

Broiler Response to Open Field Test in Early Ontogeny

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Abstract

The aim of this study was to observe the development of exploratory behaviour in growing broilers, i.e. their reaction to a novel environment (habituation) during the post-incubation period. Twelve males and twelve females of ROSS 308 line were kept from hatching until 42 days of age in separate pens, under identical husbandry conditions. To study their behaviour, we used the open-field test (OFT). Individual 10-min tests were initiated at the age of 3 days, and were repeated at weekly intervals, i.e. a total of 6 tests were carried out. The results were evaluated using analysis of variance (ANOVA) for repeated measures and Tukey post-hoc test. Broilers of both sexes were most active during the first OFT on day 3 after hatching. In male broilers, we found a significant decrease in all exploratory activities between the first and 2nd OFT; duration of their horizontal locomotor activity was reduced from 104 ± 51.86 s to 3.50 ± 3.50 s ($p < 0.05$), duration of visual orientation decreased from 327.50 ± 55.94 s to 125.00 ± 31.94 s ($p < 0.001$), and duration of vocalizations decreased from 412.50 ± 63.12 s to 90.00 ± 34.25 s ($p < 0.001$). In females, a significant decrease was observed between the first and 2nd OFT only in duration of horizontal locomotor activity that decreased from 234.25 ± 52.07 s to 14.25 ± 8.36 s ($p < 0.001$) and between the first and 3rd OFT in duration of vocalization (decreasing from 430.00 ± 66.30 s in 1st OFT to 60.00 ± 23.35 s; $p < 0.001$). On the other hand, a significant increase in duration of comfort behaviour was noted between the first and second OFT in both sexes (in males from 95.00 ± 40.76 s to 462.50 ± 38.81 s; $p < 0.001$; in females from 85.00 ± 31.73 s to 437.50 ± 67.74 s; $p < 0.001$). Changes in defecation numbers were not significant in either sex. Our findings show that the most profound changes in behaviour of broiler chickens occurred between the first and second OFT, that male broilers used visual orientation in the open field arena more than females in the first test, they vocalized less in the second test, and they seem to have habituated to the novel environment earlier than females.

Development, locomotor activity, visual orientation, vocalization, comfort behaviour, post-hatching changes

The behaviour of chickens in the post-incubation period is subject to rapid age changes (Rogers 1995). Its development is determined by both genetic and environmental factors. Innate behaviour mechanisms are brought into effect at certain stages of ontogeny on the basis of inherent stimuli. However, the environment may influence this cascade and induce an earlier onset of behaviour or alter the meaning of the original stimulus (Hogan and Bolhuis 2005).

Intensive genetic selection in the past five decades for rapid growth (i.e. for a high food intake) and improved feed efficiency in broiler-type chickens has led to a highly efficient bird capable to attain a body mass of about 2 kg in 40–42 days (Barbato 1994; Julian 1998). However, the genetic selection aimed at high muscle to bone ratio and apart from the more than doubled growth rate resulted in many unforeseen and unwanted adverse effects and problems such as e.g. sudden death syndrome or pulmonary hypertension syndrome. Another well-known problem is severe lameness due to pain caused by bone defects and deformities (e.g. Julian 1998; Sanotra et al. 2001). According to Kestin et al. (1992) leg weakness in commercial broilers is related to rapid juvenile growth rate. Metabolic imbalance may cause some of these conditions (Julian 1998). Skeletal abnormalities thus influence gross locomotor skills and activity of the birds (Lilburn 1994). Their

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behaviour is also affected by conditions in large-scale husbandry systems that require high adaptability. Broilers are mostly kept indoors in large flocks counting more than 10 000 birds and exposed to artificial light for 23 h with 1 h darkness.

From the behavioural point of view, meat-type chickens in general show very low locomotor activity and spend about 80-90% of the time lying (Lewis and Hurnik 1990; Bessei 1992), they are described as calm, and increasingly inactive especially after 3 weeks of age (e.g. Bokkers 2004). However, when given the opportunity, they show motivation to explore their environment (Newberry 1999).

Growth and development of the domestic chick, a precocial species, is rapid; the organism undergoes morphological and physiological changes from hour to hour both before and after hatching (e.g. Freeman 1964, 1965, 1970; Baranyiová 1972; Rogers 1995).

We were interested in the early development and in possible differences in exploratory behaviour between the two sexes.

Materials and Methods

Twelve male and twelve female broilers of ROSS 308 line were kept for 42 days since hatching in separate pens of 267 × 132 cm dimensions. Chickens of both sexes were visually and acoustically separated and kept under identical husbandry conditions on deep litter (5 cm layer of wood shavings) and were exposed to light for 23 h with 1 h darkness. During the first week the chickens had access to an electric brooder with the temperature of 35-32 °C, and the temperature in the pen at the floor level was 28 °C. It was stepwise decreased to 21 °C. Relative humidity was kept between 50-70%. Chicks were fed standard broiler diets BR1, BR2, BR3 as recommended by manufacturer from metal food troughs, water was available *ad libitum*.

Open-field tests (OFT) were initiated on day 3 after hatching and were repeated at weekly intervals (6 tests were carried out). The test was done in the room adjacent to the pen area and there was a usual background noise of poultry pens since day 1 (Jones 1977a). The temperature in the test room was 20 °C. During the first three weeks, the test arena had the dimensions of 80 × 40 cm with squares of side length of 10 cm. For older birds, the arena was enlarged to 100 × 100 cm with squares of side length of 20 cm. Individual tests took 10 min. The following behaviours were observed and recorded using a video camera: duration of horizontal locomotor activity, visual orientation – looking around in the arena, vocalizations, comfort behaviour – relaxed body postures (i.e. preening, sitting, lying, sleeping), and vertical locomotor activity (i.e. jumping, flying), eliminations were counted.

The results were evaluated by analysis of variance (ANOVA) for repeated measures with Tukey post-hoc test, using SYSTAT program.

Results

A brief, 10-min exposure to a novel environment lead in the youngest chickens to intensive horizontal locomotor activity, visual orientation, vocalization, defecation and comfort behaviour (Fig. 1). Interestingly, no vertical locomotor activity such as attempts to fly or jumping against the arena walls

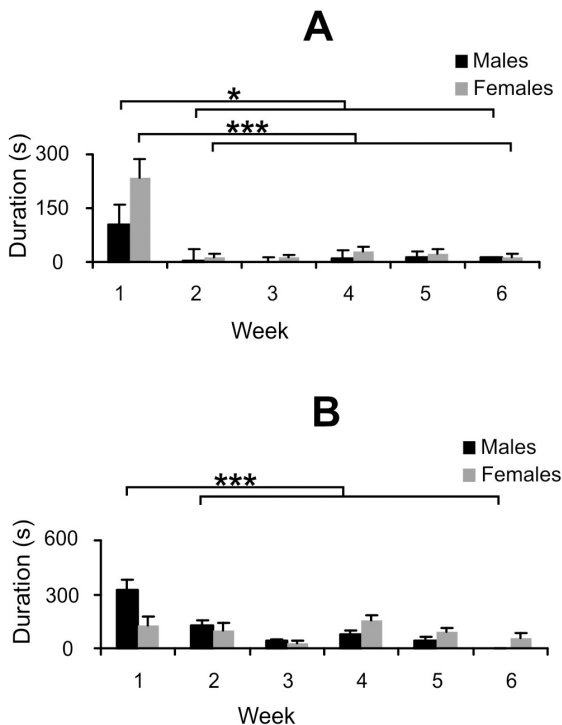


Fig. 1. Open field behaviours of broilers. A: horizontal locomotor activity, B: visual orientation;

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

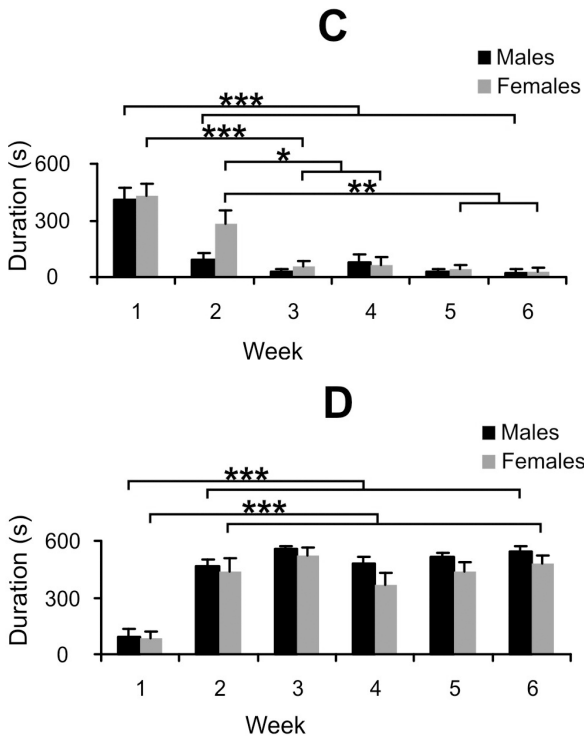


Fig. 1. Open field behaviours of broilers. C: vocalizations, D: comfort behaviour

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

a decrease in the horizontal locomotor activity between the 1st and 2nd OFT (to 14.25 ± 8.36 s; $p < 0.001$). The differences between the 1st OFT and all other tests were significant ($p < 0.001$) (Fig. 1A). During the OFT we observed no vertical locomotor activity in the birds under study.

Visual orientation

In males, a decrease in the duration of visual orientation occurred between test 1 and 2 (327.50 ± 55.94 s versus 125.00 ± 31.94 s; $p < 0.001$), (Fig. 1B). During the 3rd test, it further decreased to 40.00 ± 11.28 s. In further tests, however, visual orientation decreased again (to 0.00 ± 0.00 s in the 6th OFT). The differences between the 1st OFT and all other tests were significant ($p < 0.001$) (Fig. 1B).

Females showed significantly shorter visual orientation than males in tests 1 ($p < 0.01$) and 6 ($p < 0.05$) but none of the changes between tests 1 and 6 were significant.

Vocalization

In male broilers, a decrease in vocalization time was recorded between test 1 and 2 (412.50 ± 63.12 s versus 90.00 ± 34.25 s; $p < 0.001$). This decrease continued through the following test (3rd to 6th) repetitions (Fig. 1C).

The situation was slightly different in females: a considerable decrease in vocalization time occurred as well, but not until the 3rd OFT (i.e. from 430.00 ± 66.30 s in 1st OFT to 60.00 ± 23.35 s; $p < 0.001$). The differences between the 1st OFT and all other tests were significant ($p < 0.001$) (Fig. 1C). Significantly shorter vocalization times were also found between the 2nd and 3rd test (282.50 ± 71.46 s versus 60.00 ± 23.35 s; $p < 0.05$), between the

was observed and was therefore not evaluated. Both males and females were most active during the first testing. Along with OFT repetitions, their locomotor and exploratory activities decreased and the chicks habituated to the new environment.

Horizontal locomotor activity

In males, we noticed a significant decrease in the horizontal locomotor activity between the 1st and 2nd open field test (OFT), from 104.25 ± 51.86 s to 3.50 ± 3.50 s ($p < 0.05$). Low values in horizontal locomotor activity were maintained during all further test repetitions. The differences between the 1st OFT and all other tests were significant ($p < 0.05$) (Fig. 1A).

Although the females showed a considerably higher locomotor activity in the first test (234.25 ± 52.07 s) compared to males, the difference between males and females was not significant. However, also in females a very similar development followed, i.e. a

2nd and 4th repetition (282.50 ± 71.46 s versus 65.00 ± 38.70 s; $p < 0.05$), and also between the 2nd and 5th repetition (282.50 ± 71.46 s versus 40.00 ± 21.32 s; $p < 0.01$) as well as between the 2nd and 6th repetition (282.50 ± 71.46 s versus 30.00 ± 17.32 s; $p < 0.01$). The only sex difference in vocalization was observed in the 2nd test, when the females vocalized more than 3 times as long as males ($p < 0.05$). The character of vocalizations changed as well: during the first three tests they were limited to distress calls and in older birds they changed to peeps and warbles (Marx et al. 2001).

Comfort behaviour

The most conspicuous comfort behaviour we observed was resting in the squatting position, only occasionally preening occurred. The time spent resting increased considerably in both sexes between the 1st and 2nd test (in males from 95.00 ± 40.76 s versus 462.50 ± 38.81 s; $p < 0.001$; in females from 85.00 ± 31.73 s versus 437.50 ± 67.74 s; $p < 0.001$). This duration was essentially maintained throughout the following tests. The differences between the 1st OFT and all other tests were significant ($p < 0.001$) in both sexes (Fig. 1D) but no significant sex differences were found in comfort behaviour.

Defecation

In the 1st test, male defecations amounted to 1.25 ± 0.13 , in females 1.75 ± 0.25 times. Neither the differences between tests nor sex differences were significant.

Habituation

Decreased frequencies and durations of exploratory activities (locomotor activity, visual orientation and vocalization), and a marked rise in duration of comfort behaviours (resting in crouching position, sleep) in males showed a rapid adaptation to the test situation. In females, the habituation process had a similar though less conspicuous trend. Their visual orientation showed no significant decrease with test repetitions. Their vocalizations decreased significantly later, in the third test repetition.

Discussion

The physiological and behavioural development (Broom 1969) in the post-hatching period of chickens shows rapid changes from day to day; it was therefore intriguing to study their reactions to a novel situation early in life, beginning just at the end of the imprinting period.

The open-field test is a widely used and validated method of assessing reactions to a novel environment both in mammals and birds (e.g. Martínek and Lát 1968ab; Martínek and Novacký 1977; Jones 1989, Forkman et al. 2007). The ability to cope with a stressing situation reflects the stability of the nervous system and the degree of individual excitability (Novacký and Czako 1987; Novacký and Liday 1994, 1995). In general, a novel situation in the OF arena causes a certain degree of stress to an animal exposed to a bare environment offering virtually no stimuli.

Locomotor activity and vocalization are most frequently observed and analysed behaviours during OFT (Montevicchi 1973; Ginsburg et al. 1974; Jones 1977b). According to Jones (1989), the open-field response represents a compromise between opposing tendencies to regain contact with the flock and to minimize detection by potential predators. The sequence of behaviour patterns was analyzed by Faure et al. (1983) who suggest that in gallinaceous birds, phase 1 may include either panic running or, more often, immobility and silence probably reflecting the inhibition of all behavioural patterns by fear. The second phase they consider as an active phase including ambulation, jumps and distress vocalizations. In our birds, however, no such distinction was observed.

Heiblum et al. (1998), Vestergaard et al. (1993) and Rodenburg et al. (2003) observed in layers other activities like running against walls of OF arena, pecking, head

shaking, freezing (motionless stance) and comfort behaviour. In our experiment in broilers, we noted the following behaviours: horizontal but no vertical locomotor activity (such as jumping or attempts to fly), visual orientation, vocalization, defecation, and comfort behaviour, mostly resting. Reactions to the OF were essentially the same for both sexes.

Horizontal locomotor activity was highest in the first test on day 3, though males tended to exhibit less of this behaviour than females (the difference not significant) on day 3; this is in agreement with findings of Jones (1977a) who found male chicks less active in the open field.

There may be various reasons for the high locomotor activity in our trial during the first test. Since we began the OFT at the age of three days, the high activity might be the result of a higher sensitivity threshold for fear reactions, as stated by Heiblum et al. (1998) and Andrew and Brennan (1984). It might also serve as a thermoregulatory compensation mechanism, as mentioned by Heiblum et al. (1998); this may have been the case in our broilers as well. The animal in the arena is motivated to regain contact with its companions at the same time to minimize detection by potential predators (Jones 1989). Thus the high locomotor activity might have been such natural activity in social isolation. Similar results were reported in repeated tests for Japanese quail by Okuliarová et al. (2006).

Locomotor activity has also been viewed as an expression of fearful behaviour. As fear increases, chicks have higher motivation to escape. Heiblum et al. (1998) documented locomotor activity in OFT of chickens to increase between days 3 and 5 of age with vocalization and attempts to escape. Andrew and Brennan (1983) found that in male domestic chicks an early peak in fear reactions occurs on day 5 and then on days 8 to 10.

However, in the following test one week later we observed a remarkable and significant decrease to nearly zero in both sexes that continued in all following tests. Locomotion in broilers has been reported to decrease with advancing age (Lewis and Hurnik 1990; Newberry and Hall 1990; Reiter and Bessei 1998). It has also been repeatedly reported as affected by physical abilities, body mass, morphology, lameness, and pain (e.g. Danbury et al. 2000; Sanotra et al. 2001; Kestin et al. 2001, 2002). In our study, however, the broilers as young as 10 days (i.e. in the second OFT) did virtually not move in the open field arena. At this age and body mass of 126 g in males and 106 g in females no gait problems could be expected, and indeed, upon routine inspection, no bird showed gait problems.

Another explanation of this behaviour in the following tests might be that the intensive selection for production traits (i.e. high feed conversion and *ad libitum* access to food) during the past 50 years resulted in birds that show less energy-demanding behaviour (Rauw et al. 1998; Price 1999; Schütz and Jensen 2001, Lindquist et al. 2009). Although the cited authors employed layers in their studies, we assume that the situation is no different with broilers.

Vision and hearing are the most important senses used by precocious chickens in a new environment; they become of paramount importance immediately after hatching when the chickens have to follow their mother in search for food (Bateson 1966). The chickens recognise members of their own flock, gradually become familiar with the environment and learn to perceive threats (Graves 1973). If they experience uncertainty or move away too far from the mother and the flock, they emit loud distress calls of high frequency (Andrew and Brennan 1983). The broilers in our study used visual orientation as follows: on day 3, the males were visually orienting significantly longer than the females. As they grew older, however, both males and females showed less and less visual orientation, although females tended to use it in a slightly different time pattern than males.

Vocalization in 3-day-old broilers of both sexes accompanied more than half the time of the open field test; these were exclusively distress calls as described by e.g. Andrew (1964) and Marx et al. (2001), and the same type of calls was observed during the second and third test at the age of 17 days. Later on the vocalizations changed to short peeps, or other vocal types (Marx et al. 2001) and no more distress calls were recorded. Interestingly, duration of vocalization did not differ between males and females except for the second test; this

finding differs from that of Jones (1977a). The number of calls markedly decreased in Japanese quail chicks between days 2 and 9 (Okuliarová et al. (2006).

Ginsburg et al. (1974) consider distance vocalization as a fear-motivated behaviour. The greater the fear, the more the chicks vocalize. Our results have shown that vocalizations in males decreased as soon as in the 2nd test. In females, a decrease occurred in the 3rd test. When considering vocalization as a sign of fear, our chicks did not exhibit such symptoms after the third test, similar to findings of Okuliarová et al. (2006).

Bizeray et al. (2002) studied groups of behaviours in chickens after hatching and found that the behaviour class of foraging contained bouts of almost 10 min duration and high activity, especially locomotion; the authors have shown foraging in the litter to be a major motivation, similar to data of Bubier (1996). Since the OF arena is an environment with minimum stimuli and no litter available, it can be speculated that the chicks placed in OF lack one of their potent natural behavioural motivations.

The sensation of safety in chicks increases with the number of repetitions without a negative feedback, their activity declines and occurrence of comfort behaviour increases (Jones 1983). Repeated, biologically unimportant change in the environment causes gradual disappearance of primary reactions. This learning process is called habituation (Hinde 1970). Heiblum et al. (1998) observed habituation in chicks in OF test during the first 7 days after hatching. They saw habituation in locomotor activity, but not in vocalization and running to walls of the OF arena. They found as we did, a considerable decrease in locomotor activity between the 1st and 2nd test. In males, we observed habituation in the 2nd OFT in locomotor activity, visual orientation and vocalization, as well. In females, the habituation process tended to be somewhat slower and less distinct.

We also noted a significant increase in comfort behaviour in both sexes in the 2nd OFT. In OF tests, the comfort behaviour is a result of adaptation to new surroundings. The change to a bigger OF arena in the 4th test did not affect the behaviour of the birds. Quiet squatting body posture of our broilers often with closed eyes was not indicative of fearfulness as described by Jones and Mills (1983), Jones (1996) and others. Resting, preening and sleep are all behaviours expressing rather stable mood of an animal, relief in a situation and physically relaxed stance (Franck 1996). Thus the repeated exposure of our chicks to the OF arena, may have been a sort of “habituation within habituation”.

Our results show the development of habituation in broiler chickens up to the age of six weeks. The behaviours in the new environment were most conspicuous in the first test, and their occurrence decreased markedly thereafter except for the comfort behaviour that showed the opposite development. There were only few significant changes between the sexes. Thus habituation to the open field arena, once established, persisted up to the age of 6 weeks giving support to the notion about domestication resulting in animals with less energy-demanding behaviour (Lindqvist et al. 2009). Domestication and selection of poultry are associated with lesser fearfulness in chickens (Campler et al. 2009); thus further intensive selection of broilers for intensive food intake and rapid growth may have resulted in even less fearful or less excitable birds.

Správanie brojlerových kurčiat v teste otvoreného poľa počas ontogenézy

Správanie kurčiat v postinkubačnom období podlieha postupným zmenám v závislosti na veku. Cieľom našej práce bolo sledovať vývoj reakcii brojlerov na neznáme prostredie a ich schopnosť prispôbiť sa novej situácii (habituácia) a zistiť sexuálne rozdiely v exploračných aktivitách. Dvanásť samcov a 12 samíc línie ROSS 308 sme chovali od vyliahnutia po 42 dní v samostatných kotcoch, v rovnakých štandardne používaných zoohygienických podmienkach výkrmu. Na štúdium správania u brojlerov v postinkubačnom období sme využili test otvoreného poľa (open-field test, OFT). Individuálne 10 min. testovanie sme začali u 3-dňových kurčiat a opakovali v týždenných intervaloch (6 testov). Na štatistické spracovanie výsledkov

sme použili analýzu variancie (ANOVA) pre opakovane merania s Tukeyovým post-hoc testom. Zistili sme, že stres z neznámeho prostredia indukuje u oboch pohlaví rovnaké prejavy správania: motorickú aktivitu, vizuálne orientačné správanie, vokalizáciu, defekáciu a komfortné správanie. Obe pohlavia boli najaktívnejšie pri prvom testovaní. U samcov sme zaznamenali signifikantný pokles všetkých exploračných aktivít už v 2. OFT; trvanie motorickej aktivity sa zredukovalo zo 104 ± 51.86 s na 3.50 ± 3.50 s ($p < 0.05$), vizuálne orientačné správanie pokleslo z 327.50 ± 55.94 s na 125.00 ± 31.94 s ($p < 0.001$) a trvanie vokalizácie kleslo zo 412.50 ± 63.12 s na 90.00 ± 34.25 s ($p < 0.001$). U samíc sme signifikantný pokles zistili len v trvaní motorickej aktivity medzi 1. a 2. OFT (z 234.25 ± 52.07 s na 14.25 ± 8.36 s, $p < 0.001$) a medzi 1. a 3. OFT v trvaní vokalizácie (zo 430.00 ± 66.30 s na 60.00 ± 23.35 s, $p < 0.001$). Zmeny v počte defekácií boli nevýznamné u oboch pohlaví. S postupnou adaptáciou brojlerov dochádzalo u oboch pohlaví k signifikantnému predĺžovaniu komfortného správania od 2. OFT (u samcov sme zaznamenali nárast z 95.00 ± 40.76 s v 1. OFT na 462.50 ± 38.81 s v 2. OFT, $p < 0.001$ a u samíc z 85.00 ± 31.73 na 437.50 ± 67.74 s, $p < 0.001$) až do ukončenia experimentu. Naše pozorovania preukázali najvyšší výskyt zmien v správaní u brojlerov medzi 1. (3. deň) a 2. (10. deň) open field testom, pričom samci využívali na exploráciu neznámeho prostredia v 1. OFT viac vizuálnu orientáciu ako samice, v 2. OFT menej vokalizovali ako samice a mali tendenciu rýchlejšie sa adaptovať na neznáme prostredie.

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