Behavioural tests

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Introduction

To answer specific questions regarding cow behaviour or affective states, it is sometimes necessary to study the cow's responses to specific stimuli in a test situation. This can be done either in the animal's home environment or in a test arena [1]. As specific research questions determine the test design, it is not possible to provide a general guideline that fits all types of behavioural test. However, there are several considerations common for most behaviour tests in cattle. These guidelines will assist when planning a test conducted either in the home environment or in a test arena but is by no means an exhaustive description of behavioural tests in cattle.

A – Aims and hypotheses

Before conducting a behavioural test, aims and hypotheses should be formulated. These will decide on further considerations of the testing, such as what type of behavioural test to use and which responses to observe.

B – Considerations of the environment

The home environment usually incorporates activities such as feeding, milking, and cleaning, as well as other cows. These factors may have an impact on the behavioural response of the cow and need to be accounted for. Other factors that may affect the outcome and should be controlled for are, for example, lighting, the presence of other people, time of the day, and time related to feeding and/or milking or other routine procedures. It has also been shown in calves that their responses are affected by the stability of the home environment [2].

C – Type and presentation of stimuli

The type of stimuli and how to present it depends on the response you want to observe. It has been suggested that novel feed has a greater impact on the response than a novel object [3]. When deciding on what stimuli to use, it is important to bear in mind that cattle can see, hear, smell and sense tactile stimulation, and that the stimuli may affect all these senses.

Example: "An assistant holding a closed umbrella (black, 80 cm in diameter) approached the cow slowly and stopped 1 m in front of the barrier where the cow was tied up (0–2 m in front of the cow)." [4]

There are a number of ways stimuli can be presented. It is important to consider things such as:

1. Novelty: Have the cows previously been exposed to the stimuli? For a novel object test, it is important to choose an object the animals have not previously encountered, as studies have shown desensitisation of the novelty of the object after repeated exposure. For a human approach test, previous experiences with humans may interfere with the test, as positive handling of cows has been shown to change behavioural response in this type of test [5], [6]. It has been noted that even wearing gloves or a different perfume may change the responses of an animal [7].

2. Speed and direction: Should the stimuli be stationary or moving? Dependent on the research question, you may want to present your stimuli as stationary or moving. In the human approach test or the avoidance test, a person might be stationary and latency to first contact is recorded, or the person is moving towards the animals/through a group of animals and any avoidance response is recorded. The speed and direction in which the stimuli are moving are likely to affect both the behavioural response of the animals and the repeatability of the experiment [8].

   Example: “The experimenter approached the focal cow using strides of approximately 1.0 m, using the space between cubicles to gauge distance. After every step the experimenter remained motionless for 10 s to allow the cow to respond. The experimenter approached diagonally from the front towards the cow's neck, avoiding eye contact with the cow, looking towards the feet of the cow and keeping arms and hands close to the body.“ [9]

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3. Predictability: Should the stimuli be preceded by a cue to incorporate a certain level of predictability? If the aim is to observe how an animal cope with a known stressor, you might consider including a level of predictability in the test. This could include a visual, auditory or olfactory cue to be presented before the stimuli.
4. Duration and repetitions: For how long and for how many repetitions should the stimuli be presented? The question of duration relates to the type of test and the novelty of the stimuli. For example, in a novel object test, the exposure time of stimuli is usually predetermined, as the first response to the stimuli is often what is observed, and the novelty and interest of the stimuli presented will decrease with time.

   Example 1: “Four different stimuli were presented: usual food (30 min provision of 8 kg total mixed ration); novel food (30 min provision of 5 kg of carrots); novel object (30 min exposure to a white plastic container) and an unfamiliar person (5 min exposure to person dressed in hooded white overall).” [3]

   Example 2: “A device made of a 155 cm pipe perforated with 11 holes (1.5 mm diameter) fixed to the ceiling of each pen (height 2 m) enabled cold water to be sprayed onto the calves. The reaction of the calves was monitored from a remote place. After the two calves from a pen had been observed lying with their heads down for more than 2 min, a Is spray was given.” [2]

D – How to observe the response

Consider which method to use for observation of the animal’s response. Cows can be video recorded, studied by continuous direct observations or scan sampling, using sound recordings or automatic registrations of the behaviour. An ethogram should be created, with clear definitions and descriptions of behaviours bring observed to allow other researchers to identify the same behaviours based on the descriptions.

Behaviour class	**Posture**	**Definition**
Body position	Standing	Standing with at least three feet on the ground
	Lying	Lying down on the sternum or side, body to the floor
Head position	Head lifted and moving	Head lifted and moving, supported by the neck
	Head resting	Head resting on the body or ground, not fully supported by the neck
	Head lifted and still	Head lifted and still, supported by the neck
Jaw movements	Rumination	Rhythmic masticatory movements not related to eating
	Other	All other oral behaviours, such as eating, drinking, grooming or no jaw movement [10]

If the test is repeated multiple times and/or over a long time period, a reliability test should be included. Intra-observer assessment variability for tests with one observer, and inter-observer assessment variability for tests with multiple observers should be assessed.

“**The kappa statistic**

One of the most common ways of measuring reliability between two observers without the problems inherent in percentage agreement is by using Cohen’s kappa, which takes into account the chance agreement of two observers [11]. It is thus a far more useful measure of inter-observer reliability; kappa is defined as

$$\text{Kappa}\ (\kappa) = \frac{O-E}{N-E}$$

Where O is the number of times both observers agree, E is the number of times they would be expected to agree by chance, and N is the total number of observations.“ [12]

E – Testing in a test arena

Some behavioural tests require a more controlled environment where influences of conspecifics, temperature, lighting and other activities can be managed. There are various types of tests that may be performed in a test arena; cognitive tests such as learning tests; fear tests such as the open-field and novel arena tests; locomotor motivation tests to examine play motivation; and restraint tests.

1. The test arena - design, acclimatisation and training: The design of a test arena should match the physical characteristics of the experimental animals, the number of animals tested at the same time, and the behaviours aimed to be registered. The design and size of a test arena may vary widely between and within species.

   Example 1: “A novel arena test varies from 10–12 m ^{2} for calves and heifers up to 100 m ^{2} for cows.” [13]

   Example 2: “Our results show that both size and shape of the arena influenced the amount and type of play seen during the arena test. The duration of running was increased in both larger and longer arenas. [...] Conversely, calves performed more jumping in the smaller arenas, possibly because less space is required for this behaviour compared to running.” [14]

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2. Transport to the test arena: Moving animals from the home environment to the test arena should be done in a calm manner, respecting the animals’ pace. The test arena should be as close as possible to the home environment to avoid excessive energy use or disturbances along the way. The walkway between the home environment and the test arena should be comfortable for the animal to walk on, slip-resistant, and free of obstacles.

   Example: “… moving the cows in groups and testing them with visual social contact to pen mates avoided the burden of social isolation and/or being in a different environment that otherwise could have compromised the performance of the tasks.” [15]

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3. Acclimatisation and training: Animals may react to a test environment and/or human-animal contact prior to testing by showing signs of stress and excitement such as increased heart and respiratory rate, agitation, increased frequency of urination, defecation and vocalisation, vigilant body posture such as erect ears and head, occurrence of red eyes and an increased exploratory behaviour [16]. As these responses to the environment would interfere with any responses to the test, a period of acclimatisation to the test environment may be needed. An acclimatisation period needs to be of sufficient duration to allow the experimental animal to settle in the new environment, as well as to minimise any confounding effect between environment and response, if this is not part of the experiment [15].
4. A training period may be included. The duration of the training period depends on the task, test apparatus, and animal status prior to training as well as adopted learning criteria. Franchi et al. [15] demonstrated that dry cows learnt to perform operant tasks to obtain feed rewards in a relatively short time. The reason for the quick learning process is believed to be the high motivation for feed, as the experimental cows were feed deprived for approx. 90 min prior to the initial training. The period designated for acclimatization and/or training depends on factors such as the chosen experimental animal (species, age, status etc.), type of arena test (fear tests, novel

tests, and motivation tests etc.), type of stimuli, expected behavioural responses. Because of the many factors for determining the appropriate acclimatization and/or training period, a pilot study is often conducted where the attributes of the training can be evaluated.

Example: "… Subsequently, each cow went through a 3-step training process to learn to open the gate of the feeder. The first step the cows needed to fulfil was to feed on the concentrate for 3 s. If successful, the experimenter closed the gate and initiated the second step, where the cows needed to repeat step 1, but with the gate initially closed. First, the experimenter opened the gate and smoothly swung it by holding the external metal bars leaving a 5-cm gap between the gate and the trough. If the cow succeeded to push the gate until it reached the hook, which held the gate fully open, and to feed, the cow advanced to the third and final step, which was a repetition of step 2." [15]

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