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A variety of welfare indicators which can be used to assess the welfare of animals which are being handled or transported are listed in Table 1. Some of these measures are of short-term effects whilst others are more relevant to prolonged problems. Where animals are transported to slaughter it is mainly the measures of short-term effects such as behavioural aversion or increased heart-rate which are used but some animals are kept for a long period after transport and measures such as increased disease incidence or suppression of normal development give information about the effects of the journey on welfare.

Table 1

Measures of welfare

Physiological indicators of pleasure

Behavioural indicators of pleasure

Extent to which strongly preferred behaviours can be shown

Variety of normal behaviours shown or suppressed

Extent to which normal physiological processes and anatomical development are possible.

Extent of behavioural aversion shown

Physiological attempts to cope

Immunosuppression

Disease prevalence

Behavioural attempts to cope
Behaviour pathology
Brain changes, e.g. those indicating self narcotisation
Body damage prevalence
Reduced ability to grow or breed
Reduced life expectancy

Details of these and other measures may be found in Broom (1988), Fraser and Broom (1990) and Broom and Johnson (1993).

Behaviour measures

Changes in behaviour are obvious indicators that an animal is having difficulty coping with handling or transport. Some of these help to show which aspect of the situation is aversive. The animal may stop moving forward, freeze, back off, run away or vocalize. The occurrence of each of these can be quantified in comparisons of responses to different races, loading ramps, etc. Examples of behavioural responses such as cattle stopping when they encounter dark areas or sharp shadows in a race and pigs freezing when hit or subjected to other disturbing situations may be found in Grandin (1980, 1982, 1989).

Behavioural responses are often shown to be painful or otherwise unpleasant situations. Their nature and extent vary from one species to another according to the selection pressures which have acted during the evolution of the mechanisms controlling behaviour. Human approach and contact may elicit *antipredator behaviour* in farm animals. However, with experience of handling these responses can be greatly reduced in cattle (Le Neindre et al 1996). Social species which can collaborate in defence against predators, such as pigs or man, vocalize a lot

when caught or hurt. Species which are unlikely to be able to defend themselves, such as sheep, vocalize far less when caught by a predator, probably because such an extreme response merely gives information to the predator that the animal attacked is severely injured and hence unlikely to be able to escape. Cattle can also be relatively undemonstrative when hurt or severely disturbed. Human observers sometimes wrongly assume that an animal which is not squealing is not hurt or disturbed by what is being done to it. In some cases, the animal is showing a freezing response and in most cases, physiological measures must be used to find out the overall response of the animal.

Within species, individual animals may vary in their responses to potential stressors. The *coping strategy* adopted by the animal can have an effect on responses to the transport and lairage situation. For example, Geverink et al (1998) showed that those pigs which were most aggressive in their home pen were also more likely to fight during pre-transport or pre-slaughter handling but pigs which were driven for some distance prior to transport were less likely to fight and hence cause skin damage during and after transport. This fact can be used to design a test which reveals whether or not the animals are likely to be severely affected by the transport situation (Lambooij et al 1995).

The procedures of loading and unloading animals into and out of transport vehicles can have very severe effects on the animals and these effects are revealed in part by behavioural responses. Species vary considerably in their *responses to loading procedures*. Any animal which is injured or frightened by people during the procedure can show extreme responses. However, in most efficient loading procedures, sheep are not greatly affected, cattle are sometimes affected, pigs are always affected and poultry, which are handled by humans are always severely affected. Broom et al (1996) and Parrott et al (1998b) showed that sheep show largely physiological responses and these are associated with the novel situation

encountered in the vehicle rather than the loading procedure. Pigs, on the other hand, are much affected by being driven up a ramp into a vehicle, the effect being greatest if the ramp is steep.

Once journeys start some species of farm animals explore the compartment in which they are placed and try to find a suitable place to sit or lie down. Sheep and cattle try to lie down if the situation is not disturbing but stand if it is. After a period of acclimatisation of sheep and cattle to the vehicle environment, during which time they may stand for 2-6 hours looking around at intervals, most of the animals will lie down if the opportunity arises. Unfortunately for the animals, many journeys involve so many lateral movements or sudden brakings or accelerations, that the animals cannot lie down.

An important behavioural measure of welfare when animals are transported is the amount of *fighting* which they show. When male adult cattle are mixed during transport or in lairage, they may fight and this behaviour can be recorded directly (Kenny and Tarrant 1987). Calves of 6 months of age may also fight (Trunkfield and Broom 1991) and fighting can be a serious problem in pigs (Guise and Penny 1989, Bradshaw et al 1996c). The recording of such behaviour should include the occurrence of threats as well as the contact behaviours which might cause injury.

A further, valuable method of using behaviour studies to assess the welfare of farm animals during handling and transport involves using the fact that the animals *remember aversive situations* in experimentally repeated exposures to such situations. Any stock-keeper will be familiar with the animal which refuses to go into a crush after having received painful treatment there in the past or hesitates about passing a place where a frightening event such as a dog threat occurred once before. These observations give us information about the

welfare of the animal in the past as well as at the present time. If the animal tries not to return to a place where it had an experience then that experience was clearly aversive. The greater the reluctance of the animal to return, the greater the previous aversion must have been. This principle has been used by Rushen (1986a,b) in studies with sheep. Sheep which were driven down a race to a point where gentle handling occurred traversed the race as rapidly or more rapidly on a subsequent day. Sheep which were subjected to shearing at the end of the race on the first day were harder to drive down the race subsequently and those subjected to electroimmobilization at the end of the race were very difficult to drive down the race on later occasions. Hence the degree of difficulty in driving and the delay before the sheep could be driven down the race are measures of the current fearfulness of the sheep and this in turn reflects the aversiveness of the treatment when it was first experienced.

Physiological measures

The physiological responses of animals to adverse conditions, such as those which they may encounter during handling and transport, will be affected by the *anatomical and physiological constitution* of the animal as mentioned later. Some physiological measures are detailed in Table 2.

Table 2 Commonly used physiological indicators of stress during transport

Stressor	Physiological variable
Measured in blood or other body fluids	
Food deprivation	↑ FFA, ↑ β -OHB, ↓ glucose, ↑ urea
Dehydration	↑ Osmolality, ↑ total protein, ↑ albumin, ↑ PCV
Physical exertion	↑ CK, ↑ lactate
Fear/arousal	↑ Cortisol, ↑ PCV
Motion sickness	↑ Vasopressin
Other measures	
Fear/arousal and physical	↑ Heart rate, heart rate variability ↑, ↑ respiration rate
Hypothermia/hyperthermia	Body temperature, skin temperature

FFA, free fatty acids; β -OHB, β -hydroxybutyrate; PCV, packed-cell volume; CK, creatine kinase. (modified after Knowles and Warriss 2000).

Whenever physiological measurement is to be interpreted it is important to ascertain the *basal level* for that measure and how it fluctuates over time (Broom 2000). For example, plasma cortisol levels in most species vary during the day and tend to be higher during the morning than during the afternoon. A decision must be taken for each measure concerning

whether the information required is the difference from baseline or the absolute value. For small effects, e.g. a 10% increase in heart rate, the difference from baseline is the key value to use. The large effects where the response reaches the maximal possible level, for example, cortisol in plasma in very frightening circumstances, the absolute value should be used. In order to explain this, consider an animal severely frightened during the morning and showing an increase from a rather high baseline of 160 nmol l^{-1} but in the afternoon showing the same maximal response which is 200 nmol l^{-1} above the lower afternoon baseline. It is the actual value which is important here rather than a difference whose variation depends on baseline fluctuations.

Heart rate can decrease when animals are frightened but in most farm animal studies, tachycardia increase in heart rate has been found to be associated with disturbing situations. Heart rate increase is not just a consequence of increased activity; heart rate can be increased in preparation for an expected future flight response. Baldock and Sibly (1990) obtained basal levels for heart rate during a variety of activities by sheep and then took account of these when calculating responses to various treatments. Social isolation caused a substantial response but the greatest heart rate increase occurred when the sheep were approached by a man with a dog. The responses to handling and transport are clearly much lower if the sheep have previously been accustomed to human handling. Heart rate is a useful measure of welfare but only for short-term problems such as those encountered by animals during handling, loading on to vehicles and certain acute effects during the transport itself. However, some adverse conditions may lead to elevated heart rate for quite long periods Parrott et al (1998a) showed that heart rate increased from about 100 beats per minute to about 160 beats per minute when sheep were loaded on to a vehicle and the period of elevation of heart rate was at least 15 minutes. During transport of sheep, heart rate remained elevated for at least

nine hours (Parrott et al 1998b). Heart rate variability has also been found to be a useful welfare indicator in cattle and other species (van Ravenswaaij et al 1993, Minero et al 2002).

Observation of animals can provide information about physiological processes animals without any attachment of recording instruments or sampling of body fluids. *Breathing rate* can be observed directly or from good quality video recordings. The metabolic rate and level of muscular activity are major determinants of breathing rate but an individual animal which is disturbed by events in its environment may suddenly start to breathe fast. *Muscle tremor* can be directly observed and is sometimes associated with fear. *Foaming at the mouth* can have a variety of causes, so care is needed in interpreting the observations, but its occurrence may provide some information about welfare.

Changes in the *adrenal medullary hormones* adrenaline (= epinephrine) and noradrenaline (= norepinephrine) occur very rapidly and measurements of these hormones have not been used much in assessing welfare during transport. However, Parrott et al (1998a) found that both hormones increased more during loading of sheep by means of a ramp than by loading with a lift.

Adrenal cortex changes occur in most of the situations which lead to aversion behaviour or heart rate increase but the effects take a few minutes to be evident and they last for 15 min to 2 h or a little longer. Another example comes from work on calves (Kent and Ewbank; 1986; Trunkfield et al 1991; review by Trunkfield and Broom, 1990). Plasma or saliva glucocorticoid levels gave information about treatments lasting up to 2 h but were less useful for journeys lasting longer than this.

Saliva cortisol measurement is useful in cattle. In the plasma, most cortisol is bound to protein but it is the free cortisol which acts in the body. Hormones such as testosterone and cortisol can enter the saliva by diffusion in salivary gland cells. The rate of diffusion is high enough to maintain an equilibrium between the free cortisol in plasma and in saliva. The level is ten or more times lower in saliva but stimuli which cause plasma cortisol increases also cause comparable salivary cortisol increases in humans (Riad-Fahmy et al 1982), sheep (Fell et al 1985), pigs (Parrott et al 1989) and some other species. The injection of pilocarpine and sucking of citric acid crystals, which stimulate salivation, have no effect on the salivary cortisol concentration. However any rise in salivary cortisol levels following some stimulus is delayed a few minutes as compared with the comparable rise in plasma cortisol concentration.

Animals which have substantial adrenal cortex responses during handling and transport show increased body temperature (Trunkfield et al 1991). The increase is usually of the order of 1° C but the actual value at the end of a journey will depend upon the extent to which any adaptation of the initial response has occurred. The body temperature can be recorded during a journey with implanted or superficially attached temperature monitors linked directly or telemetrically to a data storage system. Parrott et al (1999) described deep body temperature in eight sheep. When the animals were loaded into a vehicle and transported for 2.5 h, their body temperatures increased by about 1C and in males were elevated by 0.5C for several hours. Exercise for 30 minutes resulted in a 2C increase in core body temperature which returned rapidly to baseline when the exercise finished. It would seem that prolonged increases in body temperature are an indicator of poor welfare.

The measurement of *oxytocin* has not been of particular value in animal transport studies (e.g. Hall et al 1998). However, plasma *β-endorphin* levels have been shown to increase during

loading (Bradshaw et al 1996b). The release of corticotrophin releasing hormone (CRH) in the hypothalamus is followed by release of pro-opiomelanocortin (POMC) in the anterior pituitary which quickly breaks down into components, including adrenocorticotrophic hormone (ACTH) which travels in the blood to the adrenal cortex, and beta-endorphin. A rise in plasma beta-endorphin often accompanies ACTH increases in plasma but it is not yet clear what its function is. Although beta endorphin can have analgesic effects via mu-receptors in the brain, this peptide hormone is also involved in the regulation of various reproductive hormones. Measurement of beta-endorphin levels in blood is useful as a back up for ACTH or cortisol measurement.

Creatine kinase is released into the blood when there is muscle damage e.g. bruising, and when there is vigorous exercise. It is clear that some kinds of damage which effect welfare result in creatine kinase release so it can be used in conjunction with other indicators as a welfare measure. *Lactate dehydrogenase* (LDH) also increases in the blood after muscle tissue damage but increases can occur in animals whose muscles are not damaged. Deer which are very frightened by capture show large LDH increases (Jones and Price 1992). The isoenzyme of LDH which occurs in striated muscle (LDH5) leaks into the blood when animals are very disturbed so the ratio of LDH5 to total LDH is of particular interest.

On long journeys animals will have been unable to drink for many times longer than the normal interval between drinking bouts. This lack of control over interactions with the environment may be disturbing to the animals and there are also likely to be physiological consequences. The most obvious and straightforward way to assess this is to measure the *osmolality* of the blood (Broom et al 1996). When food reserves are used up there are various changes evident in the metabolites present in the blood. Several of these, for example *beta-hydroxy butyrate*, can be measured and indicate the extent to which the food reserve

depletion is serious for the animal. Another measure which gives information about the significance for the animal of food deprivation is the delay since the last meal. Most farm animals are accustomed to feeding at regular times and if feeding is prevented, especially when high rates of metabolism occur during journeys, the animals will be disturbed by this. Behavioural responses when allowed to eat or drink (e.g. Hall et al 1997) also give important information about problems of deprivation.

The *haematocrit*, a count of red blood cells, is altered when animals are transported. If animals encounter a problem, such as those which may occur when they are handled or transported, there can be a release of blood cells from the spleen and a higher cell count (Parrott et al 1998b). More prolonged problems, however, are likely to result in reduced cell counts (Broom et al 1996).

Increased adrenal cortex activity can lead *immunosuppression*. One or two studies in which animal transport affected T-cell function are reviewed by Kelley (1985) but such measurements are likely to be of most use in the assessment of more long-term welfare problems. The ability of the animal to react effectively to antigen challenge will depend upon the numbers of lymphocytes and the activity and efficiency of these lymphocytes. Measures of the ratios of white blood cells, for example the heterophil to lymphocyte ratio, are affected by a variety of factors but some kinds of restraint seem to affect the ratio consistently so they can give some information about welfare. Studies of T-cell activity e.g. in vitro mitogen stimulated cell proliferation, give information about the extent of immunosuppression resulting from the particular treatment. If the immune system is working less well because of a treatment, the animal is coping less well with its environment and the welfare is poorer than in an animal which is not immunosuppressed.

Carcass and mortality measures

Death during handling and transport is usually preceded by a period of poor welfare. *Mortality records* during journeys are often the only record which give information about welfare during the journey and the severity of the problems for the animals are often only too clear from such records.

Amongst extreme injuries during transport are *broken bones*. These are rare in cattle and sheep. *Bruising*, scratches and other superficial *blemishes* can be scored in a precise way and when carcasses are down-graded for these reasons, the people in charge of the animals can reasonably be criticized for not making sufficient efforts to prevent poor welfare. There is a cost of such blemishes to the industry, as well as to the animals. The cost, in monetary and animal welfare terms, of dark firm dry (DFD) and pale soft exudative (PSE) meat is great. DFD meat is associated with fighting in cattle and pigs but cattle which are threatened but not directly involved in fights also show it (Tarrant, personal communication).

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