BREEDING AND HATCHERIES

Exploring animal genetic resources of the domestic chicken and their behavior in the open field

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Primary Audience: Researchers

SUMMARY

Animal welfare is of general interest, and in the farming arena, efforts have increased to create optimal conditions for poultry production. Detailed and standardized studies on specific behavioral issues, such as fear, exploration, and social reinstatement behavior, are needed to support these initiatives. The variables mentioned can be directly queried from the animal in the open field to provide information on welfare-related behavioral traits, such as general fearfulness and balanced emotionality. Animal genetic resources help us get an insight into the possible diversity of behavioral responses. The majority of these responses can be identified as breed-specific and thus genetically correlated, providing a starting point for future breeding objectives. The behavioral responses of a chicken can be related to the breeding history of the population or to the experience gained during its lifetime. Both may go hand in hand to enable the animal to adapt to its environment, contributing to animal welfare.

Key words: behavior, open field test, exploration, fear, local chicken breeds

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DESCRIPTION OF PROBLEM

Animal welfare, as defined today, encompasses not only an absence of fear but also the provision of positive affective experiences (Mellor et al., 2020). To achieve positive welfare, the environment should encourage so-called behavioral diversity (Miller et al., 2020). Behavioral diversity covers a wide range of species-specific behaviors, for example, exploratory behavior, and describes the need and ability of an animal to exhibit its behavioral repertoire. While the behavioral diversity model has, to date, only been applied to zoo animals (Ramis et al., 2020; LaDue and Schulte, 2021), it is becoming appropriate and even necessary to transfer the approach to farm animals in terms of the fifth domain of welfare, which addresses mental health and emotional experiences (Mellor et al., 2020).

Rats were the first animals to be observed for their emotional behavior in an unfamiliar field situation (Hall, 1934). Since then, a wide range of animals has been tested in the so-called open

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field test, including horses (Wolff et al., 1997), pigs (Haigh et al., 2020), and chickens (Faure et al., 1983). This test is designed to reveal an individual's emotionality in terms of general fear (Forkman et al., 2007) and personality traits that can be addressed by selection (Boissy, 1995). The correlation between emotional reactivity and a selectable matrix of expression in an individual animal offers fundamental ways to optimize animal welfare.

Chickens have been tested in the open field in various contexts. Besides the comparative measurement of general fear and its genetic background (Ishikawa et al., 2020), the open field test has been extensively applied to feather-pecking lines (Jones et al., 1995; Hocking et al., 2001; Rodenburg et al., 2003; Kops et al., 2013), just as it has also been utilized in other species, for example in stereotypic tail-biting in pigs (Haigh et al., 2020). The open field test has been discussed as an early predictor of feather-pecking lines, with "number of steps" being an indicator in chicks (Rodenburg et al., 2003). However, while environmental enrichment might lower feather-pecking events, the triggering factors have not been fully elucidated (Fijn et al., 2020). Some studies have found a correlation between open field behavior and feather pecking (Jensen et al., 2005), while others have not (Albentosa et al., 2003). Recently, range use has been correlated to open field behavior (Campbell et al., 2019), although other studies did not support this (Ferreira et al., 2020). Whereas Campbell et al. (2019) found that hens that preferentially stayed indoors exhibited longer latency and shorter track length, Ferreira et al. (2020) found no correlations, possibly because of the younger age of the chickens (immature) at testing.

Due to increasing consumer demands for improved welfare, more free-range products, and a desire for more local chicken breeds for production, producers are now also searching for options within the animal's (chicken's) genetic resources (FAO, 2015; Del Bosque et al., 2020; Tiemann et al., 2020). Animal genetic resources are of present or potential value, and are or may in future be used for food production or other agricultural branches. Whether local breeds are predisposed to higher welfare is unknown, but there is much interest in the topic (Sakaguchi and Ishikawa, 2019; Rozempolska-Rucińska et al., 2020; Yoshidome et al., 2021). Additionally, while consumers express a desire for free-range husbandry systems, there is a need to identify those breeds, and individuals, that make use of the outdoor area, as it is often the case that large portions of a flock will stay inside (Petterson et al., 2016; Taylor et al., 2020; Bari et al., 2021). Using the open field test, advantageous and disadvantageous behavioral traits of animal genetic resources could be identified in terms of welfare, for example, emotionality and exploration/foraging behavior.

Foraging behavior has been a part of the domesticated phenotype. Both Agnvall et al. (2012) and Katajamaa and Jensen (2020) showed that activity in the open field, and therefore, low fearfulness, are side-effects of selection toward reduced fear of humans in the red jungle fowl. Interestingly, in the study of Katajamaa and Jensen (2020), there was a correlation with sex, whereby females with low fear of humans ranged over longer distances and males over shorter distances, while there was no effect of sex in the study by Agnvall et al. (2012). Furthermore, Henriksen et al. (2020) found behavioral variability to be identifiable in a quantitative trait locus mapping analysis, as did Agnvall et al. (2012) for distance moved in the open field (heritability, $h^2 = 0.32$). These findings question how local breeds behave in standardized testing procedures, and hint at the diversity of behavioral and especially emotional responses.

Testing emotionality in the open field covers 2 major aspects of welfare research: 1) the measurement of fear, which is in opposition to the positive welfare state to be achieved and 2) the examination of exploratory behavior, to reflect the increase in the extent of free-range husbandry in chickens Mahboub et al. (2004). reported on the genetic impact on fearfulness and free-range behavior. Behavior in the open field is a balanced behavioral response to a novel environment that includes an individual's expression of general fearfulness, ranging from fear (shown by pronounced immobilization or flight response) to exploration representing the animal's vested interest in foraging, gathering information, and social reinstatement (Faure et al., 1983; Jones, 1989). Open field testing has seldom been applied to local chicken breeds, a research gap that this study aimed to address.

Different breeds of domestic chicken (*Gallus domesticus*), ranging from bantams and local adapted breeds to dual-purpose hybrids, were tested in the open field to observe how they responded to a novel environment with respect to behavioral traits, such as general fear on one hand and exploration on the other. We hypothesized that breeds adapted to extensive housing systems would be more active, indicating a low general fear level and high exploration drive. The overall objective was to characterize the different breeds tested for their predisposition to show positive behaviors, such as exploration and less fear in a stressful situation within a novel environment. Balanced behavioral responses are thought to predispose toward a high welfare state.

MATERIALS AND METHODS

Animals

Six different breeds of the domestic chicken were employed, with the experiments performed on naïve mature hens (N = 65, Table 1). The breeds observed were Augsburger (**AUG**), Bergischer Schlotterkamm (**BS**), East Frisian Gull (**EFG**), German Empire Breed (**GEB**), Japanese Bantam (**JB**), and Lohmann Dual (**LOH**). All chickens were incubated and hatched at the Poultry Research Centre, Rhein-Kreis-Neuss, Germany (Table S1). The chickens were housed in separate hutches containing stable social groups that included all the tested hens plus 1 or 2 cocks of the same breed (which were not tested due to low sample size). The ages of the hens ranged from 19 wk to 7 y (mean 37.55 wk). After the experiment, the chickens remained on the farm for breeding purposes or were given to private breeders supporting animal genetic resources.

Housing

All chickens of the same breed were kept in one hutch of area 6 m^2 and were provided with bedding (wood shavings), perches, and nests. Each hutch allowed free exit to the outdoors area (200 m^2), that is, all chickens could range freely every day, which they did extensively. This extensive free-ranging behavior is typical for native breeds, and these chickens will almost exclusively go indoors only for egg laying, feed intake, and roosting. The stocking density equated to 0.3 to 0.7 m^2 $chicken^{-1}$ indoors, and ~12 m^2 $chicken^{-1}$ outdoors. Visual inspection and cleaning were ensured once per day. To ensure consistency, a 12L:12D artificial light program was maintained within the hutches throughout the year, although all chickens had access to natural daylight every day through free-ranging activity. Chickens were fed a conventional diet (Deuka all-mash "Zucht"; composition for breeders; pellets; crude protein 16.5%; methionine 0.4%; calcium 3.6%; phosphorus 0.5%; energy 12.4 MJ ME/kg; no coccidiostat; Deutsche Tierhaltung Cremer, Düsseldorf, Germany). Animals were fed ad libitum, including water and grit,

Table 1. Breeds of chickens tested in the open field (**OF**, N = 65). The table shows sample size and age and means (± SE) for body weight, the proportion of active time in the OF, and the track length covered in the OF.

Breed	Sample size [N]	Age [wk]	Body weight [g]	Activity in the OF [%]	Track length covered in the OF [cm]
Augsburger	9	86	1,632 ± 53	78.33 ± 3.85	28,843 ± 8,472
Bergischer Schlotterkamm	15	20	1,427 ± 58	15.73 ± 3.48	1,387 ± 430
East Frisian Gull	9	41	1,883 ± 28	6.41 ± 2.17	1,023 ± 378
German Empire Breed	13	21	1,708 ± 64	42.77 ± 7.23	2,100 ± 417
Japanese Bantam	10	47	639 ± 19	5.16 ± 3.33	512 ± 301
Lohmann Dual	9	28	1,733 ± 64	27.56 ± 7.63	2,043 ± 473
Mean				31.46 ± 3.89	5,378 ± 1,632

and were vaccinated against Newcastle Disease at 3-mo intervals.

Testing Procedure

The open field (dimensions 1.80 × 1.80 × 0.72 m, W × D × H) was made of grey Trovidur PVC and was located in a separate room without daylight. The floor was covered with green PVC sheet to ease cleaning. The room had a daylight-emitting fluorescent tube with UV spectrum (Arcadia Products, Redhill; UK) and electronic ballast (Relco Group Germany GmbH, Hilden; Germany) to adjust flicker frequency to the animals' demand. An Eyseo EcoLine TV 8750 camera (ABUS Security Center GmbH & Co. KG, Affing Germany) was installed above the arena and connected via an Advanced Dv Converter (ADVC) 55 (Canopus Co, Ltd., China) to a computer installed with the tracking software Viewer 3.0.1.241 (BIOBSERVE GmbH, Bonn, Germany; Schwarz et al., 2002; Tiemann and Rehkämper, 2009) located outside the room. The videos were analyzed for time spent on various activities (proportion of active time) and for track length (distance covered). The entire arena was covered by a black, coarse-meshed net (mesh size 19 × 19 mm) to prevent the chickens from leaving.

Tests were always started one hour after the animal caretaker had vacated the hutch (late morning) and continued until one hour before sunset. This time period was chosen to meet the animals' natural behavior and to exclude time periods of egg laying or resting/roosting. Chickens were used to frequent handling and catching and showed no major flight responses, indicating minor stress. Chickens were carried in an upright and close-to-body position (Herborn et al., 2015) and were pseudo-randomly chosen out of the group to ensure that each one was caught only once. For transport to the experimental room, boxes of size 20 × 40 × 50 cm (W × D × H) were used. Four boxes were simultaneously carried on a small wagon to limit catching events in the groups and the duration of time in the box. The testing schedule ensured that chickens spent no more than 1 h in the box, and the duration of stay was balanced across all individuals of a given breed.

For the open field test, the animal was placed in the middle of a side wall facing the center of the arena. The tracking software recorded the distance traveled by the chicken in cm (track length) and the proportionate period of activity. The starting position was rotated clockwise for the chickens to be observed. After the chicken was positioned, the experimenter left the room, started the tracking software, and the behavior of the animal was recorded for 10 min. After the experiment, the chicken was taken out of the arena, the ID of the leg band was recorded, and the chicken was weighed using a Kern HDB hand scale (Kern und Sohn GmbH, Bahlingen, Germany). The chicken was then placed back into the transport box and returned to its social group. Before the next animal went through the experiment, the arena was cleaned with water and a sponge.

Statistics

Graphical representations of the data were created using Sigma Plot 14 (Systat Software Inc., Chicago, IL). Statistical analysis was performed using SPSS Statistics 27 (IBM Corporation, Armonk, NY) and R 4.0.3 (R Core Team, 2019). The significance level α was set at $P \leq 0.05$ (indicated as *), $P \leq 0.01$ (**), and $P \leq 0.001$ (***). A P-value of ≤ 0.1 was regarded as a trend. Analyses of the parameters "activity" (>1 cm s^{-1}) and "track length" (cm; calculated based on prior pixel-to-track setting) were performed using Poisson-distributed generalized linear models (**GLM**) using the lme4 package. Prior to the actual analysis, we tested the 3 covariates breed, weight, and age for correlations. For this purpose, aŋ-correlation was calculated between the nominal variable breed and the metric variables weight and age, respectively. This analysis revealed a strong ($\eta \geq 0.8$) and significant ($P \leq 0.05$) correlation between the variables breed and age ($F(5, 59) = 29.596$, $P < 0.001$, $\eta = 0.977$). This, in turn, led to the exclusion of the variable age from the GLM. There was no significant correlation between breed and weight ($F(58, 6) = 1.982$, $P = 0.198$, $\eta = 0.767$), resulting in both covariates being included in the analysis. To generate an integrated perspective on the variables measured within the open field, we used a parameter

OF_{score} that combined the measured values, as follows

$$OF_{score} = \textit{track length}\ [\mathrm{cm}]/\textit{activity}\ [\%] \quad (1)$$

as a means to describe open field behavior (Table 1). A Poisson-GLM on the variable score was conducted including the covariate breed. To prove convergence and the fit of the model, the null deviance was checked to confirm that it was higher than the residual deviance. Furthermore, global P-values of the car package and post-hoc pairwise comparisons (corrected for multiple testing by Bonferroni) of the emmeans package were conducted.

Ethics Statement

The animal husbandry complied with the order on the protection of animals and the keeping of production animals (Tierschutz-Nutztierhaltungsverordnung, 2006 (last revision 2017)). The Campus Frankenforst of the Faculty of Agriculture, University of Bonn was approved as the trial farm (39600305-547/17). The experimental procedure was approved by the responsible authority (North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection, AZ 81-02.04.2019.A372) and was conducted in accordance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching (4th edition, 2020).

RESULTS AND DISCUSSION

Activity

Whereas the global P-value for weight showed no significant impact (P = 0.658), breeds differed in their activity in the OF ($P \leq 0.001$; null deviance: 2164.51 on 64 df; residual deviance: 850.08 on 58 df; Table 1). Pairwise comparisons revealed further differences between breeds (all $P \leq 0.001$; excluding JB −EFG P = 1.0 and GEB−LOH P = 0.987, Table S2). Proportions of activity ranged from AUG with the highest amount of activity and JB with the lowest.

Increased activity in the open field is thought to indicate decreased fear (Katajamaa and Jensen, 2020) although an alternative flight response might cause increased movements. Based on Forkman et al. (2007) and Powell et al. (2004), there might be 2 contradictory motivational states impacting open field responses: fear, which causes immobility and social reinstatement; and exploration, which causes activity. The open field responses revealed low activity in JB and high activity in AUG. This is contradictory to the assumptions of Katajamaa and Jensen (2020), which correlated tameness to general fearfulness and increased open field activity. Bantam breeds have been bred for human−animal interaction (Bortoluzzi et al., 2018) and tameness, implying high activity scores in open field testing (Katajamaa and Jensen, 2020). Therefore, an alternative argument could be that JB have reached a level of (human-associated) tameness at which social reinstatement to conspecifics is not of high priority. JB might also be less exploratory as they are usually not kept as extensively as AUG (JB are kept for the purpose of winning exhibition contests, requiring complete and clean plumage). AUG's origins are in the Mediterranean laying breeds, bred for foraging behavior and egg-laying performance, presuming a higher level of physiological activity. High egg productivity has been correlated with lower sociality, from which one might predict AUG to show less-pronounced open field activity (Dudde et al., 2018). This was not reflected in results from our study, as AUG showed the highest level of activity. As the first intermediate conclusion, it can be stated that breed-specific activity levels were shown by the animals tested. For further interpretation, it would be necessary for additional variables to be taken into account, such as the velocity during movement which could be an indicator of a flight response. The distinction between exploratory and fear-related movements within the open field is already discussed in rodent research (Yang et al., 2020) and should also be more consciously integrated into the data collection and analysis in future poultry research.

Track Length

The GLM for track length (Poisson distribution) with the covariates of breed and weight revealed the impact of both weight and breed (both $P \leq 0.001$; null deviance: 897940 on 64 df; residual deviance: 219372 on 58 df). Pairwise comparisons were significant for each pair of breeds (all $P \leq 0.001$, Table S3). By far, AUG showed the longest track length, whereas JB covered the shortest distance in the open field (Table 1). The overall picture on track length is congruent with the interpretation of breed-specific activity level. Again, AUG showed the longest track length and JB the shortest. Ranging in between, in ascending order, were EFG, BS, LOH, and GEB. Whereas the first 2 breeds are kept extensively for egg laying, LOH and GEB are both dual-purpose breeds (Dürigen, 1932; Tiemann et al., 2020). Dual-purpose chickens are bolder than laying hens (Giersberg et al., 2020), a result reflected here in terms of the longer track length covered by LOH and GEB during open field testing compared to EFG and BS. Although the interpretation for these four breeds might be in line with the literature, our results for JB and AUG do not seem to follow this argument.

In this study, general influences (other than genetic) were controlled as far as possible, such as brooding (Riber and Guzman, 2016) and handling (Jones and Waddington, 1992) differences; however, the influence of age cannot be excluded. The availability of population sizes that are rather small often limits animal genetic resources; however, from a researcher's perspective, it is still necessary to seek out subject groups with balanced or comparable characteristics other than the one in focus. This is also true for the animals tested here, in which age and breed were correlated. Age might affect open field behavior in terms of exploratory drive or the motivation for social reinstatement (Forkman et al., 2007); however, Hocking et al. (2001) found that older chickens traveled less in the open field, and exhibited prolonged latencies. This could explain the difference between Augsburger and Japanese Bantam in our study; yet, Hocking et al. (2001), who studied 2 lines of brown laying hens, found behavioral traits were stable over time, which would account for individual (genetic) differences. Studies in rats showed age-related changes in open field behavior, with younger rats being more active (Bronstein, 1972). The questions surrounding the impact of age on fear and exploration remain unresolved in our study, and it is recommended that they be addressed in future studies using experimentally naïve animals. This knowledge gap becomes even more interesting in the research field of animal personalities and their development (Cabrera et al., 2021). To address this research objective, an integrative view of the

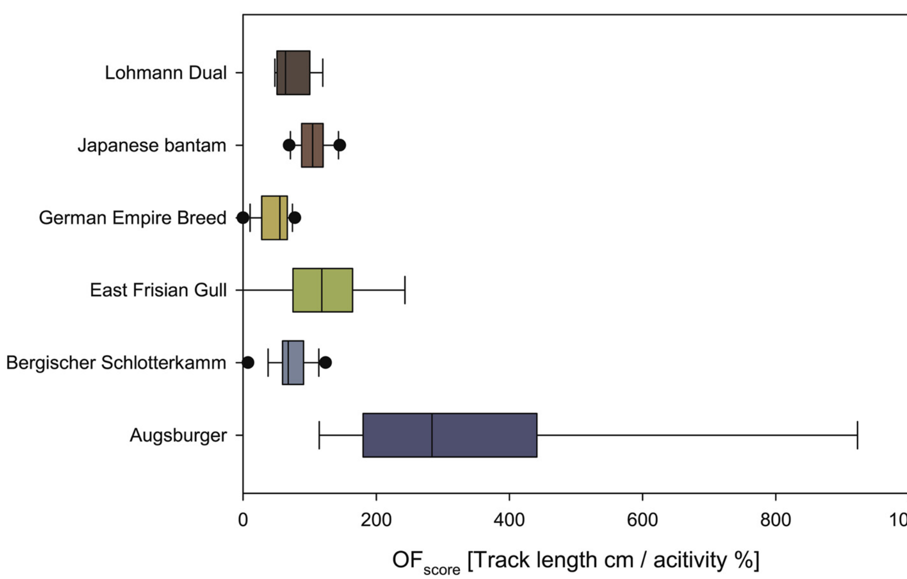

Figure 1. Open field scores (OF_{score}) based on track length and activity^{-1} of the six chicken breeds tested. Values are median, 10th, 25th, 75th, and 90th percentile of the OF_{score} per breed. Breeds are sorted alphabetically.

quantified variables was implemented in the calculation of the OF score.

Open Field Score

Analysis revealed a significant impact of the genetic background of the animal on the OF_{score} ($P \leq 0.001$; null deviance: 5715.5 on 63 df; residual deviance: 1890.8 on 58 df; Figure 1). Except for the pairwise comparison between BS and LOH, all pairs of breeds showed significant differences (all $P \leq 0.001$, Table S4). Looking at the average scores, AUG showed, by far, the highest score, indicating that individuals of this breed covered longer distances within their activity time (compared to GEB, LOH, and BS), whereas the scores for JB ranged between those of EFG and AUG. We suggest that a high OF_{score} indicates flight and/or social reinstatement behavior rather than a high level of exploration behavior. This proposal extends the perspective of Forkman et al. (2007), who reported that the jumps and peeps of chicks are indicating social reinstatement, and of the analogous proposal of Godwin et al. (2012) for Zebra fish, which has not yet been extended to chickens. In our study, chickens that showed extreme OF_{scores} would exhibit fear and/or social reinstatement, whereas chickens with low OF_{scores} would show adapted, non-fearful behaviors that reflect coping abilities in novel environments, which do not cause distress (Väisänen and Jensen, 2004).

Oka and Bungo (2014) reported that short-legged and bantam-sized JB responded less fearfully during a manual restraint test, a test also addressing the individual's fear response. This could be due to the historical target to breed fearless bantams with an affinity for humans. Future studies should cover a wider range of breeds of animal genetic resources that include different morphological characteristics, especially in the case of comparative studies.

The diversity of chicken breeds provides a unique insight into their varied behaviors. This variety is of particular interest when it comes to assessing what range of behavioral responses is even possible, especially in a standardized experiment on emotionality. Emotionality and emotional reactivity, including the opposing emotions fear and curiosity, determine the expression of the fifth domain of animal welfare, mental health (Mellor, 2016; Kozak et al., 2019). The desired behavioral phenotype is one that is balanced between appropriate levels of fear and exploration, and one in which new situations do not trigger distress.

It is this phenotype that should be used for future breeding objectives. Simultaneously, the breed-specific phenotype should be considered when repurposing animal genetic resources in the domestic chicken.

CONCLUSIONS AND APPLICATIONS

1. Variables measured in the open field should include activity and track length to permit mapping of a superordinate system of breed-specific behavior.
2. A wider variety of breeds with different breeding backgrounds and morphological characteristics should be tested in the open field for additional variables (for example, duration of stay in center vs. peripheral areas) to establish a more detailed system of behavioral diversity.
3. Future research should address the possibility of deriving genetic correlations from extended open field testing for use in breeding.
4. The added value of these findings from standardized test procedures with a large number of breed-specific evaluations to increase animal welfare is undisputed. If we know which adaptations are possible, which behaviors promote good animal welfare, and which precise mental and emotional demands the animals make on their environment, we can further increase and optimize animal welfare management procedures and husbandry systems.

DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.japr.2022.100237.

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