



# *Poultry Extension Collaborative newsletter*

A collaboration between Purdue University, University of Arkansas,  
University of Georgia, and Virginia Tech

## *Fear Tests in Poultry*

### What are Fear Tests?

Animal welfare encompasses the entirety of an animal's life, i.e., day-to-day activities and behaviors, and emotions that can be difficult to interpret (Mononen, 2013). Emotions can be either long-term, relating to cognitive traits of the animal, or short-term, relating to a specific stimuli in their environment. These emotional states and feelings can be perceived as either pleasant, unpleasant, or something in between (Fraser, 2008), so the use of behavior tests helps to interpret them (Hemsworth et al., 2015).

In this newsletter, we will focus on fear responses, which are the animal's reaction or perception of a potentially dangerous stimuli (Boissy, 1995 & Gray, 1987). An increased fear response in poultry can lead to reduced production which can be detrimental to farmers (Boissy, 1995). Behavior tests are used for verifying theories of cognition or emotion for a specific animal (Belovicova et al., 2017) in this case, poultry. Fear tests are types of behavior tests that are used to determine an animal's fearfulness based on behavioral responses in a standardized test. The scientific community has made advances to indirectly and objectively measure affective states and emotions of animals by using these fear tests.

### What can Fear Tests measure?

Fear tests measure the actions and behaviors of an animal in the specific fear test used. For example, vocalizations, body movements, distance from an object, eye contact, defecation, or other interactions with the stimuli introduced in the test are measured (Keer-Keer et al., 1996). Researchers conducting fear tests will observe these behaviors either during the test or from video recordings. The major differences between the tests lie in how the tests are conducted and interpreted. General interpretations of fear test measures are that greater durations, distances, and frequencies indicate a greater fear response. However, it is ultimately up to the researcher how these behaviors will be analyzed.



This newsletter will cover 4 fear-based behavioral tests: the Novel Object Test (NOT), Tonic Immobility (TI), the Emergence Test (ET), and the Novel Arena Test (NAT). We will also highlight the test environment, advantages and disadvantages, and why these tests matter in the context of animal welfare.

# Tonic Immobility (TI)

## How to set up & conduct:

1. The researcher places a bird on its back in a v-shaped cradle or simply a cardboard box with a cloth in it.
2. The researcher then restrains the bird for ~15 seconds, the most common time to restrain the bird, to induce TI. To restrain the bird, the researcher holds one hand over the bird's head and neck, with the other hand gently pressing on the sternum (breast bone) (Herrera-Castillo et al., 2022) and then gradually releases.
3. After the restraint, the bird is gently let go, while still laying in the cradle.
4. Behavioral measures are recorded.
5. The test ends when the bird rights itself or the designated period of time elapses, usually 5-10 minutes

(Adapted from Gallup et al., 1971; Gallup et al., 1972; & Forkman et al., 2008)



\*Photo credit: Karlsson (2016)

## What is TI and what can it measure?

The Tonic Immobility Test is a “fear-potentiated response induced by physical restraint and characterized by reduced responsiveness to external stimuli” (Jones & Faure, 1981). This test is used as an indicator of fearfulness for an individual bird by measuring the latency to right itself. If the bird does not right itself in the time allotted, the test starts over with a new bird and that bird will have a latency score of 0. The longer the latency, the more perceived fearfulness of the bird.

### Behavioral Measures:

- Latency to right (seconds)
- Frequency of vocalizations
- Frequency of defecation
- Frequency of eye movements



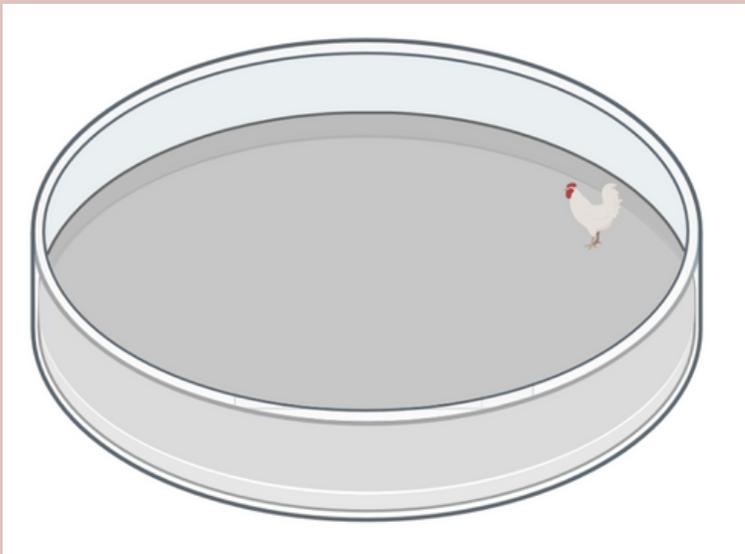
\*Photo credit: Carolyn Cole and Alex Gumas (2016)

# Novel Arena Test (NAT)

## *How to set up & conduct:*

1. Determine test duration. This could range from 1 minute to 30 minutes.
2. An individual bird is placed in a novel arena, commonly circular or rectangular. The bird is placed either in the center of the testing arena or against a wall.
3. The behavioral measures are then recorded.
4. After the designated time has passed, the researcher ends the test.

*(Adapted from Forkman et al., 2008)*

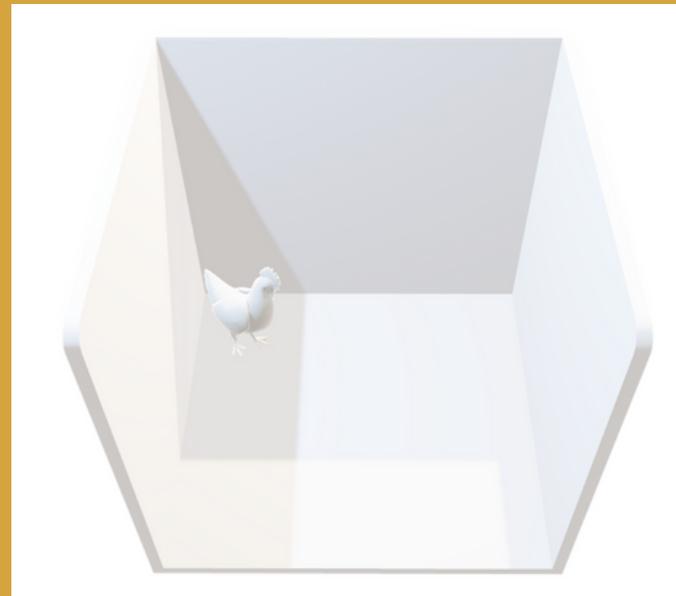


## What is the NAT and what can it measure?

In the Novel Arena Test, sometimes referred to as the Open Field Test, the novelty is the environment. The NAT involves placing the bird in a new environment that they have not been in contact with or have seen previously and researchers assess the fear response of the bird.

### Behavioral Measures:

- Latency to enter and leave the initial area (seconds)
- Frequency of defecation
- Frequency of vocalizations
- Frequency of escape attempts



# Emergence Test (ET)

## How to set up & conduct:

1. Individual birds are placed inside a closed emergence box located in a testing arena.
2. After a period of acclimation (usually 30 seconds), the box door is opened. The bird then has a choice to emerge from the box or to remain inside the box.
3. Researchers record behavioral measures.
4. After a designated time (3 -5 minutes) has passed, the researcher ends the test.

*(Adapted from Forkman et al., 2008)*



*\*Photo credit: Weimer Lab (2022)*



*\*Photo credit: Weimer Lab (2022)*



*\*Photo credit: Weimer Lab (2022)*

## What is the Emergence Test and what can it measure?

The ET has novelty in both the testing environment and the emergence box, so the resulting behaviors are a multiplicative response to two new stimuli. The latency to exit the box is a measure of how fearful the bird is when put in an isolated, dark novel area. The second action of exiting the box is a measure of how the bird weighs potentially dangerous stimuli: remain in the dark box or investigate the new environment in front of them. The environment where the box is placed could be very similar to their home pen, but if the bird has never seen the testing pen, it is considered novel.

### Behavioral Measures:

- Latency to exit the emergence box into the open arena (seconds)
- Frequency of vocalizations
- Frequency of movements the bird takes towards the opening of the box



*\*Photo credit: Weimer Lab (2022)*

# Novel Object Test (NOT)

## *How to set up & conduct:*

1. The NOT can be conducted in the home space or a separate testing area. Conducting the NOT in a familiar area is ideal because a novel environment may distract the bird from the novelty of the object (Forkman et al., 2008).
2. The NOT can be conducted on individuals or groups of birds.
3. The novel object is typically placed in the testing area or hung from the ceiling.
  - a. By hanging the object from the ceiling, the test must be set up before the birds are in the testing area.
  - b. Novel objects could theoretically be any object that the birds have not seen before.
4. After the designated period of exposure, usually 3-4 minutes, the experimenter takes the object away and the test ends.

*(Adapted from Forkman et al., 2008 and Brantsæter, et.al., 2017)*



*\*Photo credit: Magnaterra (2021)*

## What is the NOT and what can it measure?

The Novel Object Test is used to evaluate the level of fearfulness of poultry by recording reactions and behaviors of the birds after the placement of a new, never seen object in the testing area. An assumption of the test is that the birds will be neophobic, or fearful of the new object (Greenberg, 2003; Mettke-Hofmann et al., 2009).

### Behavioral Measures:

- Latency to contact or duration of contact with the object (seconds)
- Frequency of contact with the object
- Distance (cm) from the object
- Frequency of explorative behaviors while in contact with the object



*\*Photo credit: Karlsson (2016)*

### Novel object ideas:

- Plastic balls
- Empty water bottles
- Colorful shapes



## Advantages of Fear Tests

- The ease of setup and data collection.
- The tests are inexpensive and easily adaptable to different species.
- Researchers have many options for novel objects and environments to conduct the NOT and NAT multiple times in the same study.
- The NOT does not require any physical manipulation of the bird by humans.



*\*Photo credit: Garrigus (2022)*

## Disadvantages of Fear Tests

- Tests could have habituation effects if repeated with the same bird. Objects, arenas, and procedures are no longer novel to the bird and will not have a naïve fear response.
- To induce TI, the bird must be physically restrained.
- These tests infer the bird's emotional states, they are not a direct measure.
- These tests require a large sample size to create enough contrast for producing valuable results because of individual bird differences.
- The tests can be time consuming to conduct.



## Main Take Aways of Fear Tests

- Behavior tests provide valuable insights in poultry's emotions and affective states.
- Most fear tests require that an object or environment is novel and the bird simply is reacting to that new stimuli.
- Most fear behavior tests are inexpensive and can easily be adapted to different species, making them generally accepted in different fields of animal research.
- Fear-based tests tell us how an animal perceives its environment, allowing poultry researchers and farmers to make informed management decisions about their birds' environment, providing them with increased welfare.

## References

- Belovicova, K., Bogi, E., Csatosova, K., Dubovicky, M. (2017). Animal tests for anxiety-like and depression-like behavior in rats. *Interdiscip Toxicol.* 10(1), 40-43. doi: 10.1515/intox-2017-0006. PMID: 30123035; PMCID: PMC6096862.
- Brantsæter, M., Tahamtani, F., Nordgreen, J., Sandberg, E., Hansen, T., Rodenburg, T., Moe, R., & Janczak, A. (2017). Access to litter during rearing and environmental enrichment during production reduce fearfulness in adult laying hens. *Applied Animal Behaviour Science*, 189, 49–56. <https://doi.org/10.1016/j.applanim.2017.01.008>
- Gallup, G.G., Nash, R.F., & Wagner, A.M. (1971) The tonic immobility reaction in chickens: Response characteristics and methodology. *Behav. Res. Meth. & Instru.*, 3, 237–239. <https://doi.org/10.3758/BF03208389>
- Gallup, G.G., Rosen, T.S., & Brown, C.W. (1972) Effect of conditioned fear on tonic immobility in domestic chickens. *Journal of Comparative and Physiology Psychology*, 78, 22-25. <https://doi.org/10.1037/h0032833>
- Greenberg, R. S. (2003). The role of neophobia and neophilia in the development of innovative behaviour of birds. *Animal innovation*.
- Forkman, B., Boissy, A., Meunier-Salaün, M., Canali, E., & Jones, R. (2007). A critical review of fear tests used on cattle, pigs, sheep, poultry and horses. *Physiology & Behavior*, 92(3), 340–374. <https://doi.org/10.1016/j.physbeh.2007.03.016>
- Fraser, D. (2008). Understanding animal welfare. *Acta Veterinaria Scandinavica*, 50(S1), S1–S1. <https://doi.org/10.1186/1751-0147-50-S1-S1>
- Herrera-Castillo C. M., Geiger M., Núñez-León D., Nagashima H., Gebhardt-Henrich S., Toscano M., Sanchez-Villagra M. R. (2022). Skeletal variation in bird domestication: limb proportions and sternum in chicken, with comparisons to mallard ducks and Muscovy ducks. *PeerJ* 10:e13229 <https://doi.org/10.7717/peerj.13229>
- Hemsworth, P., Mellor, D., Cronin, G., & Tilbrook, A. (2015). Scientific assessment of animal welfare. *New Zealand Veterinary Journal*, 63(1), 24–30. <https://doi.org/10.1080/00480169.2014.966167>
- Karlsson, L. (2016). The rowan ranger chicken breed - a suitable alternative for the organic chicken meat industry (Dissertation). Retrieved from <http://urn.kb.se/resolve?urn=urn:nbn:se:liu:diva-129085>
- Keer-Keer, S., Hughes, B. O., Hocking, P. M., & Jones, R. B. (1996). Behavioural comparison of layer and broiler fowl: measuring fear responses. *Applied Animal Behaviour Science*, 49(4), 321–333. [https://doi.org/10.1016/0168-1591\(96\)01055-6](https://doi.org/10.1016/0168-1591(96)01055-6)
- Mettke-Hofmann, C., Lorentzen, S., Schlicht, E., Schneider, J., & Werner, F. (2009). Spatial Neophilia and Spatial Neophobia in Resident and Migratory Warblers (*Sylvia*). *Ethology*, 115(5), 482–492. <https://doi.org/10.1111/j.1439-0310.2009.01632.x>
- Mononen, J. (2013). What is animal welfare and how can we measure it? *Review on Agriculture and Rural Development*, 2(1), 5-10. Retrieved from <https://www.proquest.com/scholarly-journals/what-is-animal-welfare-how-can-we-measure/docview/2382649977/se-2>