



Simple but Complex—A Laying Hen Study as Proof of Concept of a Novel Method for Cognitive Enrichment and Research

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Enrichment can reduce stress and stereotypic behavior and therefore enhance captive animal welfare. In cognitive enrichment, cognitive tasks engage and challenge the animals' natural behavioral repertoire and provide mental stimulation. Enrichment with similarities to "puzzle boxes" in cognitive research is widespread in zoos but rarely applied in commercial farming, as it requires costly time and effort. Here, we introduce a flexible method for cognitive enrichment and research. The test battery apparatus (TBA) is a configurable cubic box with frames for interchangeable test panels, each holding a problem-solving task that must be solved for a food reward. As a proof of concept, we report observations and first results from two groups of laying hens (Gallus gallus forma domestica; 52 birds in total) to show the TBA's feasibility in commercial farming and to investigate the animals' spontaneous interaction with four test panels. While we could not reliably identify individuals, we found the majority of the hens highly motivated to engage with the device. At least five individuals in each group were successful and there was a significant gradient of success rates across the four panels. As the implementation and maintenance required little time and effort, the TBA is promising as a cognitive enrichment device in farm settings. Its potentially limitless configurations allow diverse opportunities for cognitive and behavioral engagement in the long term. While further studies will be crucial to validate welfare effects and problem-solving tasks, the TBA is simple in its application but complex in its possibilities.

Keywords: cognitive enrichment, problem solving, laying hen, *Gallus gallus*, chicken, proof of concept, test battery apparatus

INTRODUCTION

Enhancing the welfare of captive animals is an important societal concern and research topic. Animal welfare does not only include physical health but essential behavioral and mental needs, stimulating environmental challenges and agency (Broom, 1986; Dawkins, 1990; Špinka and Wemelsfelder, 2011). In commercial farming, practical issues such as costs and feasibility complicate animal welfare improvements (Webster, 2001). Legally, animal husbandry must be appropriate to the animals' "physiological and ethological needs in accordance with established experience and scientific knowledge" (Council of Europe, 1976),

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Schmelz M and Krause ET (2021) Simple but Complex—A Laying Hen Study as Proof of Concept of a Novel Method for Cognitive Enrichment and Research. Front. Anim. Sci. 2:671905. doi: 10.3389/fanim.2021.671905 implying the necessity to adapt to novel scientific developments. One approach to meet animals' ethological needs is environmental enrichment (Newberry, 1995; Shepherdson, 1998) that has been linked to positive welfare effects such as mental stimulation, improved fine motor skills and, if applied correctly, reduced negative stress and stereotypic behavior in numerous species (Carlstead and Shepherdson, 2000; Swaisgood and Shepherdson, 2005).

A subset of environmental enrichment is cognitive enrichment targeting the animals' cognitive skills. As defined by Clark (2011), cognitive enrichment "(1) engages evolved cognitive skills by providing opportunities to solve problems and control some aspect of the environment, and (2) is correlated to one or more validated measures of wellbeing" (p. 6). The need for an intrinsic or extrinsic reward was later added as a third condition (Clark, 2017). One important aspect of any effective enrichment is variation and change. Enrichment devices that are installed once and permanently only reward the same behavior do not offer long-term challenges needed for positive eustress and less boredom (Meehan and Mench, 2007; Selye, 2013).

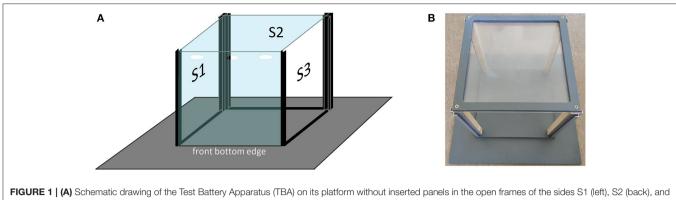
A meta-analysis of publications from 1985 to 2004 found variable environmental and cognitive enrichment to be absent or at least very rare in farm animals (de Azevedo et al., 2007), most likely due to feasibility and costs. Enrichment in commercial poultry farming commonly consists of inflexible items such as pecking stones, alfalfa bales, strings or straw (Schreiter et al., 2019). In recent years, the appraisal and discussion of cognitive skills of farm animals has received more attention, implying an important need for more mental stimulation for livestock in farm settings (Nawroth et al., 2019). Operant conditioning has been utilized as cognitive enrichment (Meyer et al., 2010) and while its direct impact on animal welfare is difficult to correlate and quantify, there are some promising studies showing positive effects on some species of farm animals (e.g., pigs: Ernst et al., 2005; Manteuffel et al., 2009; goats: Langbein et al., 2009; Kalbe and Puppe, 2010; Zebunke et al., 2013). Chickens (Gallus gallus forma domestica) are an underrepresented species in the increased research interest in farm animal cognition of recent years (Nawroth et al., 2019) and numerous welfare problems are known in this species (for a review, see Janczak and Riber, 2015). While cognitive enrichment in poultry is virtually non-existent, a number of cognitive skills have been shown in chickens that principally allow, if not require, it to be implemented (Krause et al., 2006; Smith and Johnson, 2012, Tahamtani et al., 2015; Dudde et al., 2018; Garnham and Løvlie, 2018).

In contrast to farm settings, cognitive enrichment is more common in zoos (e.g., Clark et al., 2013; Yamanashi et al., 2016). Additionally, providing zoo animals with hidden food that can be obtained by overcoming obstacles or opening containers is widespread and, while not being validated scientifically, can involve cognitive challenges. Interestingly, typical tests of problem-solving abilities in animal cognition research share some similarities with this kind of enrichment: "puzzle boxes" with special food rewards that can be accessed by solving a cognitive problem (e.g., Benson-Amram et al., 2016; Borrego and Gaines, 2016). However, puzzle boxes with a single solution are not variable and flexible enough for long-term application as cognitive enrichment. A more complex and variable version of a puzzle box is the multi-access-box (MAB) (Auersperg et al., 2011; Huebner and Fichtel, 2015; Johnson-Ulrich et al., 2018; Williams et al., 2021). Instead of only one solution to obtain food there are several solutions in a MAB that can be discovered, explored and learned stepwise. In this paradigm, results can be discussed with regard to problem solving, cognitive flexibility and (repeated) innovation (Auersperg et al., 2012). Importantly, the MAB approach allows diverse behavioral engagement with a single relatively small apparatus-an aspect of interest for lowmaintenance enrichment devices. Another approach to diversity and flexibility in cognitive enrichment is a modular maze idea of the "Gorilla Game Lab" (Gray et al., 2018; Clark et al., 2019). Different problem-solving modules can be connected and flexibly arranged to create a changing enrichment device that is challenging in the long term. However, in contrast to the MAB approach, this device has to be elaborately assembled outside of the animals' enclosure with high demands of space and effort.

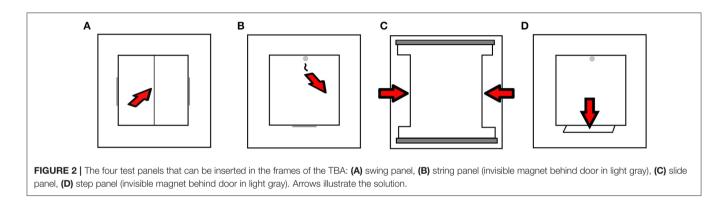
Here, we introduce a novel device called the "Test Battery Apparatus" (TBA) for cognitive enrichment and animal cognition research. Inspired by the MAB, the TBA is a cubic box baited with a food reward that allows multiple different problemsolving tasks at the same time to single animals or groups. But similarly to the modular maze approach, it is also flexible and expandable in a way that it supports, in principal, an almost limitless number of additional tasks that can easily be exchanged. The TBA is closed on the bottom and with transparent top and front surfaces (Figure 1). The remaining three sides of the cube (S1-S3) are open frames into which test panels containing problem-solving tasks can be inserted and easily relocated and exchanged for other panels. The tasks can be specifically adapted to the animals' natural and ecologically-relevant behaviors in species-specific ways. The TBA approach aims toward bolstering cognitive enrichment in farm animals and the development of a low-cost and low-effort method for a variety of species while potentially improving animal welfare in a diverse and complex manner over the long term.

The variable configuration of the TBA with its interchangeable test panels has the potential for a test battery or "mini test battery" approach (see Shaw and Schmelz, 2017) in a single basic apparatus. It is therefore not only of interest as a cognitive enrichment device but also as a behavioral research apparatus, potentially targeting a variety of topics: Problem-solving abilities can be investigated on a group level and between species and on an individual level within species. Other potential tests include novel object tests, commonly applied to measure shyness/boldness in animals (e.g., Wilson et al., 1994; Coleman and Wilson, 1998; Stöwe et al., 2006; Herrmann et al., 2007), a detour test, commonly applied to measure inhibitory control in animals (e.g., MacLean et al., 2014; Nawroth et al., 2016), a persistence test with an unsolvable problem (e.g., Rao et al., 2018) and several repeated measures of activity and exploration-all in one apparatus. The open-frame design of the TBA is not limited to a specific research topic but can be creatively expanded and adopted in various ways for future research.

In this study, we applied the TBA as potential cognitive enrichment to two separate groups of untrained and naïve



S3 (right). (B) Photo of TBA from the opposite side. The open frame of S2 can be seen on the front, the frames of S1 and S3 are on the right and left side, respectively. The far side and top are permanently covered with transparent acrylic glass.



laying hens as a proof of concept. We aimed to (1) investigate the interaction of the groups with four test panels, presenting different problem-solving tasks adapted to the birds' behavioral repertoire (see **Figure 2**) and (2) show the basic feasibility in a commercial farm setting. We expected the laying hens to spontaneously engage with the TBA in general and the test panels in particular whenever it was baited with a food reward and to be able to successfully solve the tasks of these panels at differing success rates. Furthermore, we expected the use of the TBA to be simple from the humans' point of view and therefore applicable to a farm setting with minimal disturbance of the daily routines.

METHODS

Animals

We tested two groups of producing laying hens that were available for testing at the Friedrich-Loeffler-Institute for Animal Welfare and Animal Husbandry in Celle, Germany, between October 2019 and April 2020. In total, 52 laying hens (~20 months old at the beginning of the study) were allocated in the two separate groups that were housed next to each other and tested sequentially. These animals had individually participated in a previous learning study with no methodological similarities to the problem-solving tasks presented here (Dudde et al., in prep.). They were kept in standard litter floor system pens of about 11 m² with wood-shavings, perches, and a group nest.

About 2.5 \times 3 m of each pen were an unobstructed open ground area.

Apparatus and Procedure

The TBA box was made of PVC (thickness 1 cm), transparent acrylic glass (thickness 0.4 cm), and wooden posts (thickness 2 cm) on the four corners (see Figure 1B). Its dimensions were $30 \times 30 \times 30$ cm attached to a 50 \times 50 cm heavy platform to preclude it from being moved or toppled over by the animals. The TBA with open frames, that is without inserted panels in sides S1-S3 (as depicted in Figure 1), was placed in the middle of the ground floor area of each of the pens of the laying hen groups continuously (4 and 2 months, respectively). A handful of wheat grains, a food reward laying hens have been shown to be motivated to work for in previous studies (see Dudde et al., 2018), was put inside the open TBA at irregular intervals (ranging from daily to ca. weekly) to habituate the animals to the box in the beginning and then establish it as a part of their pen and a feeding location. The TBA remained in place inside each of the pens throughout the course of the experiment and was roughly cleaned before each test session and thoroughly cleaned and disinfected when it was moved to the other group.

In the test sessions, one of four different test panels was inserted into the frame of side S2, while the other two frames, S1 and S3, always held plain opaque panels with no opening. The food reward inside the TBA could therefore only be accessed by solving the problem of the respective test panel on the side S2 in these sessions. All test panels were designed to allow the hens to use behaviors within their natural behavioral repertoire but in a specific way not encountered before (pulling string with beak, pushing door forward or sideways with beak or body, pressing pedal downwards with beak or feet). In particular, the four test panels were:

Swing Panel

An opening in the panel (20×20 cm) was blocked by an opaque two-winged door with hinges on both sides. To access the food, the door had to be pushed inside (**Figure 2A**).

String Panel

An opening in the panel $(20 \times 20 \text{ cm})$ was blocked by an opaque hatch of the same size that was attached with a hinge on the bottom and held in place by a magnet on the top back side. On the top front a short string with a knot was attached to the hatch. To access the food, the string had to be pulled to disengage the magnet, causing the hatch to fall open to the outside (**Figure 2B**).

Slide Panel

An opening in the panel $(20 \times 20 \text{ cm})$ was blocked by an opaque specifically-shaped hatch (ca. 28 cm wide on the top and bottom and $20 \times 20 \text{ cm}$ in the middle) that was inserted into a frame system on the top and bottom of the panel. To access the food, the hatch had to be slid sideways in the frame, either to the left or to the right (**Figure 2C**).

Step Panel

An opening in the panel $(20 \times 20 \text{ cm})$ was blocked by an opaque hatch of the same size that was attached with a hinge on the bottom and held in place by a magnet on the top back side. On the bottom side an elongated step pedal $(10 \times 5 \text{ cm})$ was attached to the hatch at a 90° angle. To access the food, the pedal had to be stepped on or pushed down to disengage the magnet, causing the hatch to fall open to the outside (**Figure 2D**).

In the test sessions, the food reward was put into the TBA, the two plain opaque panels were inserted into frames S1 and S3 and one of the four test panels was inserted into frame S2 so direct access to the food was blocked. In general, there was no consistent test protocol for this proof-of-concept study with regards to the order of the presented test panels and the exact length of each test session. We aimed to present each test panel 10 times to each group. The order was altered between both groups and decided ad hoc with the stipulation that the same panel was not used twice on the same day. Test sessions had a minimal duration of 30 min when the problem was not solved but sometimes ran longer (success after 30 min did not occur). For practical reasons, sessions were sometimes, but not always, stopped as soon as a hen was successful and the problem was solved. Whenever a successful test session was not stopped, the TBA merely became a food location for the remainder of the time. The side S2 holding the respective test panel and its immediate surrounding were filmed in each test session (Aiptek AHD H12 Extreme Camcorder).

 TABLE 1 | Overview of success rates, mean solving times, and goal-directedness in each group.

	Test panel	Success rate [%]	Mean solving time [min:sec]	Goal- directedness [%]
Group 1	String	100	13:48	20
Group 1	Swing	80	10:17	75
	Slide	70	07:12	100
	Step	0	-	_
Group 2	String	100	01:38 [†]	37.5 [†]
	Swing	90	06:26	100
	Slide	60	21:12	100
	Step	10*	23:54	0

Note that solving times and goal-directedness only apply to successful sessions. *Malfunction of hatch; [†]Missing video did not allow analysis of two sessions.

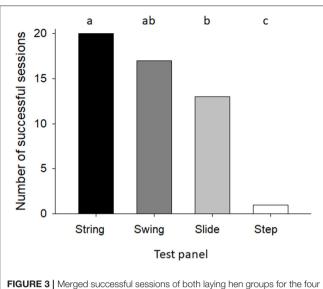
Analysis

We analyzed 10 sessions per test panel per group, so that there were 40 test sessions per group in total. Analysis of the videos included success (yes/no), time of success (min:sec; starting point was always when the test panel was inserted and the experimenters left the TBA after baiting), and whether a successful individual proceeded to obtain the food reward within a few seconds after solving the problem ("goal-directedness": yes/no). The behavior and engagement with the TBA of the groups could not be analyzed in detail due to the limited camera angle but informal live observations were made. We tried tentatively to identify successful individuals of the groups from the videos. However, this was only possible in very rough terms, for example by clear differences of plumage color or conspicuous bald spots in the plumage.

To analyze successful problem-solving, we first compared the success rates on the four panels between the two groups using a Pearson's Chi-squared test in a 4×2 matrix (i.e., 4 panels \times 2 groups). As no significant differences (P-value threshold 0.05) appeared across the success rates between the groups, we merged their data for the subsequent tests. Then, we tested whether the success rates across the four panels differed using a Pearson's Chi-squared test in a 4×2 matrix (i.e., 4 panels \times success yes/no). For pairwise post-hoc comparisons we compared the success rates on the different panels with each other using Fisher's Exact Test for Count Data in 2×2 matrixes (i.e., 2 respective panels x success yes/no). All tests were calculated with R 4.0.3 (R Core Team, 2020; R code of the analyses is provided in the Supplementary Material). Behavioral observations were reported and the latencies and the "goal-directedness" of the successful birds were presented in a descriptive manner (see Table 1).

RESULTS

In both groups of laying hens, the animals habituated to the TBA with open frames immediately and learned quickly that it held a preferred food reward. A majority of hens was highly



test panels: swing panel (black), string panel (dark gray), slide panel (light gray), and step panel (white). Different letters above the bars indicate significant differences in pairwise *post-hoc* comparisons between the test panels. The single success with the step panel was due to a malfunction of the hatch.

motivated to approach and engage with it to obtain the food. During baiting in test sessions, we observed that almost all hens were in close proximity to the TBA, limited only by the crowded space. Numerous birds explored and pecked the device with their number declining over the course of a session when no bird successfully solved the problem. Conservatively, there were at least five individuals in each group that solved at least one problem and there were single individuals that solved more than one problem. The problems were always solved by a single successful individual, not by a "group effort." However, considerably more than five individuals approached and engaged with the TBA in every session.

We found a similar gradient of success levels across the four test panels in both groups and the success rates between the two groups did not differ ($\chi^2 = 1.17$, df = 3, p = 0.77).

The string panel was solved in every session in each group but successful animals proceeded to obtain the food reward afterwards in only 20 and 37.5% of successful string panel sessions, respectively. The success rates with the swing panel and the slide panel lay between 60 and 90% and successful individuals had a high rate of immediate reward in these sessions (75-100% of instances). The step panel was solved only once in one group and this was due to a malfunction when the hatch opened after a light touch (see Table 1 for more detailed results). The success rates for the four test panels differed significantly ($\chi^2 = 26.85$, df = 3, p < 0.0001; Figure 3). The pairwise(*post hoc*) comparisons revealed that success rates between string panel vs. slide panel (p = 0.008), string vs. step (p < 0.0001), swing vs. step (p < 0.0001), and swing vs. step (p < 0.001) were significantly different from each other, while string did not differ from swing (p = 0.23) and swing did not differ from slide (p = 0.27) (see Figure 3).

DISCUSSION

Our study aims have been met in the first application of the TBA device. We found that (1) three of the four test panels were solved at high success rates. At least 10 individuals had the agency and control to spontaneously solve at least one novel problem to gain a reward. We observed the majority of hens to be highly motivated to approach, explore, and engage with the TBA whenever it was baited. We also found (2) that the exchanging of panels and baiting proved to be quick and easy for the experimenters and hardly affected daily caretaking routines. As a proof of concept, it was a promising success and demands further exploration of this device as a cognitive enrichment and research method.

There was a significant gradient of success rates with the string panel being the easiest (100% success rate in both groups), followed by the swing panel (80 and 90%), the slide panel (60 and 70%), and finally the step panel that was never solved by means of the intended mechanism. While adjustments might be needed if the difficulty of the step panel turns out to be too high in general, a gradient of success rates is promising for more controlled studies as it can reveal variety and individual differences. The avoidance of ceiling and floor effects is a prerequisite for the design of validated cognitive test batteries (see e.g., Völter et al., 2018), for example with a "mini test battery" approach as a first building block (Schmelz et al., 2015; Shaw and Schmelz, 2017).

An interesting finding of the current study was the fact that successful animals with the string panel more often than not did not obtain a food reward immediately afterwards. In comparison, successful individuals with the swing panel and slide panel almost always proceeded to obtain the food reward. This is most likely due to the hatch of the string panel opening to the outside, so that bystander animals could enter the TBA faster than the ones opening it. However, this also (by chance) created an interesting variation across the different panels, as successful animals were mostly, but not always the ones being rewarded immediately. Because of this, monopolization was precluded and it suggests that the engagement with the TBA might potentially have been intrinsically rewarding without a direct food reward (Clark, 2017). Future studies should investigate if and to what extent animals keep on engaging and solving problems when there is consistently no food reward or when they can choose to obtain identical food without "working" for it (see Langbein et al., 2009).

As our approach (1) engaged the laying hens' cognitive skills by offering problems to solve and control over their environment while (2) providing extrinsic and potentially also intrinsic rewards, the TBA fulfilled at least two of the three conditions of Clark's (2011, 2017) definition of cognitive enrichment. With regards to the third—validated measures of wellbeing—we did not observe any aggression or injuries during test sessions. Any heightened arousal and spatial competition was arguably rather an indicator of positive eustress and challenge and therefore of successful enrichment (Špinka and Wemelsfelder, 2011). However, for the application of the TBA as a cognitive enrichment device in commercial farming, the validation and quantification of welfare benefits will be the most crucial aim of further studies. Aggressive group competition, monopolization by dominant animals, negative stress, and other adverse effects of its application must be excluded. One approach could be a comparison of behavioral and physiological measures between groups with and without access to this device. Additionally, the TBA is adjustable with regard to the size and the panels to be applied to other farm animals for a validation of its wider use as cognitive enrichment, for example to different species of poultry such as turkeys (*Meleagris gallopavo* domesticus) or mammalian species such as pigs (*Sus scrofa* domesticus).

In laying hens, our proof of concept must be expanded with a more standardized and controlled study protocol than the current study and the possibility to identify individuals, for example by wing tags that can be differentiated on video. We could not analyze in detail how many hens of each group engaged and were actually successful with the TBA in this initial study. Based on our informal observations a majority of the hens approached and engaged with it at least during baiting but a formal confirmation and quantification is crucial for an enrichment and research method as both are only effective when more than a small subset of animals is included.

Finally, further research must validate which cognitive or noncognitive factors were targeted by our four problem-solving tasks and thereby add stronger insights to our knowledge of chicken cognition. This also contributes to the ongoing discussion of operant problem-solving studies and what they actually test (see e.g., Griffin and Guez, 2014; van Horik and Madden, 2016). While group-level testing has advantages (e.g., the familiar environment causing lower stress, ease of testing, and application in farm settings), individual tests of single animals separated from the group to engage with the TBA on their own will be needed. It allows a comparison of differences on an individual level to investigate if the hens engage in similar ways with the TBA and if individuals show consistent success rates across tasks and over time. With additional behavioral observations of non-cognitive factors like activity and exploration we can then correlate these to their problem-solving skills and success rates (see van Horik and Madden, 2016). Individual tests compared directly to group tests can also reveal how many individuals are successful with free access to the TBA and more time before a problem is already solved by another individual.

In conclusion, the laying hens in this study could have been unsuccessful or avoiding the TBA altogether. The practical application could have been complicated and time-consuming. However, this was not the case. Even though further research is needed, the TBA is a promising novel approach both for animal cognition research and for cognitive enrichment in farm animals in general and laying hens specifically. In our proof of concept, we could show that it is cheap to build and easy to apply with minimal disruption of daily routines in farming. By design, it is flexibly expandable and its potentially limitless

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DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the study device was merely provided as enrichment and none of the animals was handled or restricted at any time for this study. Laying hens were kept in accordance to the German laws (TierSchNutzV) and housing and management were conducted to respective farming procedures with the permit no. DE276033510060555. Commercial layer feed and water were provided *ad libitum*.

AUTHOR CONTRIBUTIONS

MS designed the basic idea, apparatus, and procedure, collected and analyzed the data, and wrote the manuscript. EK contributed to the apparatus and procedure, collected and analyzed the data, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fanim. 2021.671905/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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