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# Genetics of the Novel Object Test outcome in laying hens

Iwona Rozempolska-Rucinska[a], Lucyna Kibala[b], Tomasz Prochniak[a], Grzegorz Zieba[a], Marek Lukaszewicz[c,*]

[a] *Department of Biological Bases of Animal Production, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland*
[b] *Centre for Nucleus Breeding MESSA Ltd., Mienia 100, 05-319 Ceglow, Poland*
[c] *Institute of Genetics and Animal Breeding, Postepu 36A, 05-552 Jastrzebiec, Poland*

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ABSTRACT

The purpose of the study was to answer the question whether the behavioural reactions to a novel object can serve as a criterion in selection toward lowering the fearfulness level in laying hens. Fear can be a harmful emotional state resulting from perceiving of an experience as a potential danger. High level of fearfulness is negatively correlated with the overall laying performance. Reactions to the Novel Object Test (NOT) of 9483 Rhode Island White (RIW) and 4326 Rhode Island Red (RIR) hens were camera recorded for 30 s per hen. Six reactions were defined following the analysis of the footage: escape, observing, avoidance, approach, avoidance-approach, and pecking. A hen was attributed only one reaction, and no other combinations were encountered. The variance components of the reactions regarded the animal additive genetic and the residual effects and were estimated with Gibbs sampling software accounting for binomial character of the NOT output. Two groups of reactions positively correlated within a group and negatively between the groups allowed for distinguishing of two behavioural profiles − "exploratory" (observing, approach, pecking) and "fearful" (avoidance-approach, avoidance, escape). The rearranged into profiles output of NOT (two-trait) was processed with the same model to estimate the genetic parameters for the profiles. The genetic correlations between the profiles were negative at ca. 0.70 while the heritabilities reached 0.17/0.19 for the exploratory profile and 0.19/0.08 for the fearful profile, in RIW/RIR. Except for the heritability of the fearful profile in RIR, the profile heritabilities were higher than heritability of any of the 6 basic reactions. Our recommendation is to indirectly lower the fearfulness level by selecting for exploratory behaviour based either on the exploratory profile or on the basic behaviour of pecking alone as this is a reaction of a sufficiently high heritability (0.13–0.16) and easy to define.

## 1. Introduction

Fear is one of the basic emotions regulating the way the animals react to the stimuli of the surrounding. However, fear can be a harmful emotional state resulting from perceiving of an experience as a potential danger (Boissy, 1998). The research conducted as early as in 1960′ (Jones, 1996) stated that fear is a phenomenon exerting negative impact upon an organism, in particular, when it is frequent or intensive. In hens, high level of fearfulness is negatively correlated with laying rate, eggshell quality, growth rate, feed conversion, and plumage condition (Jones, 1996; Jones et al., 1997; Schütz et al., 2004). Excessive fear also blocks natural drives' systems impairing the ability of birds to adapt to changing environmental conditions and proper interaction with other individuals, as illustrated by an anomaly of feather-pecking (Jones, 1996; Hocking et al., 2001; Rodenburg et al., 2004). Simultaneously, fear can easily degenerate into panic in the flock which brings about the jeopardy of physical injury or even death. Such behaviours caused that extensive research on how to lower the level of fearfulness in hens have been conducted for long, from both the bird's and the breeder's standpoints (Kjaer et al., 2001; Rodenburg et al., 2004; Uitdehaag et al., 2006, 2008a,b; de Haas et al., 2013). So far, it has been shown that opportunities to lower the level of fearfulness through environment enrichment, regular taming, and diet supplementation exist (Jones, 1996). Suggestions to hire genetics to help improve the adaptability of birds to stressful environment emerged a couple of decades ago (e.g. Mench, 1992; Craig and Swanson, 1994; Jones, 1996) but we still lack a clear and simple criterion that would be easy to evaluate under the commercial farm conditions.

The Novel Objects Test (NOT) evaluating the fearfulness level in chickens has been shown to be the easiest to perform, while remaining reliable (Jones, 1996; Forkman et al., 2007; Uitdehaag et al., 2008b). A question then arises, whether the NOT results, as specific responses of

* Corresponding author.
*E-mail address:* m.lukaszewicz@ighz.pl (M. Lukaszewicz).

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the chickens, can represent the phenotype in the process of breeding value prediction in the context of the fear emotions. The answer to this question depends largely on the level of genetic determination of particular responses to NOT, as only sufficiently heritable traits can be efficiently ameliorated genetically.

The study aimed at getting the answer to the question whether the behavioural reactions to a novel object can serve as a criterion in selection toward lowering the fearfulness level in laying hens.

## 2. Materials and methods

### 2.1. Characteristics of the stock

The analyses regarded two hen lines: Rhode Island White (RIW) and Rhode Island Red (RIR). The birds were caged separately in a windowless, artificially lit building equipped with a mechanical ventilation system. The cages were furnished with nipple drinkers and a mechanical feed delivery system. The hens received veterinary supervision. The birds were assessed individually for performance traits. The mating system employed in the farm minimized the level of kinship between the mated individuals to control the inbreeding increase in the flock. In each season, the reproduction stock was formed by setting up family flocks of 12 hens and a cockerel, each. Hens were artificially inseminated and hatch eggs were collected for 14 consecutive days.

Since the data were obtained within the routine performance recording in a commercial farm, Animal Care and Use Committee approval was not warranted.

### 2.2. Behavioural test

Birds' behaviour was assessed with NOT, which is the most useful tool for estimation of birds' emotional state; in particular for assessment of fear-related emotions (Jones, 1996; Forkman et al., 2007; Uitdehaag et al., 2008b). It consists in defining animals' behaviour as response to a novel object appearing in their sight. The test evaluated emotional reactions of the birds in circumstances similar to those encountered by the hens every day − while recording the birds' reaction, the staff repeatedly approached the cages, in an analogous manner to the routine reading of the individual bar codes with a laser scanner. The choice of this test was supported by the fact that the results obtained should eventually have a practical application, therefore, they should characterize hens' behavioural responses in conditions similar to the standard management procedures. A novel object, which was a shiny pencil, was inserted at a distance of 1 cm from the cage wall towards the hen and held still in that position for 30s. The test was carried out in turns, every four cages apart, so that the birds from the neighbouring cages could not see the object beforehand. The test was performed in two consecutive generations and the behaviour of 9483 RIW and 4326 RIR hens was recorded. The youngest hens were in their 33rd week of laying. Given the large number of the examined hens, the test was performed in the same environmental conditions for several consecutive days. Birds' behaviour was video-recorded by the farm staff and the footage was analyzed for the behavioural responses by one person, afterward.

The distinct behavioural reactions, their description, and the percentage of individuals manifesting those reactions are presented in Table 1.

The behavioural reactions were recorded as binary outcomes, where 0 denoted the lack and 1 the presence of a behaviour. Only one of the reactions could have been assigned to a hen and no other possible combinations of behavioural reactions were encountered.

Having analyzed the (co)variance components of particular reactions, two overall behavioural profiles were defined based on either the fearfulness or the exploration notions.

### 2.3. Statistical analyses

As the first step the estimation of (co)variance components of the six traits describing the behavioural reaction of the birds to NOT (Table 1) were computed based on the model fitting the fixed effect of year-of-hen's-hatch × hatch-within-year and random genetic additive effect of an animal combined with the numerator relationship matrix. At the second step the same model was used for variance component estimation of the resulting behavioural profiles (two-trait) concluded from the results of the first step (see the "Discussion" section). The applied model was decided following an introductory ANOVA, at which we excluded the effects of the cage battery and the floor within a battery as insignificant for explanation of the behavioural reactions' variance. Since only one behavioural reaction was ascribed to a hen the remaining reactions were set to zero within a hen, what required zeroing of the residual covariances between the behaviours.

The pedigree was three generations deep (including two behaviour recorded generations). Estimation of the (co)variance components was performed with the THRGIBBS1F90 software (Tsuruta and Misztal, 2006), which accounts for the discrete character of the behavioural profile denotation. Three hundred thousand samples were obtained with 100,000 discarded as burn-in, following graphical inspection of the posterior chain, inspection of the effective sample size of the parameter of interest as well as checking of the diagonal of the error variance which all elements mixed/converged to unity.

## 3. Results

The behavioural reaction of escape is moderately (0.35) to highly (0.61) correlated genetically with the behaviours of avoidance and avoidance-approach, in both RIW and RIR (Table 2). Similarly, the trio of observing, approach, and pecking is moderately to highly correlated positively within. At the same time, these two groups of reactions are negatively correlated with each other. This pattern is less pronounced in the RIR population.

The heritabilities of the two behavioural profiles built on the six basic reactions to NOT reach 0.19 and have decently low standard errors (Table 3). Only the fearful profile in RIR is low heritable, at 0.08. In both breeds the genetic correlations between the profiles are high and negative.

The heritability estimates for distinct behaviours in reaction to NOT are given in Table 4. The highest ones are found for the observing and pecking behaviours, while majority of them are lower than 0.10, in both breeds.

## 4. Discussion

The behavioural reactions are indirect information about the experienced emotions and, excluding an assessment at the physiological level, are the only indication if an animal perceives a given situation as potentially significant (Desire et al., 2002).

Frightened animals express different behavioural reactions rather than just a single one, common to all animals. There is no doubt that the escape and the avoidance point to the fear emotion (Jones, 1996; Stankowich and Blumstein, 2005). In the current study the genetic correlations between those reactions were moderate in both breeds, reaching 0.47 in RIW and 0.35 in RIR (Table 2). Thus, it can be concluded, that the escape and the avoidance point to different intensities of experienced fear.

The second group of traits collected reactions evidencing exploratory behaviour of the hens and their lowered fearfulness: namely, the approach and pecking. The lack of fear alone, with no exploration involved, could manifest itself solely by no escape or by avoidance, and lack of interest in the object. In both breeds the genetic correlation between approach and pecking was very high at 0.69. This may mean that both reactions genetically denote almost one trait − exploration −