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These articles are organized into three topics: articles on how to identify and measure stress behaviours, transportation-specific articles and articles focused on the human interaction aspect. Within the categories, the articles are organized by the number of times each one has been cited, as a measure of their significance to subsequent research.

## **Identifying and Measuring Stress Behaviours:**

Boissy, A., & Bouissou, M. F. (1995). Assessment of individual differences in behavioural reactions of heifers exposed to various fear-eliciting situations. *Applied Animal Behaviour Science*, 46(1–2), 17–31. https://doi.org/10.1016/0168-1591(95)00633-8

**Summary:** This article investigated fear responses in cattle when exposed to various stressful conditions. Boissy and Bouissou (1995) aimed to identify and record these responses as previous research in this field was very limited in scope. This was accomplished with fear tests to explore the range of reactions that individual cattle express. Fourteen Friesian heifers, all raised together, were tested individually over an eight-day period. Four fear-eliciting trials were chosen and conducted in a closed room that had a remotely controlled gate. Observations were taken from behind one-way glass.

The first trial was an open field test where cattle were separated from the herd and exposed to a poor environment. The second was exposure to a novel object that was previously unfamiliar to the cattle. The third was a conflict test where the cattle were offered familiar food in an unfamiliar setting. The fourth was a surprise test which was set up the same as the conflict test but this time with a gust of air that would shoot out of the bucket 30 seconds after the cow started to eat. The behaviours in the open field test were compared to each of the subsequent tests to identify fearful behaviour.

It was determined that behaviours indicating significant signs of fear included hesitation to approach or engage in a scenario, immobilization and keeping the head upright. Lack of fear included vocalizations and investigating or engaging in a scenario. This study found that heifers had individual inclinations towards a level of reactivity across all situations. The researchers then made connections to the physiological side of fear behaviour, which was an area they highlighted for future research as more information is required to confirm and expand upon their findings.

**Contribution:** This article laid some of the critical foundation documenting fear responses in cattle and has since been cited hundreds of times. The research in this article significantly advanced our understanding of what constitutes fear in cattle and began the daunting task of connecting the variables to check for consistency in the response of individuals. The connection between the physiological and physical components of behaviour has been critical to the field of animal behaviour. This also provided the framework for future research to repeat these experiments and test the findings among other breeds of cattle.

Chen, Y., Arsenault, R., Napper, S., & Griebel, P. (2015). Models and methods to investigate acute stress responses in cattle. *Animals*, 5(4), 1268–1295. https://doi.org/10.3390/ani5040411

**Summary:** Measuring stress in cattle is a difficult undertaking as there is no definitive sign of it and even the definition has continued to evolve over time. Chen et al. (2015) delved into processes on a cellular level, examining the hormonal aspects of stress to compile existing information that can assist in quantifying stress in cattle. The results of stress research have demonstrated the negative effects on the health, well-being and productivity of cattle, yet there are many aspects of stress that have not yet been thoroughly researched. Stress-related hormones, like cortisol and vasopressin, are released by the hypothalamus and the regulation of the response has been linked to two signaling axes or, chains of interactions that utilize various parts of the central nervous system. The stress-inducing situations discussed in this review included transportation, food deprivation, temperature fluctuations, the weaning process, restraint and social situations including isolation.

In order to measure stress, experiments have used observations of entry order, pulling, speed, vocalization, reluctance and utilized scores to grade behaviour in various situations. The specific effects of stress are elusive and unpredictable, with the response to a given situation having a wide variety of possible behaviours displayed, making it difficult to predict. Behaviours indicating stress, included weight loss, increased susceptibility to diseases, increased heart rate, decreased milk production and changes in hormone and protein levels. It is very difficult to isolate sources of stress in cattle, as a single stressor can be a complex mix of stimuli that all affect the cattle in different ways. Evidence also suggests that stressors in different combinations can produce altered stress responses. More research is needed to explore the effects of cold stress and future research could also investigate the effects of stress on gut flora.

**Contribution:** This article is critically important as it delves into the hormones and brain chemistry behind stress responses in cattle, relating the physical to the physiological. Understanding how stress has been measured in the past is also a great way to generate questions that could lead to improved experimental designs. As a comparison, it also shows how far the research has come since the experiment by Boissy and Bouissou (1995) and has a much more technical focus.

Grignard, L., Boivin, X., Boissy, A., & Le Neindre, P. (2001). Do beef cattle react consistently to different handling situations? *Applied Animal Behaviour Science*, 71(4), 263–276. https://doi.org/10.1016/S0168-1591(00)00187-8

**Summary:** In this article, Grignard et al. (2001) studied whether a cow will react the same in different situations and investigated whether there is a genetic component to reactivity. It has been established that socializing animals while they are young can help with acclimatization to humans, resulting in a less reactive animal. A genetic element has been proposed, however, previous experiments didn't account for the variation in experimental methods, so conclusions about genetic connections required many assumptions to be made.

This study observed 245 Limousine heifers from ten different sires. The heifers were raised together for a month before the first test, for docility, was conducted on the then nine-month-old cattle. This test consisted of one cow in a pen with a human who attempted to contain the animal in a corner for 30 seconds and, if successful, then stroke the animal. The second test, a crush test, conducted at one year of age, tested each cow in a squeeze chute with or without a human who was or was not touching the animal. The researchers then recorded heart rate, movement of the legs, head and tail as well as vocalization, the release of excrement or urine and, regarding the human, sniffing and licking.

The results found that cattle from the same sire had similar levels of reactivity during the tests, which supported the existence of a genetic component of cattle behaviour. The presence or absence of people also had a significant effect on the individual reactions of the cattle. The effects of selecting for docility on other desirable traits, like maternal abilities, needs further study. These findings are important as they highlight the effects of human behaviour, which experimental methods need to account for, and selection of sires when attempting to minimize reactivity in a herd.

**Contribution:** This research was done with the aim of producing repeatable results, increasing confidence in the findings, and allowing further questions to be posed. The use of cattle from a limited number of sires adds an important control to help isolate the genetic component and the results supported previous findings and hypotheses. Another important aspect of the design was that it was arranged to mimic real situations, providing results that could be applied to current working conditions.

## **Transportation-Specific Articles:**

Grandin, T. (1997). Assessment of stress during handling and transport. *Journal of Animal Science*, 75(1), 249. https://doi.org/10.2527/1997.751249x

**Summary:** In this review, Grandin (1997) explores existing research that investigates stress in cattle and the effects that handling can have on how extreme the resulting behaviours are in addition to why those behaviours are present. Cattle, prey animals that they are, need fear as a survival mechanism and it is a powerful motivator that can instill behaviours that are hard to change after the fact. Fear in one animal can also stimulate other nearby cattle to display stress behaviours. When cattle encounter something they have never seen before, they can often react with fear. A slow introduction to new structures or procedures can help to acclimate the animals before a fear response is established, such as starting with short, painless procedures when introducing a cow to a squeeze chute. Starting the acclimation process with calves allows a nurture effect to be observed in the adult animal, influencing the physiology and behaviour. With cattle, repetition appears to be vital to building routine and more predictable responses.

Nature is also a component as reactivity is a heritable trait that can and has been selected for. There are certain breeds, physical markers, body types and bloodlines that are known to indicate a generally more reactive animal. This is important to be cognizant of, as highly reactive animals also tend more towards injury than less reactive animals when exposed to stressful conditions. There are also potential problems if over-selection occurs, which could be detrimental for a variety of factors including animal health and meat quality. Behavioural indicators of stress include escape attempts, vocalization, kicking and struggling. Researchers will also commonly monitor heart rate, cortisol levels and beta endorphins as a measure of stress. Stress behaviours and physiology in cattle are closely linked and should be studied together to obtain the most accurate results.

**Contribution:** Fear responses are a major concern for animal welfare and have implications for the handlers. This review succinctly describes the foundation of research into stress behaviours of cattle and how combining physical responses with closely linked physiological responses can lead to a better understanding of results. Cattle vary widely in both their behavioural and physiological responses to stressful situations. There are still many questions to be answered as to what the threshold is for a stress response and why drastic, sometimes contradictory results are obtained. There were many good primary sources cited within this article regarding livestock behaviour.

Minka, N. S., & Ayo, J. O. (2007). Effects of loading behaviour and road transport stress on traumatic injuries in cattle transported by road during the hot-dry season. *Livestock Science*, 107(1), 91–95. https://doi.org/10.1016/j.livsci.2006.10.013

**Summary:** Minka and Ayo (2007) examined the rate of injury and the effect of transport on the health of cattle in the hot-dry season in Northern Nigeria. Cattle often need to be transported away from where they are raised to where they are slaughtered and, while injuries during transport are known to be common, there were no previous studies regarding the loading and unloading of cattle for transport.

A mix of 150 healthy male and female cattle belonging to three West African breeds of *Bos indicus*, all between the ages of three and five were selected. Each cow was inspected for injuries and well-being before, during and after transportation on a ten-12 hour journey in seasonally hot, dry conditions. A total of six trips were included in the study. During loading and unloading, behaviours that could cause injury were recorded. These included aggressive acts or fighting, mounting another animal, falling, slipping, jumping and cases where the cattle needed a "pat" from a handler with a club to encourage them to move. Each behaviour was assigned a point value and an average per cow was calculated and compared to the rate of injury in each group.

There was a significant, positive relationship between more reactive cattle and how prone they were to injury. Loading was found to take far more time and had a higher occurrence of injury when compared to unloading. There were also significant differences found between the breeds, with both horns and increased instances of stress behaviours causing more injuries in the cattle. This indicates that multiple factors need to be considered when transporting cattle in order to minimize the cost to welfare, including the length of the trip and the breed. More research should be done on different lengths of trips as well as other climate conditions.

**Contribution:** This is the earliest research specifically focused on loading, unloading and transporting cattle, connecting known behaviours to transportation, which is both a common occurrence in a cow's life and one known to cause problems and reduce profits. It was also very interesting that this article compared different breeds of cattle in the same situations. While not the main focus of this article, it does also incorporate the human effect on cattle transportation which ties in well with other articles in this collection.

Schwartzkopf-Genswein, K. S., Faucitano, L., Dadgar, S., Shand, P., González, L. A., & Crowe, T. G. (2012). Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: A review. *Meat Science*, *92*(3), 227–243. https://doi.org/10.1016/j.meatsci.2012.04.010

**Summary:** For production animals, at least one instance of transportation is highly likely to be experienced during that animal's lifetime. Schwartzkopf-Genswein et al. (2012) conducted this review to explore research, specific to North America, that details the detrimental effects that cattle, pigs and poultry exhibit as a result of transportation. These effects have monetary consequences, such as the meat and carcass quality, but also impacts the welfare of the animals. There is a balance that should be struck for the density of a load to maximize the number of cattle that can be sent to market at once while maintaining the quality and welfare of the animals so that each animal is worth the highest possible amount and arrives at the destination in the best possible condition.

Distance is also a consideration when transporting animals as longer distances have been found to increase the negative effects of travel on the animals. The inside of the trailer that animals are transported in, or the microclimate, can expose transported animals to extreme conditions as there is often poor ventilation and no climate control. Transporting animals long distances or in poor trailer conditions has been found to result in shrinkage of the meat, or weight loss, animals coming up lame or even death.

Animals that start the process of transportation in good health have been found to tolerate the journey better overall. It is most profitable for the producer and healthiest for the animals when they are not too young and have a good store of fat.

It is still unknown what the most detrimental factors of transport are on livestock or if there is a factor that is more harmful than the rest. This is an area for future research, as well as exploring connections with other species that are often transported.

**Contribution:** Transportation is a multi-faceted and complex issue that most production animals are exposed to. This review compared several different production animals, highlighting similarities in the challenges faced during transportation and outlining several areas for future research. This article was also great because it was specific to North America and identified current transportation practices and standards. The information was also organized by species so it was very simple to find things pertinent to my research question.

Bravo, V. M., Knowles, T. G., & Gallo, C. (2020). Transport, associated handling procedures and behaviour of calves marketed through Chilean auction markets. *Animals*, *10*(11), 2170. https://doi.org/10.3390/ani10112170

**Summary:** Cattle often require transport at some point in their lives and the effects of the trip can have severe consequences for the welfare of the cattle up to and including death. Bravo et al. (2020) investigated compliance with regulations regarding the transport and handling of cattle in Chile, in addition to cattle welfare and behaviours during these processes. A survey was conducted at 20 markets to assess driver and handler training and preparedness along with an observational study to assess cattle welfare and handling before, during and after transport. Much of the data was collected verbally as initial loading at individual farms could not be observed.

Cattle behaviours observed included slipping, falling, balking or turning back, aggression, mounting and vocalizations. Drivers and market staff loaded and unloaded cattle, with their behaviours classified as positive if handling was done without force or excessive noise. Negative handling practices, such as hitting and tail tweaks, were also recorded.

A level of trained personnel is required in markets but there are challenges due to high turnover. The majority of drivers claimed to have the proper required training. Loading was found to be far more stress-inducing and time-consuming than unloading. It was also more common to observe negative behaviours, from both handlers and cattle, during loading. The density of cattle in the trailer is important as either too few or too many cattle can have negative effects and cause more injuries. A slippery floor or obstacles in the loading and unloading process are also associated with an increased rate of injury. Longer journeys have a larger negative effect on cattle, potentially due to the fact that food and water are unavailable to the cattle for the duration of the journey. This is seen as a major stressor, especially for calves.

**Contribution:**. One new aspect that this article introduces is examining cattle in an open and unfamiliar environment after a high-stress event, transportation. The findings are consistent with the previous articles and compliance with existing regulations is introduced as a variable. An area of future research would be to repeat this study but place trained observers at both ends of the trip and fact-check credentials and travel and emergency plans.

## **The Human Interaction Aspect**

Ceballos, M. C., Sant'Anna, A. C., Boivin, X., de Oliveira Costa, F., Carvalhal, M. V. de L., & Paranhos da Costa, M. J. R. (2018). Impact of good practices of handling training on beef cattle welfare and stockpeople attitudes and behaviors. *Livestock Science*, *216*, 24–31. https://doi.org/10.1016/j.livsci.2018.06.019

**Summary:** The effects of handlers, also called stockpeople, have been established as significant on cattle but the practical results of training stockpeople have not been investigated. Ceballos et al. (2018) wanted to study the effects of proper handling training on both the health of the cattle and the actions and consistency of the handlers. 150 stockpeople working on 24 beef cattle farms in Brazil with both stockpeople and farms categorized by training level were observed. Simultaneous observations were made on the same day during the same vaccination procedure. Specific desirable and undesirable techniques were assigned a point system for evaluation. A 26 question survey was later conducted among the stockpeople to determine the attitude towards cattle, the job and training.

The cattle behaviours recorded were jumping, lying down, balking, which is refusing to walk forwards, attacking, falling, contact with structures and being trampled by another animal. Positive behaviours of the handlers observed were using a handling flag to move the animal in the desired direction with no contact, gentle touch contact with the cattle and gentle vocalization. Negative behaviours of the handlers observed were negative contact with the cattle, which included the use of a cattle prod or twisting the tail and harsh vocalizations.

Farms with more training had more positive and fewer negative behaviours for the cattle and attitudes and actions of the stockpeople. How stockpeople interact and respond to the cattle is important. Unlike dairy cows, beef cattle are not usually handled daily. This provides fewer opportunities for the handlers to practice and develop their skills and less habituation for the cattle themselves. The long-term effectiveness of beef cattle training programs is still unknown but has many potential benefits. Further studies could be done regarding the people working with cattle; not just on the cattle themselves.

**Contribution:** This research is the first to conduct an observational study comparing training levels of handlers and farms. It supports the importance of human-cattle interactions and lays the groundwork for showing the positive effects of good and recurring training. One question this raises that is not addressed in the article is the experience of each of the stockpeople. A future study could compare trained and untrained stockpeople with varying amounts of time on the job.

Sorge, U. S., Cherry, C., & Bender, J. B. (2014). Perception of the importance of human-animal interactions on cattle flow and worker safety on Minnesota dairy farms. *Journal of Dairy Science*, *97*(7), 4632–38. https://doi.org/10.3168/jds.2014-7971

**Summary:** This article investigated the attitude of dairy farmers in Minnesota towards the training of handlers, its importance and effects in day-to-day handling, safety and efficiency, in cattle welfare and the workplace. It has been found that proper training, called stockmanship, reduces the number of injuries when working with cattle and that when injuries do happen, it is most often the fault of the handler, not the animal. Sorge et al (2014) concentrated on gaining a deeper understanding of what farmers currently believe to be the most important and effective strategies. To obtain this information, a voluntary, 28 question survey was sent out to randomly selected operations that had been sorted into categories by herd size. A prepaid return and an option to participate online was included to encourage responses and, out of 620 sent, 108 participated.

It was found that many participants, over 40%, learned handling from family members and then pass down the information. This was particularly prevalent in small herds. There was a general trend that the larger the herd was, the further the cows had to be moved each day for milking and the more likely it was that at least some of the handlers had attended a stockmanship course. Around 70% of respondents were either uninterested in attaining further training or were ambivalent towards it, despite the majority of respondents identifying improved efficiency for moving cattle as a result of the handler's actions.

Challenges identified with stockmanship training included language barriers, lack of access to opportunities and a shortage of time. The researchers acknowledged that there can be issues with voluntary participants as it may not represent a true mean of the data collected. Further research is needed to identify what the most dangerous parts of handling are and how to mitigate those risks.

**Contribution:** This research is vital for developing more techniques in cattle behaviour management. New findings will need to be applied by the farmer if long-lasting, positive effects are to be felt in the industry. Gaining insight into what is important to farmers and what is feasible for them is vital to the implementation of any new regulations, techniques or training that is proposed. This research also points out the need to make training programs more accessible and desirable to farmers. It also addresses a new element; are the most efficient handling methods the same between herds of different sizes?

Kosako, T., Fukasawa, M., Kohari, D., Oikawa, K., & Tsukada, H. (2008). The effect of approach direction and pace on flight distance of beef breeding cows. *Animal Science Journal*, *79*(6), 722–726. https://doi.org/10.1111/j.1740-0929.2008.00586.x

**Summary:** The existence of substantial influence of a handler's behaviour on cattle has been established, however, the effects of a handler's approach has not been studied in a standardized, repeatable and clearly defined manner. Kosako et al. (2008) aimed to establish a precedent for studying the effect of approach, both direction and speed, on the flight of cattle. Some previous experimental designs failed to account for confounding factors such as the colour of a tester's clothing or their familiarity with the cow being tested. The effects of these variables could explain some of the variation in the results of previous literature.

A total of five Japanese Black breeding cows were subjected to flight tests for three different speeds of approach, one km/h, four km/h and seven km/h applied to three directions of approach, front, side and rear leg. Each test was repeated five times on each cow, for a total of 75 trials.

The direction of approach, often unreported or poorly standardized in previous tests, had a significant effect on the flight of the cows. Cattle will flee sooner when approached from the rear or the side than they will when someone approaches from the front. The pace at which a cow is approached was also significant. Moving toward the cow faster will cause it to flee sooner. This was also poorly standardized previously and pace would be greatly affected by the height of the tester as it was defined with steps rather than a speed. Future experiments could use a standard speed and direction of approach and test for the effects of different colours of clothing or familiarity of the handler.

**Contribution:** In order to understand stress responses in cattle and apply that knowledge to real situations, it is important to understand what variable is intentionally being manipulated and what variables may have been unintentionally overlooked and still have an effect on the outcome. This paper defined some variables that are very easy to overlook if you are not a cow. It is a reminder that to study the effects on behaviour, researchers must be able to see what the subject sees.