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Effects of age and disturbance on fear in pheasants kept in a rearing facility

Eva Voslářová, Dora Olejníková, Vladimír Večerek, Veronika Vojtkovská, Iveta Bedáňová

University of Veterinary Sciences Brno, Faculty of Veterinary Hygiene and Ecology,
Department of Animal Protection and Welfare and Veterinary Public Health, Brno, Czech Republic

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Abstract

The survival of captive-reared pheasants in the wild depends primarily on their ability to avoid predators, therefore, pheasants need to maintain their innate anti-predatory behaviour. The aim of this study was to assess the effects of age and disturbance on tonic immobility (TI) in pheasants (*Phasianus colchicus*) kept in a commercial rearing facility. TI tests were performed in pheasants aged 8, 10, 12, 14 and 16 weeks. Randomly selected pheasants of the same age were tested either immediately after capture (group C) or after exposure to various stimuli/disturbance (group D). No significant differences in the number of attempts to induce TI were found between the observed groups of pheasants (the vast majority of pheasants remained immobile on the first attempt). The effect of age was found only in group D, where birds aged 14 and 16 weeks showed a reduction in the duration of TI compared to younger birds. In group C, the duration of TI in pheasants of different ages did not differ. The results document a change in the duration of TI in response to an intense stimulus depending on the age of captive-reared pheasants. Knowledge of changes in anti-predatory behaviour depending on age and habituation to the environment and new stimuli is essential when determining the optimal age for pheasants to be released into the wild considering its impact on the subsequent survival of the released birds. Birds released at an older age and thus accustomed to repeated disturbances during captive rearing may have impaired defence behaviour.

Tonic immobility, game bird, captivity, handling

Common pheasant (*Phasianus colchicus*) is one of the most popular species of non-migratory game birds in Europe (Santilli and Bagliacca 2008) and is often bred in captivity for the purpose of increasing the numbers of birds on hunting grounds (Draycott et al. 2002, 2005; Rütting et al. 2007; Butler and Davis 2010; Whiteside et al. 2016). However, the chances of survival of captive-bred birds after their release into an unknown complex environment are questionable. Studies have shown that the young reared by their biological parents exhibit more effective anti-predatory behaviour including immobility than artificially brooded birds (Santilli and Bagliacca 2019). Defensive reactions increase the fitness of wild animals, their life expectancy is prolonged if they adopt effective behaviour to avoid danger, namely predators (Jones et al. 1992; Dwyer 2004). However, in intensive captive farming, fear can impair the animal's welfare. Especially chronic fear has long-term effects (Jones et al. 1992) as it weakens the immune system, leads to panic and hysteria, injuries, reduced growth, impaired nutrient conversion, reproductive disorders, impaired skeletal and eggshell quality (Launay et al. 1993).

Fear is defined as a response to a perceived threat; anxiety is a generalized response to a potential threat to the integrity of the individual (Boissy 1998). Reactions to fear are physiological and prepare the animal to cope with danger. They can be very variable and their manifestations depend on the nature of the threat. There are active approaches to the threat (attack), active avoidance of danger (escape, flight, hiding), but also passive avoidance (immobility) (Erhard and Mendl 1999). Many animal species respond to the threat passively (Erhard et al. 1999).

Address for correspondence:
Prof. Ing. Eva Voslářová, Ph.D.
Department of Animal Protection and Welfare and Veterinary Public Health
Faculty of Veterinary Hygiene and Ecology
University of Veterinary Sciences Brno
Palackého tř. 1946/1, Brno 612 42, Czech Republic
E-mail: voslarovae@vfu.cz
http://actavet.vfu.cz/

A non-invasive tonic immobility (TI) test based on a natural defence response is used to assess the level of fear. The principle of this defence strategy of the prey is to eliminate movement and thus deactivate the attack of the predator. The duration of TI reflects the degree of an individual's fear response. The longer a bird remains in a state of TI, the more negative impact (fear, stress) the situation that caused TI had on the bird (Balážová and Baranyiová 2009). Due to the close relationship between the TI response and the level of fear, the duration of TI is a good indicator of general timidity, not just stimulus-specific fear responses (Jones and Mills 1983; Jones 1987; Jones et al. 1991).

TI manifests itself as a profound motor inhibition or paralysis-like catalepsy with intermittent periods of eyelid movement, changes in the heart and respiratory rates, tremor, and decreased reactivity to external stimuli. TI can take several seconds to more than an hour (Gallup 1974). TI is an innate, not acquired, response to fear in various species of game birds (Gallup et al. 1971; Borchelt and Ratner 1973; Sargeant and Eberhardt 1975; Gallup 1977; Thompson et al. 1981; Odén et al. 2005).

The aim of this study was to assess the effects of age and disturbance on the fear response in common pheasants (*Phasianus colchicus*) reared in a commercial rearing facility based on TI assessments.

Materials and Methods

Birds

The study was carried out at a facility rearing pheasants (*Phasianus colchicus*) for the purposes of increasing numbers of game birds on hunting grounds. The pheasants were transported to the facility as one-day-old chickens from France and kept in a fenced outdoor aviary (5 m × 30 m) with a natural soil base and with access to an indoor area where lighting and natural ventilation were provided. In both outdoor and indoor areas, wooden perches were available. The flock size per one housing unit was approx. 200 birds. The birds were fed a commercial feed mixture; water was provided in poultry drinking bowls in the indoor area.

For the purposes of the study, the birds were divided into two groups: a control, undisturbed group (group C) and a disturbed group (group D). The birds of group C were individually captured by the landing net in the aviary and transferred to the test site. Contact with the handlers' hands was limited to the minimum necessary for the transfer of each bird from the landing net to the test site. Group C birds were tested on days when there was no disturbance to the birds beyond routine operations (e.g. feeding) on the farm. Group D birds were tested on days when there was an increased disturbance on the farm caused by the catching and loading of birds selected for transport to another facility or to be released, i.e. all pheasants on the farm were exposed to an increased activity, movement of unknown people, and noise. Randomly selected pheasants were herded into a smaller aviary from where they were individually captured for examinations. Upon determination of sex, they were weighed. Blood samples were taken by a private veterinarian from the vena cutanea ulnaris superficialis for medical examinations. Previous studies have shown that these stimuli are stressful for pheasants (e.g. Chloupek et al. 2009; Bedáňová et al. 2018; Volfová et al. 2022). After exposure to these stressful situations, the TI was performed.

Tonic immobility test

TI tests (modified methodology according to Benoff and Siegel (1976)) were performed at the age of 8, 10, 12, 14 and 16 weeks in birds randomly selected from the flock. Each pheasant was caught and carried by both hands, transferred to a windowless room and turned to the right side, the left hand lightly pressing on the bird's torso, the right hand covering its head. The bird was kept in this position for 15 s, then the researcher moved 1.5 m from the table and looked away from the bird. The second researcher stood 3 m away, measuring time with a stopwatch until the pheasant stood up on both limbs, or 10 min after the test began. If TI lasted less than 10 s, the trial was considered invalid and restarted after 10 s, the maximum number of attempts to induce TI was five. In case the TI was not induced after 5 trials, the duration of TI was considered 0 s. If the bird did not stand up within 10 min, the test was terminated and TI duration of 600 s was recorded. After the end of the TI test, group C birds were weighed and their sex was determined. In each age category, 15 birds in group C and 15 birds in group D were tested.

Statistical analysis

The results were analysed using the statistical package Unistat 6.5. (Unistat Ltd., GB). At first, data were tested for normality by Shapiro-Wilk test (Zar 1999). Because of their non-normality, data were subjected to a nonparametric Kruskal-Wallis ANOVA with a combined group × age factor (group by age interaction) as the main effect. When the effect was significant, data were subsequently subjected to a nonparametric multiple comparison with t-distribution using rank sums (Zar 1999) to assess the significance of differences between all possible pairs of compared groups. A P value < 0.05 was considered significant.

Results

The results of TI examinations of the two groups of pheasants exposed to different conditions prior to testing (quietness or disturbance) at the age of 8, 10, 12, 14, and 16 weeks are given in Table 1.

Table 1. Mean (± SEM) duration of tonic immobility and number of attempts to induce tonic immobility in common pheasants tested under different conditions.

Age	Tonic Immobility			
	Induction (attempts)		Duration (s)	
	Control	Disturbance	Control	Disturbance
8 weeks	$1.07 \pm 0.07^{a,x}$	$1.00 \pm 0.00^{a,x}$	$358.0 \pm 47.0^{a,x}$	$474.5 \pm 49.0^{ab,x}$
10 weeks	$1.20 \pm 0.02^{a,x}$	$1.00 \pm 0.00^{a,x}$	$327.3 \pm 51.6^{a,y}$	$554.7 \pm 25.3^{a,x}$
12 weeks	$1.08 \pm 0.07^{a,x}$	$1.08 \pm 0.08^{a,x}$	$350.8 \pm 52.8^{a,x}$	$470.2 \pm 52.0^{ab,x}$
14 weeks	$1.07 \pm 0.07^{a,x}$	$1.00 \pm 0.00^{a,x}$	$429.3 \pm 38.3^{a,x}$	$402.9 \pm 50.8^{b,x}$
16 weeks	$1.00 \pm 0.00^{a,x}$	$1.40 \pm 0.27^{a,x}$	$314.9 \pm 44.7^{a,x}$	$403.6 \pm 60.2^{b,x}$

^{a,b} Means within the same column lacking a common letter of superscript (a, b) differ significantly ($P < 0.05$).
^{x,y} Means within the same row lacking a common letter of superscript (x, y) differ significantly ($P < 0.05$).

No significant differences were found in the number of attempts to induce TI between the two groups of pheasants during the monitored period (from 8 to 16 weeks of age of pheasants). The vast majority of pheasants remained immobile on the first attempt.

The longest duration of TI was found in group D pheasants aged 10 weeks (554.7 s). It was significantly ($P = 0.0013$) longer than the duration of TI in group C pheasants at the same age. In addition, group D pheasants aged 10 weeks showed a significantly longer duration of TI than group D pheasants aged 14 and 16 weeks ($P = 0.0291$ and $P = 0.0381$, respectively). The duration of TI was not affected by the age of pheasants in group C.

Discussion

The duration of TI is affected by a number of factors. Bilcik et al. (1998) found that when performing the TI test in a group, the duration of TI was up to 4 × shorter than that of individually tested animals. However, it is not likely that pheasants released into hunting grounds would be exposed to a predator together. In our study, TI tests were performed individually in birds separated from the flock. Gallup (1974) did not find significant differences in the duration of TI depending on sex. The absence of sex differences is to be expected from an evolutionary point of view, as there would be little reason for a trait related to anti-predator defence to be expressed as a dimorphic trait (Gallup 1974). Therefore, we did not take sex into account in our research, however, the sex ratio of the tested pheasants in both groups was almost equal. In previous studies, the effect of animal weight on the duration of TI was not found (Nowaczewski et al. 2012). In our study, the differences in the weight of the tested pheasants were mainly related to their growth during the monitored period, thus we assessed this as the effect of age on the TI response, not the effect of the weight of individual birds.

According to previous studies, the duration of TI increases with an increasing age of birds (Borchelt and Ratner 1973; Benoff and Siegel 1976; Brake et al. 1994; Vestergaard and Sanotra 1999; Nowaczewski et al. 2012). In our study, the effect of age was seen only in group D, but in the opposite way; with increasing age, the duration of TI decreased. In group C, the duration of TI in pheasants of different ages did not differ. The probable explanation is the different age range observed in our and other studies. Nowaczewski et al. (2012) tested TI in pheasants on days 10, 20, and 35 of their lives.

Vestergaard and Sanotra (1999) tested TI in broiler chickens aged 17, 24, 31 and 38 days. Brake et al. (1994) compared tonic immobility between 15 and 20-week-old pullets. Benoff and Siegel (1976) tested TI in Japanese quails at 6 and 12 days of age. Borchelt and Ratner (1973) reported increased durations of TI in bobwhite quails from 15 to 30 days. In our study, pheasants aged 8 to 16 weeks (56 to 112 days) were observed, thus they were older birds than those tested in previous studies. For comparisons, the results published by Nowaczewski et al. (2012) are particularly interesting since they tested captive-reared pheasants (while the other studies evaluated changes in the TI duration in domestic poultry). Based on their results, Nowaczewski et al. (2012) assumed that in the period from day 10 to 35 day of life, the pheasants became less resistant to stress, resulting in extended TI duration (from 62.6 s to 127.3 s). In terms of practical implications, Nowaczewski et al. (2012) concluded that while these older birds would be more susceptible to stress on farms, they may have a better defence mechanism when potentially endangered by a predator in the wild. Our results show that in older birds, the duration of TI was generally longer than that reported by Nowaczewski et al. (2012), which may support the appropriateness of the release of older pheasants. However, determining the optimal age for releasing pheasants is difficult, as the response to a potentially dangerous situation is likely to subsequently decline again. In our study, a reduction in TI duration was found in group D pheasants at 14 and 16 weeks of age compared to younger birds. Thus, the results suggest that an increase in TI duration is not linearly dependent on the pheasant age. Several factors are likely to be reflected in the response to an intense stimulus, among them also learning and habituation, as pheasants have been repeatedly exposed to disturbance and testing in our study. Habituation is fundamental in the captive breeding of animals, however, for animals intended to be released into the wild, retention of their innate responses to danger is desirable. A decrement in response as a result of repeated stimulation with no biological consequences in captivity may be a major problem for pheasants reared in captivity for their subsequent release into the wild, in contrast to animals kept permanently in captivity. It is well documented that repeated handling of tested birds, complexity, and enrichment of their enclosure have a significant effect on shortening the duration of TI (Forkman et al. 2007; Hrabčáková et al. 2012). From this perspective, it is positive that the vast majority of pheasants in both groups remained immobile at the first attempt to induce TI and the number of attempts to induce TI did not change with age. Furthermore, in group C pheasants, the duration of TI did not change with age, the basic reactions remained the same even in a stable protected environment. However, the results of TI tests in group D, which showed a reduced duration of TI at 14 and 16 weeks after repeated exposure to disturbance and more intense contact with humans (potential predators), seem more relevant to assess the response of pheasants to potentially life-threatening stimuli. Pheasants that retain their fear response are more sensitive to handling stress in captivity, but in the wild they may have better defence mechanisms against predators. How to achieve this in conditions of captive rearing is a question. A number of studies show that the survival rate of artificially brooded pheasants after their release into the wild is low. Pheasants from wild populations have a much higher survival rate in the wild than pheasants released from captivity into the same environment (Anderson 1964; Krauss et al. 1987; Hill and Robertson 1988; Brittas et al. 1992; Leif 1994). Most of the losses of pheasants released into the wild are caused by predators (Leif 1994; Sodeikat et al. 1995; Bliss et al. 2005). Thus, survival rates primarily depend on the pheasants' ability to avoid predators. The pheasants need to maintain their escape reflexes and shyness. Freezing and immobility are reactions to fear and are typical examples of predator avoidance behaviour described in various game birds species (Gallup et al. 1971; Borchelt and Ratner 1973; Sargeant and Eberhardt 1975; Gallup 1977; Thompson et al. 1981; Odén et al. 2005). They present the last stage of various anti-predatory mechanisms leading to the predator losing its interest in the prey (Jones et al. 1991). The current intensive pheasant breeding facilities use mechanical incubators for egg incubation and artificial brooders for rearing the chickens. However, the lack of parental care can have consequences on the behaviour and welfare of these birds and also affect their survival after their release into the wild. Santilli and Bagliacca (2019) compared the reaction of 4-week-old pheasants (brooded by a foster mother hen and artificially brooded) and found that the duration of TI differed significantly between the two groups of birds. Pheasants brooded by a foster hen showed a stronger response to aerial predator compared to artificially brooded pheasants.

In conclusion, the results document a change in the duration of TI in response to an intense stimulus depending on the age of the captive-reared pheasants. Changes in anti-predatory behaviour depending on age and habituation to the environment and new stimuli should be taken into account when determining the optimal age for releasing pheasants into the wild considering their impact on the subsequent survival of the released birds. Birds released at an older age and thus accustomed to repeated disturbances may have impaired defence behaviour.

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