

## **Workplan - Effect of controllability on anticipatory behaviours in lambs**

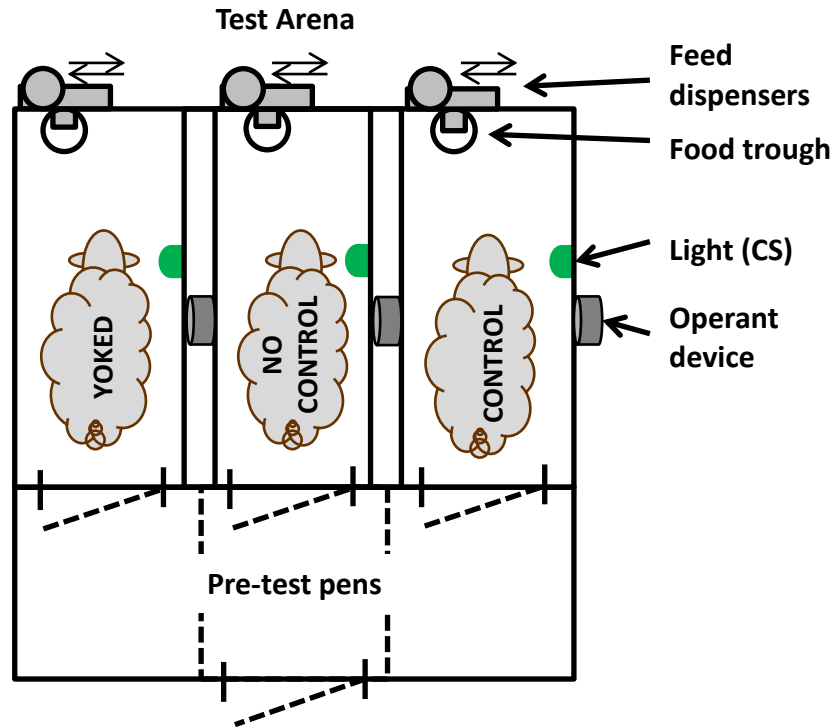
Recently several studies have investigated anticipatory behaviour in various species as a potential indirect measure of appetitive positive emotions. Many of these studies have used classical conditioning with a trace interval (a waiting period) between the presentations of the conditioned stimulus (CS), e.g. a light, and the unconditioned stimulus (US), e.g. a food reward. Such methodology however means placing animals in a situation where they have no control to affect the outcome. Contrary, instrumental conditioning means that the US is dependent on a specific behavioural response. We believe that the great variations in anticipatory behavioural responses reported from various studies, where classical conditioning with a trace interval have been used, are to some extent due to the lack of controllability and potentially frustrating, contradictory to claims of such waiting period being experienced as positive. The aim of this study is to assess how an element of controllability affects behavioural and physiological responses compared to the lack of controllability during anticipation for a reward in lambs.

36 female lambs from the dairy breed Romane will be used in this study. Initially, there will be a habituation period of approximately three weeks where the lambs will be habituated to both the presence of humans and also to having heart rate measure equipment attached (EMKA technologies). Following these three weeks lambs will be exposed to a temperament test which will assess how well they cope with being housed in solitary for a shorter period of time (approximately 60 seconds). Following this test, lambs will be allocated into three treatment groups that are balanced based on the results from this test.

Lambs will be allocated into treatment groups CONTROL, NO CONTROL and YOKED. The lambs will then be housed in three groups of twelve individuals in three adjacent larger home pens. Each group of twelve lambs will consist of four individuals from each treatment group. Each pen will consist of four trios, and each trio will consist of one lamb from each treatment group. Following treatment group allocation and the forming of the trios, lambs will be habituated to a test arena; each trio will visit the test arena regularly for a number of repetitions. Following habituation to the arena, the trios will be habituated to being isolated in three adjacent test pens, i.e. each lambs in each trio will be placed in one test pen each next to each other (see Figure below).

Following the previous step, shaping will be initiated with the CONTROL lambs. The lambs from this treatment will be trained individually to perform an operant task – putting their muzzle through a hole in the wall to break an invisible beam. When the shaping of this specific behavioural response is completed, CONTROL lambs will after putting their muzzle through the hole be presented with a signal (a light being switched on) and a food reward, consisting of barley from a feed dispenser. During shaping of the CONTROL group, NO CONTROL and YOKES lambs will on each repetition enter their individual test pens and when CONTROL is being presented with the light and the food reward, so will the NO CONTROL lamb. This will condition the NO CONTROL lambs to associate the light with the delivery of the food reward.

The YOKED lamb will be presented with the food reward at the same time as the other two lambs, but the light will be switched on at a scheduled random time to avoid forming an association between the light and the food reward, i.e. to avoid conditioning. Throughout this stage, lambs will be fitted with the heart rate belt in the pre-test pens before entering the test pens.



Following the completion of this shaping phase, there will be a gradual increase in the interval between the presentation on the light and the food reward with an approximate delay of 2-3 seconds for each subsequent repetition. This procedure will continue until the interval between the light and the food reward is approximately 40 seconds and throughout this stage lambs will have the heart rate equipment attached in the pre-test pen before entering the test pens. When this desired interval has been reached, lambs' responses will be monitored and both behavioural measures and physiological measures (heart rate and heart rate variability) will be analysed as 'anticipatory behaviours'. The behavioural responses will allow us to investigate the effect of predictability and controllability on anticipatory behaviours (see Table below).

Treatment group	Predictability	Controllability
CONTROL	YES	YES
NO CONTROL	YES	NO
YOKED	NO	NO