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Potential of application of a modified open-field test for selection of laying hens

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The aim of the study was to propose a modification to the classical open-field test (OFT) to make even more reliable and informative assessment of behavioural reactions. Birds' quick locomotion assessed with OFT does not necessarily indicate a high level of fear and an attempt to escape, but it can involve a considerable level of sociability and willingness to return to the flock. Those two motivations, fear and sociability, are not distinguishable with the classical OFT; the test does not provide unambiguous assessment of the motivation of birds' behaviour and emotions. Given this shortcoming of the open-field test, a question arose whether the use of environment-enrichment objects in the open-field test would ensure greater objectivity in assessment of hens' behaviour.

The study involved 150 laying hens – 50 Green-legged Partridge chickens (GLP), 50 Polbars (Pb), and 50 Leghorns (L), reared in a single commercial farm. All the birds were 30 weeks old at the time of assessment. The hens were subjected to a modified open-field test (MOFT). The modification involved introduction of environment enriching objects, which were novel for the birds. The box comprised containers with water, commercial feed, feed supplemented with finely fragmented cereal spikes, finely cut cereal straw and insect larvae as well as a sandpit, a mirror, and an imitation of a nest. The test lasted 600 seconds. Six different behavioural indicator traits were recorded (MOFT1-MOFT6). Spearman's rank correlations between the traits were estimated. The MOFT results enabled to distinguish such hens' traits as curiosity, inquisitiveness/exploratory behaviour, and excitability. The most important indictors include the duration of exploration of the area, the number of explored objects, time spent on exploration of the objects, and the number of squares covered. It appeared that a single behavioural indicator – the latency of undertaking physical activity – can be used for selection and breeding practice. This indicator is correlated highly with the others and is simultaneously easy and quick to assess in farm conditions. Hence, the latency of undertaking activity can potentially be used in assessment of hens' behaviour to evaluate curiosity/fearfulness and emotional excitability as correlated behaviours.

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The welfare of laying hens becomes essential concern in the poultry industry worldwide. This is associated with the constantly growing consumers' awareness of the living conditions provided to farm animals and the impact of their welfare on the production output. Birds' welfare can be improved via modification of the living environment (enlargement of the surface area, addition of enrichment elements, etc.) and assessment of temperament as one of the selection criteria in breeding programs [Rozempolska-Rucińska *et al.* 2017a, b]. Recent research has been targeted on modification of birds' behaviour at the additive genetic level, i.e. via selection and incorporation of behavioural traits into the selection criterion [Rozempolska-Rucińska *et al.* 2017a, b.] Although such suggestions were considered several years ago [Siegel 1993, Mench 1992, Craig and Swanson 1994, Jones 1996], it is still an unresolved problem on commercial farms. One of the most important traits with a significant impact on welfare is fearfulness. Farm birds should not display high levels of anxiety, which elevates stress, affects health, and causes pterophagy, as shown by research results [Schutz *et al*. 2004, Buitenhuis *et al.* 2005, Hocking *et al.* 2001, Rodenburg *et al.* 2004]. It seems that the level of fear in hens can be reduced by enhancing their curiosity. There is significant negative correlation between these traits. However, the NOT test used in earlier studies [e.g. Rozempolska-Rucińska *et al.* 2017b] seems not to be suitable enough, as it does not assess the entire range of hens' behaviours but focuses only on reactions related to curiosity/fear. However, as demonstrated in many studies, the behavioural problems of laying hens are not associated only with the fear emotion: excessive locomotor activity associated with pterophagy plays a significant role as well [Kjaer 2009, Rodenburg *et al.* 2004]. One of the best known tests for assessment of birds' temperament is the open-field test. It addresses the quantitative aspects of behaviour after placing the animal in a new open environment. Yet, there are many doubts about the assessment of the traits that are theoretically measured by this test [Carter *et al.* 2013, Pearls *et al.* 2017]. It is used successfully to measure activity and exploration [Boyer *et al.* 2010] or courage [Brown and Braithwaite 2004]. As shown by many authors [e.g. Minderman *et al*. 2010, Carter *et al.* 2013], the reactions observed may be motivated not only by fear, but also by the effect of social isolation. Birds' quick locomotion does not necessarily indicate a high level of fear and an attempt to escape, but a considerable level of sociability and a willingness to return to the flock [Forkman *et al*. 2007, Carter *et al.* 2013, Pearls *et al.* 2017]. Given the drawbacks of the open-field test, e.g. the failure to clearly assess birds' behaviour [Carter *et al.* 2013, Pearls *et al.* 2017, Boyer *et al.* 2010, Forkman *et al.* 2007], the question is whether the use of environment-enrichment objects in the open-field test will yield more objective assessment of hens' behaviour. It was assumed that hens' reactions that are normally observed in the open-field test combined with responses to the enriching objects should facilitate interpretation of birds' behaviour and assessment of emotions. The research hypothesis assumed that birds that do not exhibit a high level of fear would be willing to explore the

environment and show interest in the objects. This assumption is supported by the fact that fear blocks motivation systems, thereby impairing birds' ability to adapt to changing environmental conditions [Jones 1996, Hocking *et al.* 2001, Rodenburg *et al.* 2004]. Simultaneously, the duration of exploration of objects and locomotion in the experimental box can be evaluated easily and objectively, additionally revealing the level of excitability. If the research assumption is correct, the modification of the open-field test should facilitate assessment of fearful/curious birds with evaluation of their reactivity degree; hence, the selected indicators can potentially be used for assessment of hens' behaviour as a selection criterion.

Therefore, the aim of the study was to describe the new attributes of the modified open-field test and answer the question of a potential use of the results of this test as selection criteria in laying hens.

Material and methods

All procedures used during the research were approved by the II Local Ethics Committee for Animal Testing at the University of Life Sciences in Lublin, Poland (Approval No. 69/2017 of 28 September 2017).

The study involved 150 laying hens reared in one farm, including 50 Green-legged Partridge (GLP) chickens, 50 Polbars (Pb), and 50 Leghorns (L). The choice of several breeds was intended to provide an answer to the question whether the results are breed specific or can be treated universally. The GLP and Pb are local breeds kept as gene pool reserves, where no selection is carried out, whereas breed L is a highly productive laying breed used in commercial farms most frequently. All birds were 30 weeks old when put to test. They were kept in 6 group boxes with 25 hens in each, at a density of 0.3 m^2/individual. The boxes were equipped with nipple drinkers, a feeder, and a nest with straw bedding. A 16-hour light regime was maintained. The tests were carried out between 8.00 and 15.00 h for 6 days, and 25 birds kept in one box were assessed every day. All birds were evaluated once, as repetition of the experiment would eliminate the novelty of the field elements. Concurrently, if the potential of practical application of the test were confirmed, repetition of the test for hens would be impossible due to the organisation of farm work and the time consumption related to the test.

All birds were subjected to the open-field test [Rodenburg *et al*. 2003]; however, the environment was enriched with various objects, which were novel to the birds. These included containers with water, commercial feed, and feed supplemented with finely shredded cereal spikes, finely cut straw, and insect larvae as well as a sandpit, a mirror, and a shelter imitating hen nest. None of the objects was known to the birds beforehand. The feed/water containers were unknown to the birds, either. On the farm, feed and water were supplied from feed conveyors and nipple drinkers. Importantly, the containers with food/water had such high walls that the bird had to approach them close to see the content. This solution was intended to rule out the absence of birds' interest in the other objects at the sight of food. It was agreed that the feed should be

introduced as an enrichment object, as interest in feed and intake indicates that the animal does not experience fear or severe stress at that time [Forkman *et al.* 2007].

To carry out the MOFT, a special observation box of a 1.25 x 1.25 m area divided into 25 squares with a surface area of 25 x 25 cm each was constructed. A camera recording birds' behaviour throughout the test was mounted above the box. Each bird was recorded individually in the test box for 600 seconds, and then the video films were analysed. The experimental box was located in the same farm building where the hens were kept but constituted a separate room with identical lighting and temperature conditions as in the entire farm building. The birds were transferred to the experimental room directly from their cages. Before the experiment, the birds were not deprived of food to avoid an impact of this factor on the desire to use the feed.

The birds were placed in to the experimental box individually and placed in its central point to keep the same distance from the enrichment objects.

The enrichment objects were placed at the left and right walls of the box and arranged as shown in Figure 1.

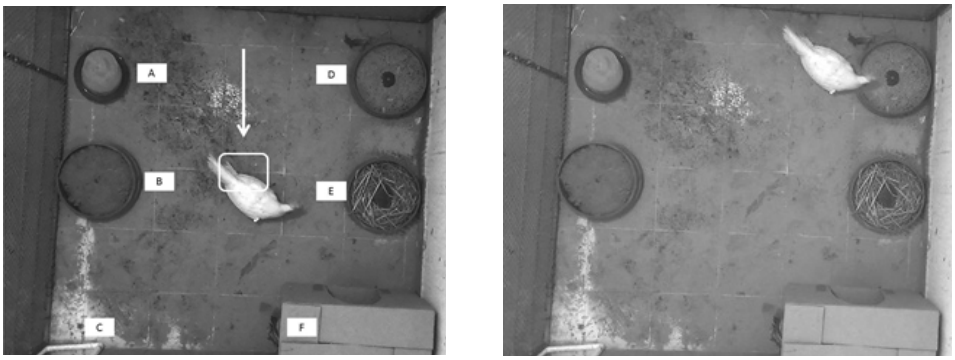

Fig. 1. Observation box. A – water container; B – sandpit; C – mirror; D – commercial feed; E – enriched feed; F – shelter.

The recording began one minute after placing the bird in the box to give the experimenter time to leave the room.

The analysis of the footage consisted in assessment of the MOFT1-MOFT6 indicators (Tab. 1) of birds' behaviour. Thirty of the 150 birds, including 9-Zk, 11-PB, and 10-L, did not explore the environment.

The mean levels of the studied traits for each breed are presented in Table 2.

The value 0 of the MOFT3-MOFT6 indicators means that the bird did not explore the objects and did not move. This behaviour was only noted in the 30 aforementioned birds that did not undertake exploration (MOFT2=600). The results of the test were analysed statistically with Spearman's rank correlations between the indicators.

Table 1. Assessment of hens' behaviour in the research procedure

Indicator	Description	Units
MOFT1	time between placing the bird on the floor in the experimental box and the first movement, e.g. head movements, looking around, but no locomotor activity	seconds (s); 600s were assigned when a bird did not undertake any activity throughout the test
MOFT2	time between placing the bird on the floor in the experimental box and the beginning of locomotor activity	seconds, 600s were assigned when a bird did not undertake exploration throughout the test
MOFT3	interest in the enrichment elements: water container, commercial feed, enriched feed, shelter, mirror, sandpit. Bird's interest was manifested by approach to the object and exploration thereof with the beak; the bird looked at the object standing next to it or entered the object	sum of point scale 1-0, where 1 denoted approach and interest in an enrichment element, 0 – no interest. The maximum value of the object depended on the number of objects in which the bird was interested. The maximum value was 6 if the hen showed interest in each object
MOFT4	number of squares covered by the bird	minimum value 0 if no exploration was undertaken by a bird; the maximum value depended on the number of squares covered by the hen
MOFT5	time spent by the hen observing and showing interest in the enrichment elements	seconds
MOFT6	duration of locomotion throughout the test, excluding the time of observation of the objects	seconds

Table 2. Mean level of traits in the modified open-field test

Indicator	Breed	Mean	Standard deviation	Min.	Max.
MOFT1 (s)	PB	92	197	1	596
	Zk	47	141	1	580
	L	81	137	1	565
MOFT2 (s)	PB	257	237	1	600
	Zk	303	244	1	600
	L	284	230	1	600
MOFT3 (number)	PB	1.7	1.5	0	5
	Zk	1.5	1.5	0	5
	L	2.4	2.1	0	6
MOFT4 (number)	PB	6	8	0	44
	Zk	5	8	0	44
	L	25	33	0	110
MOFT5 (s)	PB	163	166	0	519
	Zk	156	165	0	561
	L	163	154	0	514
MOFT6 (s)	PB	236	154	0	544
	Zk	213	159	0	507
	L	200	123	0	443

Results and discussion

In farm breeding, excessive reactivity and fearfulness is perceived as undesirable animal behaviour associated with a negative emotional state [Boissy 1998], which may lead to a number of disorders [Cocrem 2007]. In a monotonous and dull breeding environment, birds are not able to satisfy their emotional excitability, e.g. exploration and curiosity needs, which may lead to pterophagy, cannibalism, and self-destructing behaviours [Rodenburg *et al.* 2003, Ghareeb *et al*. 2008, Rose and Croft 2015]. Improvement of the environmental conditions does not solve the behavioural problems due to the multifactorial character of causes of these disorders [Lay *et al.* 2011, Rodenburg *et al.* 2008]. In terms of genetics, the genotype of birds should be adjusted to breeding systems in order to fit their repertoire of behaviours and emotional level to their living environment [Kjaer *et al.* 2001, Flock and Norman 2008]. This could facilitate reduction of the stress level and, consequently, behavioural anomalies. This is particularly important in reproduction flocks, as stress-related hormones may accumulate in hatching eggs, resulting in further behaviour anomalies in the offspring [Freire *et al.* 2006, Janczak *et al.* 2006].

The study assessed birds' behaviour by measurement of several indicators (Tab. 1) and estimation of their interrelationships (Tab. 3-5).

The highest rank correlation in each breed was found between indicators MOFT3 and MOFT4 (Tab. 3-5). Birds that were interested in many enrichment objects (MOFT3) covered a large number of squares (MOFT4), which means intensive locomotor activity during the test. This high rank correlation (over 85%) should not be surprising since hens that were interested in many objects (MOFT3) had to cover a greater number of squares on the floor (MOFT4), as they had to approach the object. To a certain extent, the MOFT4 indicator may serve as a criterion of birds' reactivity, as it reflects the intensity of locomotion during the test: the greater the number of squares covered by the bird within a given time, the faster its locomotion in the box. The locomotor activity within the experimental box is one of the traits assessed during standard open-field tests. Intensive locomotion in the test box is undoubtedly associated with emotional stimulation, which can be caused by either negative or positive emotions [Zimmerman *et al.* 2011, Crino and Breuner 2015]. Behavioural response alone does not provide explicit information about the emotions and level of stress experienced by the animal [Cocrem 2007]. Such response to a specific stimulus may be similar when the bird experiences stress and when the HPA axis is not activated [Cockrem and Silverin 2002]. Researchers emphasise that hens' reactions can be motivated by different emotions [Marino 2017, Carvalho *et al.* 2018]. However, the result of the present experiment indicates that the locomotor activity of birds during the test was motivated by positive emotions, i.e. curiosity in this case. Under strong negative emotional stimulation, animals are unable to explore objects systematically for a long time, but they move around the space rather fast and chaotically [Forkman *et al.* 2007]. Birds experiencing negative emotions would not express interest in objects present in their environment [Hocking *et al.* 2001, Rodenburg *et al.* 2004, de

Table 3. Rank correlations between parameters analysed in the Pb breed

Type of test	MOFT1	MOFT2	MOFT3	MOFT4	MOFT5	MOFT6
MOFT1	*	0.50	0.32	-0.39	-0.31	-0.16
MOFT2	0.00		-0.73	-0.80	-0.75	-0.44
MOFT3	0.02	0.00		0.85	0.73	0.13
MOFT4	0.00	0.00	0.00		0.64	0.45
MOFT5	0.02	0.00	0.00	0.00		-0.40
MOFT6	0.33	0.00	0.41	0.00	0.01	

*Above the diagonal – rank correlation estimates; below the diagonal – significance level p.

Table 4. Rank correlations between parameters analysed in the GLP breed

Type of test	MOFT1	MOFT2	MOFT3	MOFT4	MOFT5	MOFT6
MOFT1	*	0.58	0.55	-0.51	-0.55	0.01
MOFT2	0.00		-0.82	-0.81	-0.80	-0.48
MOFT3	0.00	0.00		0.92	0.81	0.07
MOFT4	0.00	0.00	0.00		0.74	0.34
MOFT5	0.00	0.00	0.00	0.00		-0.53
MOFT6	0.95	0.00	0.67	0.05	0.00	

*Above the diagonal – rank correlation estimates; below the diagonal – significance level p.

Table 5. Rank correlations between parameters analysed in the L breed

Type of test	MOFT1	MOFT2	MOFT3	MOFT4	MOFT5	MOFT6
MOFT1	*	0.74	-0.64	-0.70	-0.68	-0.20
MOFT2	0.00		-0.81	-0.86	-0.83	-0.48
MOFT3	0.00	0.00		0.85	0.79	0.25
MOFT4	0.00	0.00	0.00		0.68	0.55
MOFT5	0.00	0.00	0.00	0.00		-0.19
MOFT6	0.22	0.00	0.12	0.00	0.23	

*Above the diagonal – rank correlation estimates; below the diagonal – significance level p.

Haas *et al.* 2013]. As shown in the test, the correlation between the locomotion and the number of explored objects was positive and very high, i.e. 0.85 – PB, 0.92 – GLP, and 0.86 – L.

The duration of observation of the objects (MOFT5) was highly correlated with the number of explored objects (MOFT3). A high (nearly 80%) rank correlation implies that both indicators assess an analogous trait, i.e. probably curiosity. Curious birds are interested in many objects available in the environment and devote a significant amount of time to exploration thereof [Pearls *et al.* 2017]. The present study demonstrated that birds that were interested in many objects also spent considerable time on exploration. However, the value of the correlation in the range from 0.73 to 0.81 (Tab. 3-5) suggests some differences between the indicators. MOFT5 can also provide information about the level of inquisitiveness/ exploratory behaviour.

The value of the rank correlations between the number of explored objects and the time devoted to the exploration thereof suggests that curiosity and inquisitiveness/ exploratory behaviour are closely related to each other, but they do not have to be identical to each other. There may exist curious but little inquisitive hens/There may exist curious hens without exploratory behaviour. Excessive curiosity may not be recommended in breeding. It has been shown that the ancestor of current breeding poultry was less active and less eager to explore an unknown environment [Jensen and Andersson 2005].

A highly significant correlation was also found between the number of covered squares (MOFT4) and the duration of exploration of the objects (MOFT5). The correlation at the level above 0.65 is a good predictor, as it indicates that inquisitiveness/ exploratory behaviour does not necessarily mean excitability in hens (high MOFT4). Excitability is not a desirable trait in poultry rearing. Positive correlations have been shown between high activity/excitability in the open-field test and pterophagy in hens [Rodenburg *et al.* 2004, Kjaer 2009].

The rank correlations between MOFT1 (latency of movement without locomotion) and MOFT2 (latency of exploration) were analysed as well. Their values of 0.50-0.75 indicate that similar traits are assessed, but they are not the same personality traits. Birds that moved their head within a short time did not necessarily start moving around the object within a similarly short time. This may indicate that some birds explored the area only through head movements shortly after being placed in the new environment, but they could take longer to decide whether to move around the area. Thirty individuals did not undertake exploration at all. The assumption that MOFT1 and MOFT2 assess slightly different temperament traits is supported by the different correlations between them and the other indicators analysed. Lower rank correlations were found for MOFT1. Simultaneously, the value of the correlations between MOFT1 and the other indicators was dependent on the breed and was in the range from 0.01to 0.59 for GLP, -0.16 to 0.51 for Pb, and -0.20 to 0.74 for L. This indicator is not useful for assessment of hens' behaviour, as it is breed specific. An analogous conclusion can be formulated in the case of the MOFT6 indicator.

While analysing the results of the study, attention should be paid to the high negative value of the rank correlations between the latency of exploration of the environment (MOFT2) and the other indicators (MOFT3-MOFT5) (Tab. 3-5). Studies on other animal species have shown a very high negative correlation between the latency of entry into the open field and locomotor activity in the open field [Daniewski, Jezierski, 2003].

The latency of taking up activity can be explained differently: as a desire to escape and hide in the unknown environment and the desire to return to the flock, i.e. responses caused mainly by the emotion of fear, or as willingness to explore the environment prompted by curiosity and positive emotions [Forkman *et al.* 2007; Kopowski *et al.* 2002]. It was shown in the present study that birds that quickly began exploration approached many objects and devoted substantial time to exploration

thereof, i.e. they exhibited a high level of curiosity and inquisitiveness/exploratory behaviour. Depending on the breed, the correlations ranged from -0.76 to -0.84. This indicates that birds that quickly undertook activity experienced positive emotions.

A conclusion can be formulated that the modified open-field test refines assessment of hens' behaviour in terms of their curiosity, inquisitiveness/exploratory behaviour, and excitability. The most important indicators include the latency of exploration of the environment, the number of objects explored, the time devoted to exploration thereof, and the number of squares covered while moving. Single indicator can be used in selection and breeding practice, i.e. latency of undertaking activity. This indicator is correlated highly significantly with the others and is simultaneously easy and quick to assess in farm conditions. Hence, it can potentially be used in assessment of hens' behaviour in terms of the most important breeding traits, i.e. curiosity/fearfulness and emotional excitability.

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