights. Welfare reflects people's apprehension with the well-being of animals. Animal rights goes further arguing that animals have basic rights like to be liberated from imprisonment, suffering, barbarism, experiments perhaps even death. The paramount estimation of biological robustness is lifetime reproductive success of an animal. Impaired reproductive success is indicated by postponed onset of reproduction during development, extended intervals between consecutive breeding, reduced litter size, and early death. Such measures can be used directly for wild animals, but where breeding opportunities are under humans control these measures can be used to identify poor welfare situations in which reproduction seems unfeasible. Measures of body damage are visibly relevant to welfare assessment such as broken bones or wounds can be recognized and their frequency can be assessed. It has been reported that 27% of hens from battery cages have at least one bone broken between removal from the cage and stunning (Gregory, 1998). The major cause of this is that hens in battery cages have a modest exercise and as a consequence, their bone vitality is less than that of hens kept in surroundings in which more exercise is possible (Knowles and Broom, 1990). According to Alam *et al.* (2019) buffalo and cattle at slaughterhouses experience significant welfare compromises due to occurrences of injury, dehydration and stressful pre-slaughter casting. Further he

suggested that welfare improvements could be made by training for effective halal slaughter, and future welfare improvements could be gained by improved facility infrastructure for restraint and halal approved pre-slaughter stunning. Rahman (2018) has reported that cattle and goat were affected by a number of serious injuries and illness during handling and transportation which clearly indicate the poor condition of animal welfare in selected cattle market of Bangladesh. For this reason, greater attention needs to be paid during handling and transportation of livestock for better welfare.

Measuring Animal Behaviour to Know Animal Welfare

Alterations in animal behaviour are the most apparent indicators that the animal is having challenges in adjusting with the welfare measures and in most cases, some feature of the situation is aversive (Broom, 2003). 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 (Ostojic *et al.*, 2019). During the pre-slaughter period, animals recognize specific features through previous experience and learning (Gregory, 2008) and will subsequently react through appropriate behavioural responses that influence meat eating quality (Muchenje *et al.*, 2009). Although evidence exists that man-animal relationships during handling can have major impact on both production and welfare of animals (Munksgaard *et al.*, 2005), information on the behaviour of cattle during road transport is scarce, but it is important in indicating possible modifications to improve handling facilities (Tarrant and Grandin, 2000) and how animals acclimatize under extensive management conditions. 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 designed to evaluate housed dairy cows and thus do not address the unique challenges of pasture-based systems (Crossley, 2018). The commonly used behavioural measurement methods include temperament, flight zones, visual fields and vocalization scoring. According to Belaid *et al.* (2019) 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. Assemblage of these measurements is effortless and economical such that resource-poor farmers if trained appropriately can generate accurate on farm welfare audits.

Temperament as Welfare Indicator

Temperament refers to an animal's behavioural expressions in response to adverse situations such as human handling, the presence of novel objects, darkness or any changes in normal environment (Ferguson and Warner, 2008). Temperament and stress are strongly associated to cattle behaviour and can be used in a variety of aspects to calculate the subsequent experience of the animal and predicting most favorable welfare techniques to diminish meat defects in beef animals. Temperament can be evaluated by using crush

scores 1 (calm) to 5 (combative) scales (Campo *et al.*, 2008). Although, adaptation of animals to handling procedures makes them comfortable to handle during the preslaughter period (Gregory, 2008), certain breeds such as *Bos taurus* have calmer temperaments as compared to the *B. indicus* cattle (Fordyce and Goddard, 1984). Research on the Limousin, Red Bororo and Brahman cattle shows that *B. indicus* animals are hard to handle (Minka and Ayo, 2007) and their meat tends to be characterized by dark cuttings and toughness due to stress hormone cortisol secreted during handling and transportation. Behrends *et al.* (2009) reported that the reaction of cattle to novel objects in early life are best indicators of traits impacted by temperaments later in life such as beef tenderness. Submissive cattle are connected with greater average daily gain (ADG) compared to those agitated during routine handling (Voisinet *et al.*, 1997) and this signifies that rearing of docile cattle has positive economic implications. Generally, animals that are easily stressed are difficult to handle. Crump *et al.* (2019) indicated that pasture provides a comfortable surface and reduces competition for lying space. Furthermore, cows at pasture walked farther, with potential benefits for their physical health and psychological wellbeing. Genetic variations in temperament among and within breeds are evident. Heritability estimates as high as 61% have been reported (Gauly *et al.*, 2001) and selection pressure can be exerted in breeding programmes to improve temperament by selection. There is a need to identify and evaluate traits which are correlated with temperament, such as facial hair whorl position in beef animals to aid genetic characterization and improvement (Lanier *et al.*, 2001). The growing interest in the applied ethology of cattle in recent decades may significantly increase our knowledge of the behaviour traits of this species, which can also be applied in practice. The development and improvement of methods for the assessment of cattle behaviour facilitates the interpretation of behavioural responses observed in animals, which makes an easier and safer human interaction with them (Budzyńska *et al.* 2019).

Observation of animal behaviour may also facilitate detection of diseases. The methods of assessing temperamental traits become particularly important when such traits are used as selection indicators

Cognition as Behavioural Measure

Social isolation early in life can impair cognition in rodents and other species (Jones *et al.* 1988; Fone and Porkess, 2008). Socially isolated rodents showed deficits in reversal learning, a method often used to assess behavioural flexibility in animals (Fone and Porkess, 2008). A recent study examined reversal learning in pair-housed and individually housed calves and found that individually housed calves made more mistakes during the reversal-learning phase, indicating impaired behavioural flexibility (Gaillard *et al.*, 2014). A follow-up study, using a colour discrimination training task, showed that calves housed with social companions from an early age, either in a complex social environment (with the presence of their dam and other cows and calves) or simply pair-housed, performed better in reversal learning than did individually

raised calves (Meagher *et al.*, 2015). In rodents, it is well established that the prefrontal cortex is responsible for behavioural control, decision-making and inhibition of behaviour (Dalley *et al.*, 2004). These controls are essential for success in reversal learning. We can say that calves raised in isolation exhibit deficient social skills, difficulties in coping with novel situations and poor learning abilities, all of which may reduce the animal's ability to adjust to variable environments on the dairy farm. Neville *et al.* (2019) reported that by characterising the cognitive process we can get a better insight into indicators of welfare such as judgement bias moreover by using this approach a precise measure of welfare can be obtained which will provide better estimate of poor or improved husbandry.

Knowing Flight Zones and Visual Fields

The flight zone is the area around the animal when a person or any source of danger enters into it, the animal will maintain it or move away (Grandin, 2007). The visual field is the area of the external environment that is observable to the animal at any set position without moving the eyes or turning the head. The flight zone and visual fields label the animal's private space such that when a person or any source of danger enters into it, the animals will move away. The size will slowly reduce with avoidance of the visual fields by stockman, tameness of the animal or when animal previously received frequent and gentle handling (Campo *et al.*, 2008). Knowledge of flight zones and their association with cattle visual fields, particularly during the pre-slaughter time, is more useful in preventing stress than tameness and animal experience. Application of cattle flight zones and visual fields are more helpful in replacing conventional methods engaged in developing countries, such as using goads, sticks, dogs and making violent threats or wilful behaviours to move the animals in a desired direction as well as shouting to the animals during traditional ceremonies (Mnguni, 2006; Gregory, 2007). The manner in which animals escape can be used to identify the evasiveness of the practice, temperament of the animals and lenience of subsequent handling as well as the welfare through evaluation of flight speed (FS) and flight time (FT) (Campo *et al.*, 2008). According to Campo *et al.* (2008) flight speed is the rate of change of distance covered per unit time by an animal as it maintains its flight zone and it can be generalized through ranking scale such as 1, Walked; 2, trotted; 3, ran. Petherick *et al.* (2002) reported that animals having faster and slower FS possess "poorer" temperament and "good" temperament, respectively. Moreover, high FS indicates the brutality of the welfare method used in handling the animal. Flight time refers to the time period it takes an animal to cover a fixed distance (5 m) after release from a restraining device (Campo *et al.*, 2008) or when maintaining flight distance, prolonged durations indicate aggressiveness of welfare procedure.

Sound Analysis in Dairy Cattle by Measuring Vocalisation

Automatic animal monitoring can support the farmer in achieving and maintaining farm sustainability (Banhazi *et al.*, 2012). Precision Livestock Farming (PLF) provides a tool to support the

farmer in managing livestock when farmer-cattle interactions are decreased. PLF can combine continuously measured information with automated software tools, which can be used to control, monitor and model the health and behaviour of animals and their biological responses such as milk yield (Tullo *et al.*, 2013). Automated software tools can detect behavioural changes early, which may lead to early intervention, possibly reducing veterinary costs in case of disease (Banhazi and Black, 2009). Monitoring by PLF can be based upon variables such as weight, activity or vocalisation. The analysis of vocalisations is a promising tool for the interpretation of behaviour, health state and well-being of animals (Manteuffel *et al.*, 2004). Further development of vocal analysis may provide a low-cost tool for livestock management. Vocal analysis is well studied in pigs. It has been shown that the stress call from pigs is a rather sustained cry with high frequency bands. The relation of vocalisation to stress has been verified in various experiments where the general situations, aversive behaviour, stress hormones and brain activity were recorded parallel to the acoustic utterance (Otten *et al.*, 2001; Tuchscherer *et al.*, 2002). The association of distinct vocalisations with specific behaviour can give the possibility to recognize the state of individual animal or the whole herd by using sound analysis. Vandermeulen and co-workers found 80% correlation between video and sound analysis recorded in a piggery (Vandermeulen *et al.*, 2013). Meen *et al.* (2015) reported that the potential of applying sound analysis in dairy farms. The mean maximum frequency (Hz) of calls related to lying & ruminating behaviour, which is an indicator for good welfare, was significantly lower than that of calls uttered during other behaviours. The sound that cattle produce during lying & ruminating can be described as 'murmuring'; a low frequency (Hz) call or a low humming sound. High productive cattle should spend 7–10 h ruminating each day (Grant *et al.*, 2000). Munksgaard *et al.* (2005) found that cattle prefer to have fixed numbers of hours lying and ruminating in their routine. These findings suggest lying time and ruminating time are important factors in the daily routine of cattle. As murmuring is uttered when cattle are lying & ruminating, it might be worthwhile to further investigate the detection of murmuring, in order to use this as a welfare monitor in the future. Detection of murmuring can be applied in the same way as the pig cough monitor (Vandermeulen *et al.*, 2013), where microphones detect only vocalisations with the same frequencies as murmuring. Variation between different days may indicate an increase or decrease in cattle welfare. In all these cases, a cow was standing partly inside a cubicle, vocalising while standing still and looking around. Standing idle is one of the behaviours in the 'stress related behaviour' group, since standing idle is an indicator of decreased welfare.

In contrast to heifers, dairy cattle did have compost bedding in their cubicles, which may explain why dairy cattle stood relatively less idle. These findings are supported Cook *et al.* (2008) that showed cattle stood idle for a longer time when cubicles were not bedded. This finding may also explain the low number of times murmuring was detected, since the cattle did not lie and ruminate as much as they needed. Adult dairy cattle produced calls with a significant lower mean maximum frequency (Hz) than heifers. This difference

in frequency (Hz) of calls between young and adult individuals is common and is explained by the growth of the larynx in maturing mammals (Reece, 2004). There might also be individual variance in frequency (Hz) of calls. It might be possible that cattle from different breeds have developed a unique kind of vocalisation (Watts and Stookey, 2000).

Stereotypes as Welfare Indicators

A stereotypy is a repeated; relatively invariant sequence of movements that has no obvious purpose, and the occurrence and causation of stereotypes has been the subject of much discussion (Broom, 1981; Mason, 1991). Stereotypes are shown in situations in which the individual lacks control of its environment, especially in those that are obviously frustrating, threatening, or severely lacking in stimulation. Examples includes rocking movements and repetitive vocalizations in institutionalized humans, bar-chewing installed sows, head-swinging in zoo-housed bears and elephants (Dittrich,1984), pacing in captive fennec foxes, eye-rolling in veal calves, (Fraser & Broom, 1990) and jumping in caged bank voles. Whatever their causation, stereotypes are shown in situations that are difficult, sometimes extremely difficult, for the animal, and so they indicate that the welfare of the animal is poor. Much of stereotypic behaviour suggests poorer health than an occasional stereotypical behaviour. In humans we start to recognize a problem if the action lasts for a minute. All these environments may involve chronic conflict and frustration and hence stress, particularly if uncontrollable or unpredictable (Herbert, 1987). Thus, the acquisition of stereotypes indicates an environment that is probably inadequate, and as such, presumably aversive. It is certainly possible that current performance can also indicate current suffering, but not all agree with it.

Physiological Evaluation to Monitor Animal Welfare

Sometimes quantitative and behavioural measurements are inadequate to ascertain the welfare of animals in most cases and physiological or biochemical methods should be used to validate overall response (Broom and Fraser, 2007). Campo *et al.* (2008) suggested that, steers subjected to a high energy composition diet performed better than those on low energy diets, but showed highest levels of acute phase proteins and accurate indicators of stress. Physiological parameters demonstrate aversiveness of welfare because impulse results in the activation of the hypothalamo-adenohypophyseal- adrenocortical axis due to stimulation of the parasympathetic or sympathetic nervous system, which leads to changes in heart rate, respiratory rate, temperature, blood metabolites, electrolytes and hormonal levels and subsequently the quality of the meat. The normal heart rate for resting animals is 76.5 beats per minute. Elridge *et al.* (1988) showed that, the heart rates of cattle during transportation were 15% above those recorded, while the animals were grazing at pastures. Confinement of animals into the vehicle therefore, had direct effects on the physiological status of the cattle, so there is need for resting the animals after transportation so that they

return to their basal levels during the pre-slaughter period. Generally, in case of cattle, heart rate can be used for the estimation of advancement or wellbeing of animals particularly during situations like intra-specific grooming of young cattle with constant invasion of its flight zones or during transportation. They can also be used to assess animal welfare in animals that are used for draught power (Dube *et al.*, 2001). Temperature and respiratory rate can be recorded during handling or transportation directly through measurements of rectal temperature and by direct observation, respectively. Meat quality is associated with the rate of glycolysis and temperature both in the ante and post mortem conditions. For example, Mounier *et al.* (2006) revealed that, body temperature above normal for bulls on arrival of the truck at the slaughterhouse was associated with greater pH of the longissimus muscle. Since climatic conditions of most developing countries are characterized by hot sub-tropics, temperature of the animals has to be monitored as a welfare measure, roofed vehicles should be used and transhumance during hot weathers should be avoided at all costs.

Biochemical Measures to Monitor Animal Welfare

In recent time, there has been increasing awareness concern about animal welfare in the field of behavioural and welfare science. However, the measurement of animal well-being is a complex matter (Rushen *et al.*, 2012). According to studies environmental stimuli that lead to an imbalance of homeostasis as 'stressors' and the consequent defence reactions of an animal as 'stress responses' with the brain having the central role in linking stressors to responses (Mostl & Palme, 2002). Responses involve behavioural changes, changes to the immune system, activation of the neuroendocrine system (hypothalamic-pituitary-adrenal axis) and the autonomous nervous system (ANS) Moberg (2000). The variety and complication of changes can differ markedly between species, individuals and stressors and can vary according to prior experience and stage of life history (Cook *et al.*, 2000; Sheriff *et al.*, 2011). It is important to note that stress responses are not innately bad as they help an organism to survive with its environment and challenging situations. However, if activated too much or for too long some may have unfavourable effects on the organism, ensuing in impaired biological functions e.g. reproduction, immunity and growth (Moberg, 2000). Pallab (2019) has reported that calves show higher plasma creatine kinase and lactate due to muscle fatigue during transportation.

Non-invasive techniques for monitoring glucocorticoid metabolites in faecal samples are a useful implement for welfare assessment in various species, especially as they are easily applied at farm or group level. Biochemical properties have normal basal levels which fluctuate with differences in the severity of the welfare procedure such that any deviations from normal basal levels indicate that some aspect of the situation is aversive. To optimize the welfare of animals, it is necessary to determine the physiological response in relation to the biochemical changes and products that affect the meat-eating quality especially

with extensively kept animals (Muchenje *et al.*, 2009; Ndlovu *et al.*, 2009). Measurement of metabolites, such as acute phase proteins, hormonal concentrations, blood glucose levels, non-esterified fatty acids (NEFA), urea, meat pH and glycogen concentrations can be used to monitor beef cattle health and welfare status both on the farm and at slaughter (Eckersall, 2000; Chimonyo *et al.*, 2002; Campo *et al.*, 2008). Although, biochemical assessments are more expensive and difficult to apply in resource limited conditions, they are more accurate indicators of animal welfare than behavioral assessments. Hormones such as cortisol, adrenaline, creatine kinase, dehydrogenase, prolactin, beta-endorphin and glucocorticoid are good indicators of acute stress experienced by animals. These hormones increase substantially when cattle are exposed to various welfare procedures, such as being handled, castrated, feeding, regrouped, transported and receiving veterinary attention (Corkum *et al.*, 1994; Boe and Faerevik, 2003; Muchenje *et al.*, 2009). Quispe *et al.* (2019) has reported that there is a significant correlation of medium to low between the behavioural indicators and the physicochemical properties of the beef cattle. Fluctuations of hormonal levels are important indicators of the activity of the parasympathetic and sympathetic nervous systems, because they result from neuronal washout of tissues as the animal tries to cope or respond.

Conclusion

Recently, many in the dairy industry may have assumed that animal welfare concerns could be met by working to ensure good health and productivity for the cows and calves in their care. For achieving better production and economics, welfare of animals should be assessed in order to maintain their wellbeing. As animals are considered as sentient beings any change in their normal environment can be expressed by their behaviours. Welfare policy is not only about satisfying needs but can go beyond to allow welfare state which are valued by animals. Welfare can be assessed by measuring their behavioural activities, cognition, temperament, emotions, physiological changes, biochemical changes etc. Recording of vocalization can indicate various stages of animal sufferings and wellbeing which can help in modifying the microclimate. When animal's minimum requirements are not fulfilled, they show abnormal behaviour which indicates their miserable welfare condition. More research should be done for assessing welfare by modern methods so that animal rights will be protected as well as welfare will be maintained.

Compliance with Ethical Standards

Conflict of Interest: The authors have no conflict of interest.

Ethical Review: This review does not involve any human or animal testing.

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