

## Automated induced reward-related behaviours in laying hens in the home pen environment

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Behaviour during anticipation or consumption of a signalled rewarding stimulus is a potentially useful approach to study positive emotional states in animals. We explored the use of a specially designed computer-controlled reward-delivery device named “Chicken School” to induce reward-related behaviour in ad-libitum fed laying hens housed and tested in their home pen. A “Chicken School” was attached below the home pen ceiling of the experimental group (EX; n=6) and control group (CO; n=6), all singly housed. A light signal (conditioned stimulus, CS) was paired (EX) or not paired (CO) to a reward (meal worms; US) for five days (Association Phase). Then, the CS-US interval was increased gradually in EX to a maximum of 32 seconds (Anticipation Phase, five days). The effect of exposure to CS and reward was tested on day 7 (Test 1; CS-US 22 sec) and day 11 (Test 2; CS-US 32 sec). Behaviour and location in pen were decoded from video recordings before CS (2 min), during the CS-US interval (either 22 or 32 sec), and two min after reward on the two test days. The approach latency to reward was calculated, based on mean values from all 10 days of training (Association and Anticipation). Data were analysed using t-test. In the CS-US interval, alert behaviours were seen most often in EX hens ( $P < 0.003$ ), and no differences were seen between Test 1 and 2. Comfort behaviours were seen more in CO hens compared to EX hens during anticipation ( $P < 0.03$ ) and after reward ( $P < 0.005$ ). For all hens the mean approach latency ranged from 2.9 to 5.1 seconds. No differences were found in time spent in different areas of the pen. Our results show that hens were able to learn the CS-US association using a classical conditioning procedure, and that meal worms are rewarding in ad-libitum fed hens. A CS-US interval of 32 seconds may be successfully applied in future studies. Hens express anticipation by increased levels of alert behaviours and reduced time spent performing comfort behaviours. CO hens also responded to CS alone, indicating a stimulating property per se that needs to be taken into account in future studies. In our study location in the pen was not a good measure of anticipation. In conclusion, we have established a fully automated computer-based method for inducing anticipatory behaviour in ad-libitum fed laying hens in the home pen environment. This method will facilitate the testing of various hypotheses on reward-related behaviour in laying hens.

**Keywords:** positive emotions, laying hens, anticipatory behaviour, reward