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A gentling program decreases fear-related behaviour towards humans but not towards novel objects in broiler chickens

Theresa Stocker [a,d], Christian Nawroth [b], Birger Puppe [b,c], Michael Erhard [d], Helen Louton [a,d,*]

[a] Professorship for Animal Health and Animal Welfare, Faculty of Agriculture and Environmental Sciences, University of Rostock, Justus-von-Liebig-Weg 6b, Rostock D-18059, Germany

[b] Research Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, Dummerstorf D-18196, Germany

[c] Behavioural Sciences, Faculty of Agricultural and Environmental Sciences, University of Rostock, Justus-von-Liebig-Weg 7, Rostock D-18059, Germany

[d] Chair of Animal Welfare, Ethology, Animal Hygiene and Animal Husbandry, Department of Veterinary Sciences, Faculty of Veterinary Medicine, LMU Munich, Veterinärstraße 13/R, München D-80539, Germany

A R T I C L E   I N F O

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A B S T R A C T

The human-animal-relationship is a crucial aspect of good animal welfare. Improvements in this regard might be particularly relevant for chickens, as their relationship with humans in commercial husbandry conditions is often limited to brief animal inspections (visual contact) on a daily basis. Regular human contact can potentially reduce fear responses towards humans in chickens, but to achieve this, a long period of several weeks of habituation is usually required. In this study, we investigated whether a short period of 'gentling' (i.e. standardised human-animal interactions that the chicks are likely to perceive as positive) within the first 3 days of life would be sufficient to reduce fear responses towards humans in broiler chickens. Six groups of ca. 220 (214 – 254) day-old broiler chicks each were exposed to a gentling program by a human after arrival. The same number of control groups received no human contact apart from routine husbandry procedures. We performed three behavioural tests on the chickens to assess their fear of humans. A stationary person test (SPT) measured the voluntary approach to a standing human, an avoidance distance test (ADT) measured the avoidance behaviour towards an approaching human while a touch test (TT) measured the approach behaviour towards a squatting person followed by the measurement of the avoidance behaviour from the approaching hand. Additionally, a novel object test (NOT) was performed to assess whether a potential fear reduction would be limited to humans. The tests were conducted at three different ages of the chickens (day 7, 21 and 36). In all human-related tests, chickens of the gentled groups showed less avoidance and more approach behaviour towards the human than chickens of the control groups (SPT: $p = 0.004$, ADT: $p = 0.001$, TT: $p = 0.035$). The results of the NOT showed statistically no significant behavioural differences between the gentling and control groups ($p = 0.205$), confirming that the chicks' fear responses are not generalised but specific to human-related tests. The present study suggests that gentle handling of chickens during the first 3 days of life can improve the human-animal-relationship for at least 36 days.

1. Introduction

Animal welfare has become increasingly prominent in public discourse over the past decades. Modern animal welfare concepts have their roots in the "five freedoms" (Brambell, 1965), which were further developed into the animal welfare concepts of Mellor and Reid (1994) and Fraser et al. (1997). Both highlight the importance of a good human-animal-relationship (HAR). A poor HAR quality may result in animals perceiving human contact as a threat and thus show increased fear responses (Jones, 1996). To address this welfare issue, many studies have been conducted to develop procedures and routines (i.e. gentle stroking, talking) that improve the HAR in different farmed species

Abbreviations: HAR, human-animal-relationship; SPT, stationary person test; ADT, avoidance distance test; TT, touch test; NOT, novel object test; GP, gentling person; TP, testing person; PCA, principal component analysis; PC, principal component.

* Corresponding author at: Chair of Animal Welfare, Ethology, Animal Hygiene and Animal Husbandry, Department of Veterinary Sciences, Faculty of Veterinary Medicine, LMU Munich, Veterinärstraße 13/R, München D-80539, Germany

E-mail address: h.louton@lmu.de (H. Louton).

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(cows: Breuer et al., 2003; chicken: Jones, 1993; pigs: Muns et al., 2015).

To quantify the HAR, various behavioural tests covering different species are available that utilize approach and avoidance behaviour towards humans as validated measures of fear of humans (goats: Battini et al., 2023; chickens: Raubek et al., 2007; cows: Waiblinger et al., 2003). The so-called stationary person test (SPT) evaluates the voluntary approach to a motionless person, while the avoidance distance test (ADT) measures the avoidance reaction of a human forcefully approaching one or more focal subjects (Rault et al., 2020). The touch test (TT) combines both elements, firstly evaluating voluntary approach, then the person attempts to touch the animals that have approached (Welfare Quality®, 2009). The novel object test (NOT), in contrast, measures the fearfulness towards novel objects. It is often used to assess whether specific handling routines affect general fearfulness (Forkman et al., 2007). Regarding chickens, most tests have been developed for caged laying hens (Barnett et al., 1994; Jones, 1993). The previously mentioned tests are therefore useful, as they are reported effective when used with large groups (Bassler et al., 2013; Graml et al., 2008a; Welfare Quality®, 2009).

Particularly in the case of chickens, improving the HAR will be beneficial because they are provided with only infrequent contact to humans during management procedures in commercial husbandry settings. Many previous studies have highlighted the importance of repeated exposure to reduce fear of humans (Barnett et al., 1994; Graml et al., 2008b; Jones and Waddington, 1993). Different learning types, such as imprinting, habituation and associative learning, were possibly contributing to behavioural changes (Shettleworth, 2001). Graml et al. (2008b), for example, applied an associative learning approach by providing food rewards from humans. The existing literature on early handling in chickens is limited. However, it shows comparable or even more pronounced positive effects than regular handling when lasting for at least 5 days, starting early or later in life (Jones, 1995; Jones and Waddington, 1993). It is known that the process of imprinting is confined to the sensitive period of chickens in the first 32–36 h of life and is not reversible (Engelmann, 1969; Hess, 1958). Engelmann (1969) noted that in the absence of an imprinting stimulus, chicks began a “lighter version” (e.g. familiarisation and willingness to connect) of imprinting on the partner chicks. Given that chicks are less fearful and have a greater capacity to memorise stimuli in their environment during this period, this age period might be very important in establishing the foundations for a good HAR (Hess, 1958). The practice of gentling in early life, where humans interact with chickens in a gentle and friendly manner without forcing interaction, may be an effective way to reduce fear of humans (Rault et al., 2020).

Here, we aim to evaluate the effectiveness of a gentling program for broilers to reduce their fear of humans in modern husbandry systems (floor systems, no cages). We focus on repeated contact (twice daily) during only the first 3 days after placement of day-old chicks in the housing system. This aims to investigate whether human contact during the sensitive imprinting period alone is enough to induce behavioural change. Graml et al. (2008b) found reduced avoidance and increased approach behaviours after a two-week treatment period but used adult laying hens for this. We use the validated behavioural tests of their study to test the effectiveness of our gentling program. The SPT, ADT and TT can be considered complementary behavioural tasks in that they measure different aspects of the HAR (Passillé and Rushen, 2005) while the NOT validates the other tests by its discriminant validity (Waiblinger et al., 2006). We expect that the results of the human-related tests will show less avoidance and more approach behaviour in the gentled compared to the control groups while subjects in the NOT will not show behavioural difference between groups.

2. Material and methods

All animals were treated during care and experimental procedures according to societal guidelines (ASAB Ethical Committee/ABS Animal Care Committee, 2023). The animal experiment conducted in this paper was approved by the Committee for Animal Use and Care of the Ministry of Agriculture, Environment and Consumer Protection of the federal state of Mecklenburg-Vorpommern, Germany (Ref. No. 7221.3–1–043/21). It took place from October 2021 until May 2022 in the experimental facility of the Research Institute for Farm Animal Biology (FBN Dummerstorf, Germany).

2.1. Animals and housing

We used four compartments (3.8 m x 6.0 m each) simultaneously in three successive runs (one run corresponds with one fattening period, FP). Based on availability from the hatchery (BWE-Brüterei Weser-Ems GmbH & Co. KG, Visbek-Rechterfeld, Germany), 214 – 254 slower growing broilers (Ranger Classic) were housed (stocking density: ca. 22 kg/m^2 on day 45) in each compartment (see Supplementary Material I for exact numbers). Slower growing broilers were chosen to reduce the risk of walking impairments as this could affect the behavioural test results (Vasdal et al., 2018).

Each compartment had an additional space of approximately 4.0 m^2, separated from the main housing and testing area by a metal grid. The space was used as an anteroom, such as for storing a box with various objects for testing (“novel objects”). All compartments were set-up similarly (Fig. 1): 7 plastic perches totalling a length of 10 m (Sitzstange Siesta L3000 weiß/orange, Big Dutchman International GmbH, Vechta, Germany), 8 round feeders holding 10 kg (minimal available feeding space: 4,09 cm/animal; Voss.farming, Heist, Germany), 1 drinker line (52 nipples per compartment, nipple drinker, Big Dutchman AG (Holding), Vechta, Germany), 2 ceiling heater (Sunline Deckenstrahlheizungen GmbH, Dingelstädt, Germany), 10 infrared heat lamps (IR 150 RH IR1, Signify GmbH, Hamburg, Germany), 2 artificial light (Oleveon 258 L, Trilux GmbH & Co. KG, Arnsberg, Germany) and enrichment materials (2 pecking stones: PICKStein Duo and PICKStein Hart, Vilofoss Deutschland, Neuenkirchen-Vörden, Germany, 2 lucerne bales: Hartog Compact Lucerne EKO 20 kg, Grasdrogerij Hartog B.V., Lambertschaag, Netherlands). For video recordings, two cameras (M1135, Axis Communications AB, Lund, Sweden) were installed in each compartment in the middle of the shorter sides at 2.35 m of height, facing each other. Temperature and humidity were set following the recommended age curve of the breeding company (Aviagen, Inc., Huntsville, USA) using a climate computer (135_l2 Pro, Big Dutchman International GmbH, Vechta Germany). The light/dark period was adjusted as follows: first night: 0 h, second night: 1 h, third night: 2 h, fourth night: 3 h, fifth night: 4 h, sixth night: 5 h, seventh night: 6.5 h, eighth night: 8 h of darkness, then continued with 16 h light and 8 h darkness period until slaughter. Each compartment had a window (1.34 ×0.90 m), providing natural light. Light intensity was measured prior to housing the first chicks using a luxmeter (VOLTCRAFT MS-1500, Conrad Electronic, Hirschau, Germany) placed at the centre of each compartment. A six-sided measurement was taken, and light levels were adjusted to ca. 100 lux (including daylight and artificial light) in each compartment using the climate computer. The compartments were furthermore equipped with a negative pressure ventilation system (1x MC 31 Drehzahlregler and 2x CL 1200 Zuluftelement, Big Dutchman International GmbH, Vechta Germany). Due to technical malfunction, the ventilation failed in the third FP in compartment 3 for the first 7 days. However, ventilation flaps stayed open, allowing for natural ventilation. The broilers were vaccinated on day 10 with AviPro ND HB1 (Lohmann Animal Health GmbH, Elanco) and on day 15 with Hipragumboro CW (Laboratorios Hipra, S.A.). Antibiotic treatment (Belacol 24 % liquid, 240 mg/ml Colistin sulphate, Bela-Pharm GmbH & Co. KG) was administered in the first FP (day 3–8), due to a diagnosed coli septicaemia. Following careful visual inspection, we found no strong indications of deviations in the behaviour test results between FPs. Animals were checked twice a day, spending a maximum of 15 min per day in each compartment. Feeders were filled manually when necessary,

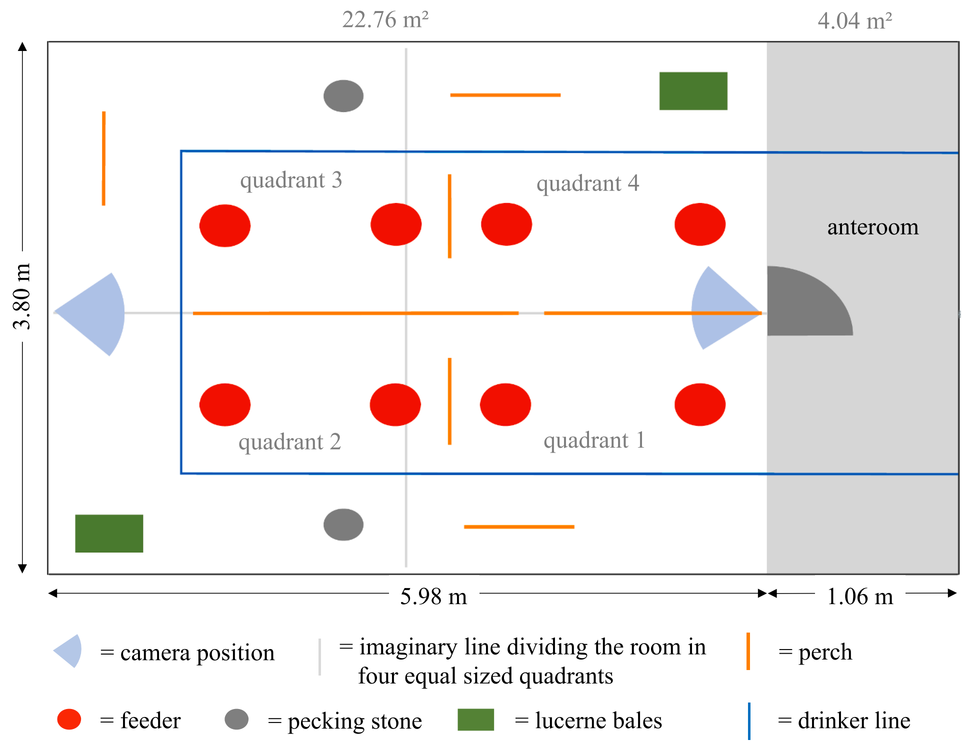

Fig. 1. Measurements and structure of each compartment used in the experiment equipped with feeders, drinkers, enrichment materials, perches divided into sections of 1 m, 2 m or 3 m in length and cameras. Grey lines show the border of the virtual quadrants that were being used for behavioural testing and analysis.

providing the animals with an *ad libitum* three-phase pellet diet (Agravis Raiffeisen AG, Münster, Germany, Supplementary Material II) containing coccidiostats in the first and second phase (monensin sodium, 4 mg/kg). Animals were kept until slaughter on day 44 (FP 3)/45 (FP 1 and 2) and carcasses were used for human consumption.

2.2. Gentling

During each FP, two of the four available groups were gentled and two remained without gentling (i.e. control groups) with only necessary care procedures. Treatments were allocated randomly across FPs to the corresponding compartments (Supplementary Material III). The gentling treatment consisted of six 1-hour phases of gentling program within the first 72 h after the chicks' arrival. The two gentling groups were treated directly one after the other in one session. The first session began 1 h after housing the chicks. On day 2 and 3, one session took place in the morning of each day (8:00–10:00 h) and one in the afternoon (14:00–16:00 h). The sixth session was in the morning of day 4. The person who administered the gentling (gentling person, GP) wore standardised clothing (blue surgical clothes) that was also worn by the caretakers over the entire rearing period. The compartment was divided into four virtual quadrants (Fig. 1) which were all treated with an identical procedure (i.e. gentling program) following the gentling routine below:

1. GP slowly entered the compartment with a small footstool (Bolmen black, Ikea Deutschland GmbH & Co. KG, Hofheim-Wallau, Germany), went to the centre of quadrant 1 (1 step/sec) and gently put down the footstool.
2. GP stood motionless for 30 s.
3. GP talked in a low-frequent voice for 1 min.
4. GP sat down on the footstool for 5 min while talking to the chicks in a low-frequent voice.
5. GP slowly stood up, took the footstool and went to the next quadrant.
6. Steps 2–6 were repeated in quadrants 2–4 in clockwise direction.
7. GP went back to quadrant 1 and repeated steps 2–7; this time interacting with the animals while sitting. The interaction was intended to be intuitive and adapted to the situation, so as not to frighten the animals. Therefore, only certain actions were allowed, always depending on the situation: reaching out a hand in direction of an individual, touch animals, stroke animals on head, back and/or chest and take an animal for a few seconds on the open hand (as proposed by Rault et al. 2020).
8. After repeating this procedure (step 7) in all four quadrants in the same order, GP slowly left the compartment.

2.3. Behavioural tests

To assess how the chickens' perception of humans and objects and consequently their behaviour, might change over time, we used four different behavioural tests, each one conducted on three testing days (day 7, 21 and 36 of each FP): stationary person test (SPT), avoidance distance test (ADT), touch test (TT) and novel object test (NOT). Three of them were used to assess the fearfulness of humans (i.e. SPT, ADT, TT) and the fourth to assess the fear of novel objects (i.e. NOT). In the tests, participation was voluntarily and animals that might have experienced stress of fear could walk away and distance from the testing area by themselves. The order was always the same from the test that is potentially least intrusive to the one most interactive with the animals: SPT – NOT – ADT – TT (according to Graml et al., 2008b). The total range of light intensities measured after the behavioural tests was between 44 and 142 lux. However, there was no difference in the average light intensity between the groups (gentling groups: 79.4 lux, control groups = 81.3 lux). Subsequent data analysis showed that the light intensity had no relevant effect on the behaviour of the chicks in the behavioural tests. The testing person (TP) was blinded regarding the assignment of the treatment groups. The TP was familiar to the chickens and wore the same standardised clothes as the caretakers and the GP. The testing order of the compartments as well as the starting quadrant were randomised across testing days and FPs (Supplementary Material

III). Two compartments were tested in the morning (8:00–12:00 h) and two were tested in the afternoon (13:00–17:00 h). The order of quadrants visited during testing was clockwise. Due to technical problems, the behavioural tests of compartments 1 and 4 in the second FP on fattening day 36 were not recorded and SPT and NOT were repeated in the following morning.

2.3.1. *Stationary person test*

Prior to housing the chicks, the middle of each quadrant was marked on the ground with a waterproof marker. For later evaluation, a circle with a diameter of 1 m was painted on the ground around each marked point and filmed with the installed cameras. After entering the compartment, TP went slowly (1 step/s) to the marked point and stood for 7 min in each quadrant in a standardised manner. The whole session was filmed with two installed cameras for later analysis. This test was done four times in each compartment, once in each quadrant on each testing day.

For analysis, a circle mimicking the one in the video was created to be laid over the video sequence using the program Golden Ratio (Golden Ratio Software, Markus Welz, Krailling, Germany). The overlay contained two circles: a larger one with a diameter of 1 m and a smaller one inside the larger one with a diameter of ca. 0.2 m. For the analysis, the space within the larger circle is called "nearby" and the space within the smaller circle is called the "contact" area. The blind spot behind the TP, which could not be seen by the closer camera, was analysed with camera recordings from the other side. The first 5 min allowed the animals to get accustomed to the arrival and movement of the person. This was necessary to make sure of testing a stationary, rather than a moving person. Between minutes 5–7 the evaluation of the animals' behaviour took place.

The analysis was done with the software BORIS v. 7.13.6 (Friard and Gamba, 2016) and contained three parts:

1. Scan sampling: every 10 s a screenshot was taken and the number of chickens in the circles was counted (13 scans in total). The average number of chickens in the two defined circles per test was calculated.
2. Continuous sampling: each test was watched continuously and the number of entries into and exits out of the circles by animals was counted. Additionally, the latency until the first chicken entered the circles was measured. This measurement started at the beginning of the video at minute 0.
3. Focus animals: two focus animals per quadrant were randomly selected by marking random animals in the first screenshot where animals appeared. The duration they spent in the defined circles was measured and an average duration in the defined circles per test was calculated.

The videos were analysed by two observers: the person who conducted the test and a second coder who was also unfamiliar with the assignment to the treatment groups. For further analysis, the mean of each parameter was calculated by averaging the values across all quadrants. This was done for every compartment in all FPs.

2.3.2. *Novel object test*

Three different objects were available to maintain novelty over the three testing days (Fig. 2): (1) a rainbow-coloured plastic ball (Regenbogen Spielball, diameter 20 cm, TOGU® GmbH, Prien, Germany) on a grey plastic ring to prevent it from rolling away, (2) an orange-coloured reflecting traffic cone (23 cm high), and (3) a 2 L blue printed beverage carton (Ice Tea peach 2 L, Pfanner Holding AG, Lauterach, Germany). The order in which the objects were used was randomised; each of them was used once in each group (Supplementary Material III).

After the SPT, the TP went into the anteroom and took the designated novel object. The object was wrapped in a dark blue cloth out of sight of the chickens. The TP went to the starting quadrant in a slow matter (1 step/s) and stood with the back to the longitudinal middle line in front of the marked point, just holding the wrapped novel object for 5 min for acclimatisation. Then, TP slowly placed the novel object on the marked point and left the compartment, moving into the anteroom. With TP out of the barn area, the chickens had two undisturbed minutes to engage with the novel object. Then, TP came back, wrapped the novel object again and carried it to the next quadrant, following the same procedure in all quadrants.

The video analysis was for this test was the same as for the SPT and was carried out by the same two observers.

2.3.3. *Avoidance distance test*

After the NOT, TP went back into the anteroom to put away the novel object. TP slowly went into the corner of the starting quadrant. From there, TP began to meander through the room along the long side of the wall. Using between 1 and 5 steps, a chicken in accessible proximity was approached by slowly extending an arm and bending over until TP either touched it or the animal withdrew. Withdrawal was defined as the chicken having lifted both legs off the ground. In case of withdrawal, the avoidance distance between the TP's fingers and the spot where the chickens' feet stood before it withdrew was estimated (10 cm increments, distances between 0 – 80 cm were possible). Per compartment, 22 animals (ca. 10 % of the group) were tested. For each compartment, the mean avoidance distance of all 22 approaches was calculated for further analysis.

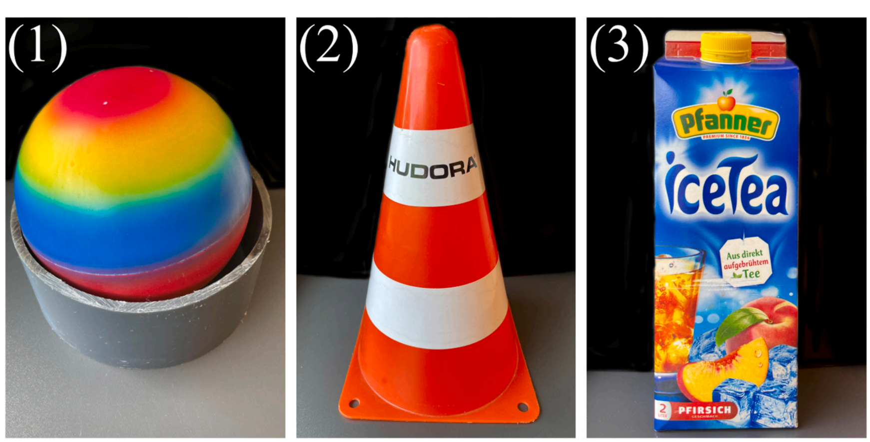

Fig. 2. Photos of the three novel objects used in the novel object tests (NOTs) (1) a rainbow-coloured plastic ball on a grey plastic ring to prevent it from rolling away, (2) an orange-coloured reflecting traffic cone (23 cm high) and (3) a 2 L blue printed beverage carton.

2.3.4. Touch test

TP went to the middle of the starting quadrant and slowly squatted down. After waiting for 1 min, the number of animals at arm's length within a 180-degree semicircle (i.e. not behind the person) were counted. Then, TP reached out with one arm and tried to touch the counted chickens, one by one. The number of animals touched as well as the number of animals not touched was recorded. As animals have moved between counts, the proportion of touched animals was calculated by dividing the number of touched animals by the total animals counted (touched plus not-touched). The proportion of touched animals has a possible range between zero and one, with zero meaning no animal of the ones in reach could be touched and one meaning all animals in reach could be touched. The TT was done four times in each compartment, once in each quadrant. For each compartment, the mean proportion of touched animals across all quadrants was calculated for further analysis.

2.4. Principal component analysis

Given the large number of parameters for SPT and NOT, a Principal Component Analysis (PCA) was performed for each test to reduce data to a single principal component (PC), based on the representation of the data in the pairwise correlation matrices. Due to the non-normal distribution of the data and the high number of zeros in the dataset, all variables were logarithmised using the natural logarithm and had 0.1 added to their initial value. This resulted in a distribution that was more aligned with a normal distribution (checked graphically via a histogram for each parameter) and an improved linear correlation between the parameters, allowing a PCA to be performed on the data. As the number of entries into and exits out of the circles was very similar (correlation coefficient: SPT = 0.97; NOT = 0.96), only the entries were considered. A PCA was performed separately for each testing day to deal with repeated measurements (PCA1, PCA2 and PCA3 for SPT and NOT). The PCAs were usually conducted with eight variables (i.e. number of animals, entries, latencies and durations, each once in the nearby and once contact circle). As the latency in the nearby circle of the SPT exhibited no variance on the third testing day (with value of only 0 s), this variable was excluded from the PCA3. The use of PCAs was contingent upon the fulfilment of two conditions. Firstly, Kaiser's measure of sampling adequacy (overall MSA) had to exceed 0.5 (Kaiser and Rice, 1974). Secondly, the eigenvalue of the principal components had to be > 1 (Kaiser, 1960). In case of small sample sizes, it is recommended that only behavioural measurements with loadings of at least 0.5 or below -0.5 should be considered (Budaev, 2010). Given that we only used behavioural measures that assess the same behavioural concept and that we will extract only one component, we expected loadings to be fairly evenly distributed across all behavioural measures with no need for a specified cutoff level (Miller et al., 2006). Finally, we calculated PC scores for each group with separate PCAs conducted for each testing day. A higher PC value indicates that more animals were in the circles, more entries were observed, the latency until the first animal entered was shorter, and the animals remained in the circles longer. Overall, this suggests that the animals showed fewer signs of fear towards the human. We decided not to label the principal components because only one was used per behavioural test. Consequently, the PC value represents the outcome of the behavioural construct measured in the test. For the ADT, the avoidance distance and for the TT the proportion of touched animals (only parameter for these tests) were used as parameters for statistical analysis. No PCA was conducted on these tests.

2.5. Data analysis

Statistical analyses were conducted using the program R v.4.2.3 (R Core Team, 2023). Two assessors analysed the videos of SPT and NOT, and the inter-observer reliability was calculated for each parameter, independent of the circle. The inter-class-correlation (ICC) was used to calculate the reliability for all assessed parameters of SPT and NOT, using 20 videos (13.9 %) per test. Reliability between observers was evaluated using the classification model.

For each of the four tests, a separate generalized linear mixed-effect model was calculated (blmer function, blme library (Chung et al., 2013)). The dependent variables used were: PC values for SPT and NOT, avoidance distance for ADT, and the proportion of touched animals for TT.

'Treatment' (factor with two levels: gentling, control) and 'testing day' (factor with three levels: fattening day 7 (1), fattening day 21 (2), fattening day 36 (3)) were included as fixed factors. The 'compartment' (1–4) nested in 'fattening period' (1–3) were included as random factors to control for repeated measurements. For the NOT, 'novel object' (1–3) was incorporated as a separate random effect. The residuals of the models were checked graphically for normal distribution and homoscedasticity (simulateResiduals function, DHARMa library (Hartig, 2022).

A full model approach was followed. Therefore, a maximum model was set up for presentation and interpretation (Forstmeier and Schielzeth, 2011). Firstly, the global p-value between the maximum and the null model was calculated. If that model reached a low p-value, each predictor variable and their interactions were tested singly by comparing the full model to the one omitting this predictor. Alpha levels were set at 0.05 for all models.

3. Results

3.1. Fearfulness of humans

In total, 144 videos of SPT and 36 samples of ADT and TT each were analysed. The ICC value between observers for video analysis of the SPT was 0.96 for the average number of chickens in the circles, 0.99 for the latency until the first chicken entered the circles, 0.96 for the number of entries into the circles, and 0.96 for the duration spent in the circles.

PC1 of testing day 1 explained 60.5 %, PC1 of testing day 2 explained 71.2 % and PC1 of testing day 3 explained 69.4 % of the variance in the data of SPT (for more information see Supplementary Material IV). Most loadings of the behavioural measurements of SPT indicated reduced fearfulness towards humans, with exception of two loadings (testing day 1: loading of latency into the contact circle, testing day 3: loading of duration of focal animals in the nearby circle, for details see Supplementary Material V).

Descriptive results of the behavioural tests are presented in Table 1 (original not-transformed data). Generally, the behavioural response to a human differed between treatment groups.

Animals of the gentled groups showed more approach behaviours towards the human in the SPT (e.g. had higher PC 1 values) than animals in the control groups independent of testing day ($p = 0.004$, Fig. 3). We found no effect of the testing day on the SPT values ($p = 1$) as well as no interaction between the testing day and the treatment ($p = 0.268$).

For the ADT, the interaction between testing day and treatment had also no effect ($p = 0.450$). The avoidance distances of the gentled groups were lower than of the not gentled control groups independent of testing day ($p < 0.001$, Fig. 3). The testing day influenced the ADT significantly ($p < 0.001$). Independent of treatment, the avoidance distance decreased between testing day 2 and 3 (means: testing day 1 = 31.29 cm; testing day 2 = 31.75 cm; testing day 3 = 21.44 cm).

The TT was the only test where an interaction between testing day and treatment was found ($p = 0.035$, Fig. 3). Across all testing days, the proportion of animals that could be touched was higher in the gentled groups than in the not gentled control groups (Fig. 3) but with differences between testing days. Visually in the graphs it was most pronounced on the second testing day, but smaller on the first and last testing day. The proportion of touched animals increased over all testing days for both treatment groups (means: testing day 1 = 21 %; testing day 2 = 46 %; testing day 3 = 74 %, Fig. 3).

Table 1
Descriptive results separated by treatment group over all testing days of the four behavioural tests: stationary person test (SPT), novel object test (NOT), avoidance distance test (ADT), touch test (TT). Latencies and durations are expressed in seconds (s).

Parameter	Gentling group Mean ± SD (min-max)	Control group Mean ± SD (min-max)
SPT		
number of animals in contact area	2.12 ± 1.99 (0 – 5.5)	1.12 ± 1.67 (0 – 4.5)
number of animals in nearby area	5.93 ± 4.92 (0 – 14.1)	3.90 ± 4.70 (0 – 12.6)
number of entries in contact area	7.46 ± 7.33 (0 – 26.0)	3.26 ± 4.71 (0 – 14.3)
number entries in nearby area	7.38 ± 5.57 (0.5 – 19.8)	4.60 ± 4.92 (0 – 16.5)
latency until first entry in contact area (s)	34.57 ± 54.92 (0 – 176.8)	73.11 ± 87.41 (2 – 213.4)
Latency until first entry in nearby area (s)	31.01 ± 61.39 (0 – 226.5)	39.23 ± 54.36 (0 – 164.6)
Duration spent in contact area (s)	34.04 ± 27.99 (0 – 81.4)	19.44 ± 26.64 (0 – 76.7)
duration spent in nearby area (s)	67.41 ± 38.35 (0.4 – 105.2)	48.64 ± 42.01 (0 – 116.3)
NOT		
number of animals in contact area	0.68 ± 1.24 (0 – 5)	0.63 ± 1.37 (0 – 5.7)
number of animals in nearby area	1.53 ± 1.70 (0–6)	1.65 ± 1.83 (0 – 6.7)
Number of entries in contact area	3.31 ± 5.23 (0 – 18.5)	3.28 ± 5.93 (0–24)
Number of entries in nearby area	7.67 ± 6.98 (0 – 21.5)	8.21 ± 8.19 (0.3 – 31.8)
latency until first entry in contact area (s)	23.42 ± 37.09 (0 – 118.7)	14.16 ± 12.10 (0–31)
latency until first entry in nearby area (s)	19.26 ± 27.74 (0 – 88.7)	18.84 ± 30.70 (0 – 97.1)
Duration spent in contact area (s)	9.56 ± 17.93 (0 – 62.1)	9.50 ± 17.11 (0 – 65.1)
duration spent in nearby area (s)	31.24 ± 25.10 (0 – 84.2)	34.40 ± 19.81 (3.4 – 71.6)
ADT		
avoidance distance	21.44 ± 8.15 (10 – 39.6)	34.88 ± 7.04 (22.7 – 45.5)
TT		
number of animals in reach	8.11 ± 4.75 (1–16)	4.60 ± 3.55 (0.3 – 10.8)
number of animals touched	5.29 ± 4.09 (0–13)	2.31 ± 2.50 (0 – 7.3)

3.2. *Fearfulness of a novel object*

In total, 144 videos of NOT were analysed. The ICC value for video analysis of the NOT was 0.98 for the average number of chickens in the circles, 1 for the latency until the first chicken entered the circles, 0.98 for the number of entries into the circles, and 0.94 for the duration spent in the circles.

PC1 of testing day 1 explained 67.7 %, PC1 of testing day 2 explained 70.1 % and PC1 of testing day 3 explained 70.6 % of the variance in the data of NOT (for more information see Supplementary Material IV). Most loadings of the behavioural measurements indicated reduced fearfulness of the novel object, with exception of three loadings (testing day 1: loadings of latencies into both circles, testing day 2: loading of latency into the contact circle, for details see Supplementary Material V).

We neither found a significant effect between gentling and control groups, nor between testing days or for the interaction between testing day and treatment in the chickens' approach behaviour towards novel objects (treatment: $p = 0.205$; testing day: $p = 1$; interaction: $p = 0.065$, Fig. 3).

4. Discussion

A poor HAR, often resulting in fear of humans, is a common welfare problem in broiler chickens (Cransberg et al., 2000). This study investigated the effectiveness of a gentling program on the fear reactions of broilers to humans. Our results support that the early gentling program improved the HAR as it reduced avoidance behaviour and increased approach behaviour towards humans in our study subjects (Graml et al., 2008b; Waiblinger et al., 2006). However, the behavioural response to novel objects did not differ between groups (Graml et al., 2008b). Hence, the gentling program has reduced the fearfulness of humans, specifically.

Our findings align with the factors influencing the HAR identified by Rault et al. (2020). These factors include gentle handling, positive reinforcement, familiarity, human attitude, skill and knowledge, predictability of the interactions and the age of the animal, e.g. younger animals bond quicker. Rault et al. (2020) highlighted that gentle interactions are particularly effective across many animal species. Especially for chickens, many studies have demonstrated that regular gentle handling is an effective method to reduce fearfulness of humans (Graml et al., 2008b; Hemsworth et al., 1994; Jones, 1993, 1994, 1995). Although the methodology varied across studies in chickens, including age at which gentling began, its duration and the specific approach used, a reduction in fearfulness was consistently observed following repeated human contact (Barnett et al., 1994; Graml et al., 2008b; Jones, 1993; Taylor et al., 2022; Zulkifli and Siti Nor Azah, 2004). However, due to these methodological differences, it remains unclear which specific learning mechanisms were responsible for this reduction in fearfulness, or whether multiple learning mechanisms were involved simultaneously.

The age of the animal is one factor that is likely to affect its responsiveness to gentling. Thompson (1976) and Zulkifli et al. (2002) found a stronger effect of repeated contact to a human when starting with day-old chicks than when starting at a later age or continuing for longer. However, Jones and Waddington (1993) did not find this effect, possibly due to the very brief interaction (only 10 s) used in their study. Although imprinting is known to occur only during the first 2 – 3 days (Engelmann, 1969; Hess, 1958, 1959, 1964), no study has focused precisely on this period. In literature, 'early handling' often refers to gentling, which starts early but continues for a much longer period of at least 10 days (Jones, 1993, 1994, 1995; Jones and Waddington, 1993), sometimes throughout the chicken's life (Hemsworth et al., 1994; Jones and Hughes, 1981; Zulkifli et al., 2002). To our knowledge, this is the first study to focus solely on the first 3 days of life of chickens, considerably reducing the required time span. We used the imprinting period, when chickens are highly receptive to new stimuli and less likely to react with fear (Hess, 1958), to increase the habituation effect to humans. Despite this shorter period, we found an effect on human-related behaviour across all three tests assessing fear responses towards humans, which further enhances the effectiveness of early gentling during the sensitive period.

There is growing evidence that visual human contact has comparable (Zulkifli and Siti Nor Azah, 2004) or even exceeding effects on the avoidance behaviour of chickens than physical human contact (Jones, 1993; Zulkifli and Siti Nor Azah, 2004 for productivity). Applying forced physical contact, such as picking up and stroking the animal (Jones, 1993; Zulkifli and Siti Nor Azah, 2004), carries a higher risk of unintentionally stressing the animal compared to visual contact alone. The way animals perceive human contact is important and might be a reason for the contradictory results regarding visual and physical contact in some of the previously mentioned studies (Jones, 1993; Zulkifli and Siti Nor Azah, 2004). Our study addressed this by applying only voluntary physical contact, allowing chickens to choose whether to engage with the human, as recommended by Rault et al. (2020). Like other studies, we also found that chickens in both treatment groups exhibited less fear with each repetition of the behavioural tests (Caroprese et al., 2006;

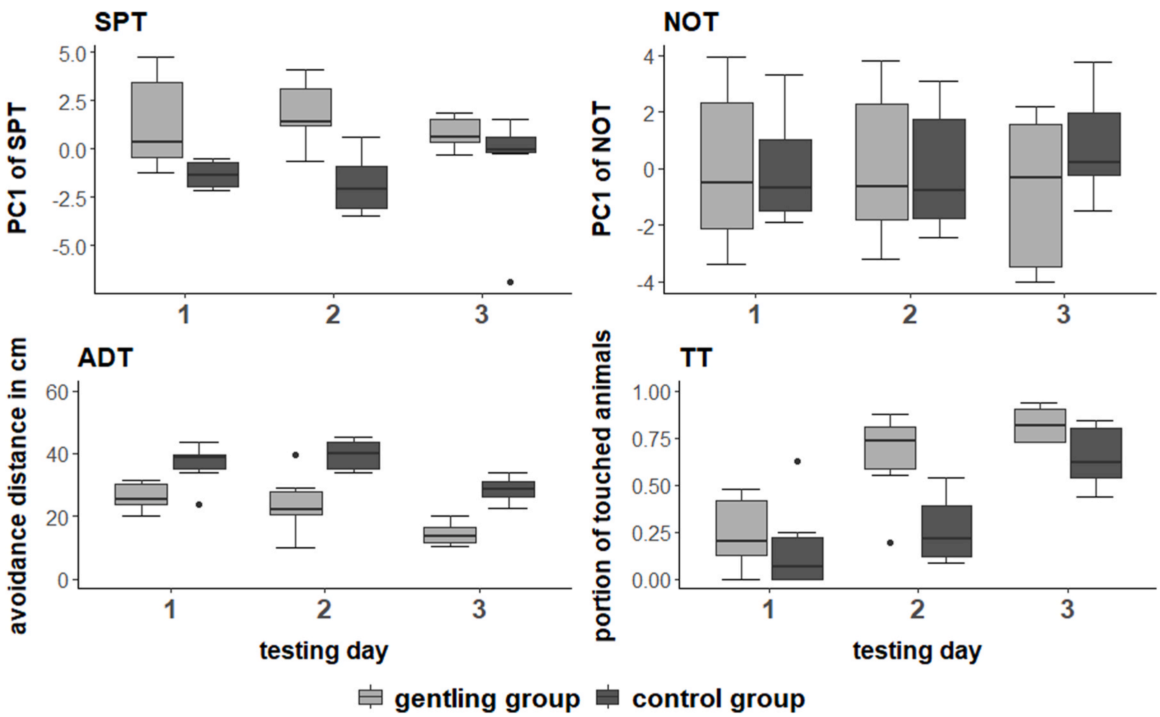

Fig. 3. Results for the behavioural outcomes in the stationary person test (SPT), the novel object test (NOT), the avoidance distance test (ADT) and the touch test (TT) per testing day. For the SPT and the NOT, the values of the first principal component (PC1) are displayed. For the ADT, the avoidance distance (in cm) and for the TT the proportion of touched animals are displayed. Data are given as boxplots with the middle line representing the median, the box representing the middle 50 % of the data, whiskers representing the range between upper and lower quartile and the dots representing the outliers.

Hakansson, 2015), which may be due to habituation to the testing procedure, growing familiarity with the human or simply affected by age, regardless of the gentling effect. Hence, it is possible that the control groups became accustomed to human presence through the required daily animal controls. The results of the TT support this assumption. While the control groups also show an increase in the TT values, it occurred later and was less pronounced than the gentling groups.

Aligning with Graml et al. (2008b), we found no effect of the gentling program on the results of the NOT, which confirms a human specific effect of the gentling program. We found no difference between testing days in the results of the NOT which is in line with Forkman et al. (2007), according to which the tests are consistent over weeks. Another possibility for a lack of a habituation effect might be that we used three different novel objects in random order in each group. As the order was the same in one gentling and one control group in each FP, it could not interfere with the effect of gentling but could still be a stronger effect than the effect of time. We could not examine potential differences statistically due to the small sample size. Upon visual expectations of the data, it was noticeable that the animals engaged much more with the traffic cone than with the ball and the beverage carton. Hence, the kind of novel object used may also be an important factor in influencing the behaviour of broilers as it is for turkeys and quail (Kulke et al., 2021; Miller et al., 2005). In other studies, often only one item was used as novel object even when the animals were tested repeatedly (Graml et al., 2008b; Hüttner et al., 2023).

The order of the behavioural tests can possibly influence the test outcome. We chose the given order in accordance with Graml et al. (2008b) from the least intrusive test to the most interacting test and omitted randomisation to minimise possible influences from one test to the other. Trying to touch a chicken as done in the last two tests (ADT and second part of the TT) can frighten animals and influence their behaviour in the first two tests (SPT and NOT) which rely on voluntary approach. An influence in the chosen order cannot be completely ruled out but is less likely than randomizing the order and is therefore a limitation of this study.

Another important factor that could influence chicken behaviour is the light intensity (Alvino et al., 2009; Blatchford et al., 2009). Usually, brighter environments make chicken more active (Newberry et al., 1988), which may increase their tendency to escape. In commercial settings, low light intensity is used to increase growth and reduce fear behaviour (Prescott et al., 2003). For this study, light intensity was levelled to approximately 100 lux before housing of the chicks. As the compartments in our study had windows and weather conditions outside might have differed between testing points, smaller differences in light intensities cannot be completely ruled out despite levelling of light intensities.

This study is the first to confirm an early gentling approach of only 3 days for broiler chickens in a husbandry system with larger groups, setting a focus on feasibility and effectiveness in larger groups rather than testing of individual birds (Jones, 1993, 1994, 1995; Taylor et al., 2022; Zulkifli et al., 2002). The comparatively smaller group size and small compartments compared to commercial settings might have made it easier to gentle the chickens as they were not able to keep much distance from the human. To be used in commercial farms, it must be tested if the program needs further optimization for practical application within the used time frame. This can impact welfare as well as productivity of commercially kept broilers (Collins and Siegel, 1987; Cransberg et al., 2000; Zulkifli et al., 2002; Zulkifli and Siti Nor Azah, 2004). According to Zulkifli and Siti Nor Azah (2004) physical human contact can positively affect body weight and the feed conversion ratio. An experiment with the use of food rewards in the early phase of life would also be conceivable, as associative learning mechanisms have been successfully used to reduce fear of humans in adult laying hens (Graml et al., 2008b) and newborn calves (Krohn et al., 2001).

5. Conclusion

Gentling of broiler chickens by humans via visual and voluntary physical contact in the first 3 days of life reduced their fearfulness of humans, but not their fearfulness of novel objects. The effect lasted during the whole fattening period until at least FD 36. This indicates that gentling is an effective tool to improve the HAR in the long-term for broilers.

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CRediT authorship contribution statement

Helen Louton: Writing – review & editing, Supervision, Conceptualization. **Michael Erhard:** Writing – review & editing. **Birger Puppe:** Writing – review & editing, Conceptualization. **Christian Nawroth:** Writing – review & editing, Formal analysis, Conceptualization. **Theresa Stocker:** Writing – original draft, Formal analysis, Data curation, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used DeepL Write (version from 14.01.2025) and ChatGPT (GPT-4-Turbo) in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2025.106710.

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