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**Sublethal effects of pesticide residues differ between strains of captive Grey partridge:
consequences in terms of rearing conditions**

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ABSTRACT

Over the last 50 years, farmland bird populations have declined steeply in Europe and North
America. Reintroduction or reinforcement for populations unable to self-maintain are popular

management tools to overcome extinction risk through captive rearing and release. However, released birds tend to have lower survival rates than their wild conspecifics. Here, we aimed to mimic the diet shift that Grey partridge (*Perdix perdix*) encounters once released (poultry food to natural grain) and compared (i) differences in responses to this transition depending on strain (wild or farm) and (ii) the consequences of ingesting organic or conventional grains on individual body mass, haematocrit and behavioural traits (anti-predator responses, activity, exploration) that are likely critical for post-release survival. Our experimental procedure involved 40 farm-strain and 40 wild-strain partridges fed with commercial poultry food during early life then with wheat and corn for 3 months, from either organic or conventional (clopyralid residues) agriculture. The results suggest that the haematocrit and body mass index of farm-strain partridges were lower at the end of the experiment, probably due to diet shift. Birds fed conventional grains (with clopyralid residues) exhibited lower flight duration and flight initiation distance, and were more likely to translocate by running rather than flying. The findings suggest that, when rearing partridges of farm-strain origin, birds fed organic grains experience better post-release survival than birds fed conventional grains containing pesticide residues.

KEYWORDS: captivity, conservation, strain, *Perdix perdix*, pesticide residues

1. INTRODUCTION

Over the last 50 years, farmland bird populations have declined by 20% in Europe (Burns et al. 2021) and 74% in North America (Stanton et al. 2018). The main causes of decline are related to agricultural intensification, including habitat loss, seasonal shifts in cultivation practices, and increased use of agrochemicals (Robinson and Sutherland 2002; Newton 2004). Reintroductions after strong decline (e.g. the process of rearing animals in captivity and

releasing them into the wild) or reinforcement for populations unable to self-maintain are two management tools that have become increasingly popular in the past 30 years to overcome extinction risk, even on a local scale (Seddon et al. 2007). Reinforcing wild populations has also been commonly used in game birds for hunting purposes, leading to game farm implementation, which bred originally wild-strain birds in captivity for decades (Buner et al. 2011). However, released farm-strain individuals such as common pheasant (Musil and Connelly 2009), chukar partridge (Yolcu et al. 2016) and grey partridge (Parish and Sotherton 2007) tend to have lower survival rates than their wild conspecifics (Sokos et al. 2009). Understanding why farm-strain birds are less fit to persist in wild environments may help us to improve reintroduction projects through changes in rearing conditions.

Captive environments are known to impair genetic diversity, lead to genetic drift, and enhance adaptation to captivity, all of which can be detrimental when such individuals are released into the wild (Frankham 2008; Keller et al. 2012). Indeed, many studies have reported behavioural deficiencies in farm-strain birds that impair survival (Rymešová et al. 2012, 2013; Homberger et al. 2014; Yolcu et al. 2016), especially in regard to excessive boldness (Madden and Whiteside 2014), inhibited escape responses (Pérez et al. 2010), lower group vigilance (Rantanen et al. 2010b) and poor anti-predator defences (Håkansson and Jensen 2008; Rantanen et al. 2010a). Upon release, animals have to cope with risk-taking, foraging and novel environment discovery (Réale et al. 2007). Fleeing quickly in life-threatening situations, and showing a high level of exploration and activity to find food, sexual partners and territory, should collectively increase fitness (Sih et al. 2004; Smith and Blumstein 2008; Homberger et al. 2013). Moreover, farm-strain birds display higher body mass, lower glycogen reserves and lower oxidative capacity than wild ones as a result of unlimited access to high-nutrient food in captivity (Putaala and Hissa 1995, 1998). In addition to making them less fit, captivity accustoms their metabolism to conditions that sharply

diverge from those that await them in nature (i.e. food unpredictability and quality, variable diet, vulnerability to predators and/or diseases). Food quality during captivity may thus also play a role in post-release survival and the success of the reintroduction process (Madden et al. 2020). Traditionally, farm-strain birds are fed with highly-digestible commercial poultry food (Liukkonen-Anttila et al. 1999, 2000) including genetically modified soya, wheat and corn that differs from their natural diet (in terms of energy supply, digestibility and quality). However, whether in processed or unprocessed form, grains used in farm-strain bird diets may contain pesticide residues used in conventional agriculture (Kumar et al. 2019). Even small quantities of pesticides in food may cause sublethal effects when eaten over an extended time (Parween et al. 2016; Moreau et al. 2021). In game birds, exposure to pesticide treatments are known to deteriorate the immune system (Lopez-Antia et al. 2015b) and haematocrit (Lopez-Antia et al. 2015a), but also induce dysfunctions of metabolic pathways associated with the ability to store fat (Lopez-Antia et al. 2013; Moreau et al. 2021), and interfere with the endocrine system (Lopez-Antia et al. 2015c; Khalil et al. 2017). The latter results in behavioural changes in general activity (Moreau et al. 2021), risk-taking behaviour (Kobiela et al. 2015) and vigilance (de Faria et al. 2018), which may have positive or negative effects on survival (i.e. foraging, reproduction, predation) depending on environmental context (Smith and Blumstein 2008).

Therefore, strain (wild or farm) and food quality may affect physiological or behavioural traits in released individuals, which could alter the fitness and long-term demographics of population though simultaneous effects that have received limited attention (but see Homberger et al. 2013, 2015 and 2021). In the present study, we used Grey partridge (*Perdix perdix*), an iconic game bird of European farmlands that is currently declining, as a test case (Sotherton et al. 2010; Aebischer and Ewald 2012; Bro 2016). Several million birds are annually reared and released in Europe, including ~2 million in France (Bro 2016;

Madden 2021). Mortality rates of released farm-strain partridges are very high at 60–80% (Parish and Sotherton 2007; Buner and Schaub 2008; Rymešová et al. 2013; Bro 2016; Homberger et al. 2021). Although many studies have investigated morphological, behavioural and physiological deficiencies, as well as post-release survival of farm-strain grey partridges (Putala and Hissa 1995, 1998; Rantanen et al. 2010a, 2010b; Keller et al. 2012; Homberger et al. 2013, 2015, 2021; Rymešová et al. 2013), none have explored the potential interactions between strain (wild or farm) and sublethal effects of pesticides. We thus aimed to mimic the diet shift that partridges encounter once released (poultry food *vs.* natural grain) to investigate (i) differences in responses to this transition depending on strain, and (ii) the consequences of ingesting organic or conventional grains on physiology and behaviour traits that are likely critical for post-release survival. Our experimental procedure involved 8-month-old grey partridges, either bred in captivity for 60 generations (farm strain) or 2 generations (wild strain). Partridges were fed from birth with poultry food. At the beginning of the experiment, they were fed with natural grain grown by either organic or conventional agriculture. Individual conditions were monitored throughout captivity and several behavioural traits were also quantified for each bird (see Methods for details). We predicted that birds that had been in captivity the longest (farm strain) would lose weight and display a lower haematocrit in comparison to wild-strain birds due to diet shift. Furthermore, we predicted that birds fed conventional grains would be even more affected in terms of physiology and behaviour due to potential pesticide residue ingestion.

2. MATERIALS AND METHODS

2.1 Experimental design

The study took place in a commercial game farm in Southwest France, and lasted 3 months, from December 2019 to March 2020. Tests were performed on 80 8-month-old captive-born

grey partridges; 40 were from a game farm strain (60 to 80 generations in captivity, ‘farm-strain’ hereafter) and 40 were from F3 generations of wild trapped birds (two generations in captivity, ‘wild-strain’ hereafter). From birth to the beginning of the experiment (corresponding to the diet shift from poultry food to natural grain), all birds had the same experience, living in two large pens depending on their strain (100 m × 10 m × 4 m) at the game farm. They were fed ground feed mixture for game birds (STARGIB entretien; see ESM 1 for composition) mainly made from imported conventional soya bean, maize, wheat and sunflower, likely containing pesticide residues (Klüche M. et al. 2020). Before experimentation commenced, each bird was sexed (according to the presence of secondary sexual characters) and fitted with an alphanumeric metal ring.

In December 2019, four experimental groups of 20 partridges were established randomly, including 10 males and 10 females, each placed in a grassy holding pen (100 m × 10 m × 4 m) including a game bird feeder, several drinkers equally spaced in the pen, and two sheets of metal providing shelter. The first group comprised 20 wild-strain partridges fed *ad libitum* with grains obtained from harvests of certified organic crops (i.e. purchased from organic producers), composed of half wheat and half corn. The second group of 20 wild-strain partridges was fed *ad libitum* with grains purchased from producers in conventional agriculture (i.e. for which different pesticides were used during the cropping season) also composed of half wheat and half corn. Similarly, the third and the fourth groups were composed of 20 farm-strain partridges each, either fed *ad libitum* with organic or conventional wheat and corn.

A previous study showed that conventional grains contain pesticide residuals whereas organic grains do not, and that the energy content for both types is equivalent (Moreau et al. 2021). In the present study, only conventional grown wheat had detectable and quantifiable pesticide residues (analysed by GIRPA, a referenced laboratory based at Beaucouze, Loire

Atlantique, France). We detected 0.014 mg/kg clopyralid, above the limit of quantification set at 0.010 mg/kg. Clopyralid is an organochlorine herbicide that is considered relatively non-toxic to terrestrial and aquatic animals, although available data concerning toxicity to birds are scarce (EFSA 2018). No pesticide residues were detected in organic grains. Birds were under constant surveillance, with water and food provided each day by the farmer from the 18th of December (week 0) to the 10th of March (week 12). All groups were kept under a natural light cycle, and 11 partridges died during experimentation (4 in the wild-strain organic group, 3 in the wild-strain conventional group, 1 in the farm-strain organic group, and 2 in the farm-strain conventional group; Fisher's exact test; $p = 0.57$).

2.2 Monitored parameters

During the experiment, birds in each group were monitored to assess (i) their physiological condition through haematocrit, which reflects nutritional state and physiological stress (Ots et al. 1998), and body mass index, (which reflects energy capital accumulated by feeding (Peig and Green 2009), and (ii) their behaviour in relation to anti-predator responses, activity, and exploration (Réale et al. 2007; de Faria et al. 2018).

2.2.1 Physiological conditions; haematocrit and body mass index

Morphological measurements and blood samples were taken the day before release, while in their pens, in December (week 0) and after 3 months of food type (week 12). To assess haematocrit, a blood sample was taken from the brachial vein using a sterile needle (\varnothing 0.06 mm) and heparinised micro-capillary tubes (10 μ L). Capillary tubes were then centrifuged at 5000 rpm for 5 min immediately afterwards to determine haematocrit levels; the proportion of the tube filled with red blood cells was divided by the total sample volume in the capillary tube. (Biard et al. 2015). Length was measured with an electronic calliper to the nearest 0.1 mm. In order to compare the body mass index of grey partridges, right and left tarsus lengths were measured twice with a calliper (accuracy 0.1 mm) at the beginning of the experiment

(week 0), since in adulthood tarsus lengths remain stable. Body mass was recorded at week 0 and week 12 to the nearest 1 g using a spring scale (Pesola 500 g). From body mass and mean tarsus length (right and left), we evaluated the body mass index with the scaled mass index (Peig and Green 2009; Moreau et al. 2021).

2.2.2 Behavioural tests

A set of behavioural tests were performed in week 9 during three consecutive days with identical weather (from the 18th to the 20th of February); 21 birds were tested on day 1, 27 on day 2, and 27 on day 3. Each evening, partridges of different groups were randomly caught, placed in cardboard boxes (five birds per box, 66 cm × 38 cm × 17.5 cm), and left inside for the night under controlled conditions (7–11°C). Catching birds the night before the experiment enabled us to decrease the capture stress that can affect behavioural tests. The following day, each bird was processed using the same sequence, which consisted in (i) the cage test and (ii) the open-field test. The cage test consisted of measuring flight initiation distance (FID) and escape attempt strategy (as birds could not escape) in a cage outside. The open-field test consisted of measuring activity, exploration, escape strategy and duration of escape in an open-field test. The behaviour of each bird was always recorded by the same observers. After the full series of measurements, birds were released into their pens.

2.2.3 Cage test

Partridges were placed by a single operator in a square, wire cage (46 cm × 32 cm × 36 cm) in a ploughed field. Another operator was hidden behind a hedge, 100 m from the cage, and they watched the bird's behaviour through binoculars. After 5 min of acclimatisation, the hidden operator approached the bird. The FID, the distance at which the individual begins to fly when a predator, or human, approaches (Frid & Dill, 2002), was estimated as the distance at which the bird reacted (tried to run or fly inside the cage) using the straight-line approach of the operator. The escape attempt strategy (running or flying) of the bird was also recorded.

Approaches towards birds were performed by the same operator to standardise approach speed and operator dress, thus limiting detectability bias. After the test, birds were caught by the first operator, placed in a cardboard box (66 cm × 38 cm × 17.5 cm) and brought directly to the open-field test in a nearby building (5 m × 5.5 m × 1.50 m, 7–11°C, 30.5 lux).

2.2.4 Open-field test

A grid was set on the floor of the building that included 110 numbered squares, each 50 × 50 cm. Partridges were brought in a cardboard box (66 cm × 38 cm × 17.5 cm) and given 3 min for acclimatisation to the box. The operator, hidden in a corner behind a sheet, then opened the cardboard box to let the bird out. The exploration behaviour of the partridge was assessed by counting the number of different squares visited during 7 min. Activity, considered any behaviour except resting, as measured as the number of times each square was visited. At the end of exploration and activity assessment, a stuffed fox (40 cm × 80 cm) on castors, hidden with the operator, was launched towards birds at a speed of ~1 m/s. The responses to this stimulus were then assessed as (i) their escape strategy (running or flying) and (ii) their duration of flight (recorded with a chronometer). After the test, partridges were caught with a landing net and returned to their cage (see Fig. 1).

2.3 Statistical analysis

We analysed the effects of strains and treatments on the physiological conditions of birds at three different times. First, at the beginning (week 0) and at the end (week 12) of the experiment, we tested whether strain (wild vs. farm), food type (organic vs. conventional), and their combination affected haematocrit and body mass index, accounting for sex differences using analysis of variance (ANOVA). Although no effect of food type was involved at week 0 since all birds were fed with the very same food until that date, food effect from the start of the experiment was included in the model to test for possible initial sampling bias. A third model investigated whether trends existed for haematocrits or body mass index

between week 0 and week 12, including the effects of strain, sex, and food nested within strain. Pairwise post-hoc comparison tests between the four groups were tested using Bonferroni correction in the case where interactions between strain and food type were significant. We chose to nest the effects of food and strain because at the beginning of the experiment we already had a strain effect (see the results below). Similarly, we analysed the effects of strain and food type on behavioural traits, including their interactions and sex differences. Activity and exploration were square root transformed before analysis. Both escape strategy during cage and open-field tests could be in the form of flight or running, hence we used a binomial modelling framework in the logistic regression model and escape strategy (0 for running, 1 for flying) as a response variable. Finally, for birds that flew away during the open-field test, duration of flight was also square root transformed before analysis. The normality of residuals and the distribution of residuals against fitted values were checked for each model. Effect of blood sample hours on haematocrit variation was also checked at week 0 using simple linear model (LM). For all tests, *p*-values were calculated at the 5% significance level. All statistical tests were performed using R software (v. 3.5.0, R Core Team 2018) and the *smatr* package (Warton et al. 2012) for scale mass index computation (Peig and Green 2009). Results are presented as means \pm standard error (SE).

2.4 Ethical approval

All experiments complied with French laws on animal experimentation, and all experimental protocols were approved by the Committee of Animal Experimentations of the Deux-Sèvres French Department (APA FIS#9465-201703101551625).

3. RESULTS

Numbers of monitored partridges differed depending on the test undertaken (this is specified on each figure). Concerning physiological condition comparison, 65 partridges were

monitored (11 deaths at the end of the experiment and four without all data). Concerning behavioural tests, 73 partridges were monitored because at the time of the test, seven partridges were already dead.

3.1 Partridge physiological conditions

Time of blood samples did not affect haematocrit variation (LM $\chi^2 = 1.77$, $df = 1$, $P = 0.19$). Farm-strain partridges had slightly but significantly lower haematocrits than wild birds at the onset of the experiment, with haematocrits of birds in the conventional food type group significantly lower than in the other groups (i.e. highlighting a sampling bias, Fig. 2.a, Table 1). At week 12, farm-strain partridges still had lower haematocrits (Fig. 2.a). Haematocrits of all four groups increased with both food types (significant intercepts when comparing week 0 and 12) with a significant effect of sex (Fig. 2.a, Table 1). Indeed, haematocrits of males (mean \pm SE at week 0; $42.96 \pm 3.17\%$ at week 12; $46.91 \pm 3.53\%$) increased more than those of females ($43.33 \pm 3.37\%$ and $44.85 \pm 3.27\%$; Fig. 2.a, Table 1). Thus, there was no statistically significant effect of food type on haematocrit.

Conversely, the body mass index of farm-strain partridges was significantly higher than that of wild birds at the onset of the experiment (Fig. 2.b, Table 1), and female body mass index was higher than that of males (week 0, 388.8 ± 31.8 g for females, 357.5 ± 28.7 g for males). At week 12 a significant sex effect was evident, with female body mass index remaining higher than that of males (week 12, 365.1 ± 37.1 g for females, 331.2 ± 22.8 g for males). In addition, body mass index decreased during the food type experiment only for farm-strain partridges regardless of food type, whereas this index stayed stable for wild birds (Fig. 2.b, Table 1).

3.2 Partridge behaviour

Strain and food type affected behavioural traits in different ways (Table 2). During the cage test, farm-strain partridges fed conventional grains showed lower FID (46.1 ± 22.2 m) than other birds (farm-strain birds fed organic grains = 63.1 ± 13.2 m; wild-strain birds fed organic

grains = 56.7 ± 16.9 m; wild-strain birds fed conventional grains = 65.2 ± 18.9 m; Fig. 3.a). Moreover, strain and food type interacted significantly to impact escape strategy; farm-strain partridges fed conventional grains were less likely to escape by flying in comparison to other groups (Table 2, Table 3). Concerning the open-field test, farm-strain partridges were less active (Table 2, Fig. 3.b) and less likely to try to escape by flying in response to the stuffed fox in comparison with wild-strain birds (Table 2, Table 3). Finally, for birds that escaped by flying, duration of flight was higher for wild-strain partridges fed organic grains, and farm-strain birds that consumed conventional grains displayed the shortest flight duration among the four groups (Table 2, Fig. 3.c).

4. DISCUSSION

In this study, we compared the effects of captive-reared bird strain (farm vs. wild) and interactions with potential sublethal effects of low doses of pesticide ingestion through food (organic vs. conventional grains), though only clopyralid was detected. Regardless of food type, the body mass index of farm-strain birds was higher at the onset of the experiment, and thus decreased as the experiment progressed, while that of wild-strain birds remained stable. Conversely, haematocrit of farm-strain partridges was lower at the onset of the experiment but increased over time for all groups. We also found that farm-strain partridges had lower activity and tended to flee by running rather than flying during the open-field test. Finally, we found an interaction between strain and food type in behavioural traits involved in anti-predator responses, with farm-strain birds fed conventional grains (containing clopyralid residues) showing lower FID and shorter flight duration, and they were less likely to try to escape by flying during cage tests than other groups. Such an interaction between strain and food type resulted in the worst performance for farm-strain birds fed conventional grains. These results suggest that these birds could be disadvantaged in the wild.

Captive-bred grey partridges are traditionally fed *ad libitum* using feeders containing commercial poultry food; however, when released, these birds have to cope with an unpredictable and strikingly different type of food, based on winter grains (Perkins et al. 2007). Our experimental design somewhat mimicked this transition as diet of birds shifted from poultry food to natural wheat and corn grains at the onset of the experiment. As predicted, farm-strain partridges showed higher body mass than wild-strain birds at the beginning of the experiment, highlighting morphological differences between the two strains. Indeed, captive-reared birds are often larger than their wild counterparts as a result of unlimited access to high-nutrient food over several generations of captivity (Putaala and Hissa 1995, 1998). Regardless of treatment type, we found that the body mass index of farm-strain birds decreased, which could suggest that those birds may be less inclined to properly assimilate new/unfamiliar food. Indeed, captivity adaptation could have led to shortened intestines, caeca and gizzards of our farm-strain partridges, in comparison with wild birds (Putaala and Hissa 1995; Liukkonen- Anttila et al. 2002), although we did not examine the digestive systems of our birds in order to keep them alive. These organs play a role in food digestion and assimilation, and the larger they are, the more effective they are at processing fibrous food such as natural grains. As expected, wild-strain partridges seemed to cope better with this dietary change, based on their relatively stable body mass index, consistent with previous studies (Putaala and Hissa 1995). An alternative hypothesis may explain the decrease in body mass of farm-strain partridges (i.e. the shift to a smaller group and/or a different group composition). Indeed, the stress responses and behaviour of individuals depends on the group to which they belong, as reported previously for body condition (Creel et al. 2013; Homberger et al. 2015). Testing this hypothesis would have required performing enough replicates for each group, which was not feasible here due to space constraints in the game farm.

Haematocrit is often positively correlated with body condition as it reflects nutritional state and physiological stress, although extrapolating from an individual's state should be done with caution (Ots et al. 1998; Fair et al. 2007). However, in the current study, we found the opposite trend between fat storage and red blood cells. Indeed, at the beginning of the experiment, farm-strain partridges (i.e. those with the higher body condition) had lower haematocrit than wild birds. Although we detected significant sampling bias at the onset of the experiment, haematocrit increased over the course of the study for all four groups. This overall increase in haematocrit may be linked to the mating period, which begins in February for partridges, since haematocrit can change according to hormones (Fair et al. 2007). This hypothesis was corroborated by the haematocrits of males, which were higher than those of females at the end of the experiment, highlighting differences between sex, even though haematocrit increased for all birds (Fair et al. 2007).

We also found that farm-strain partridges were less active and preferred to flee by running rather than by flying during open-field tests. Physiological and morphological adaptations due to long-term captivity (e.g., higher body mass for farm-strain partridges at week 0) may have induced behavioural changes in our farm-strain partridges. For example, farm-strain partridges may have weaker endurance due to smaller breast muscles, lower glycogen content, and consequently lower release and/or production of glucocorticoids in pectoral muscles (Putala and Hissa 1995). Moreover, they may have lower excretion of glucocorticoids as a response to stress, which can affect anti-predator responses (Homberger et al., 2013, 2014, 2015). As animals selected over many generations for high reproductive rates, with food provided *ad libitum*, one may expect a decrease in energy-demanding behaviours for farm-strain birds, such as escape by flying (Beilharz et al. 1993; Schutz and Jensen 2001). In addition, being isolated from predators may result in lacking appropriate anti-predator responses (Pérez et al. 2010; Rantanen et al. 2010b). Upon release, animals have

to cope with risk-taking, foraging and novel environment discovery (Réale et al. 2007). Fleeing quickly in life-threatening situations (e.g., by flying rather than running), and displaying a high level of activity to find food, sexual partners and territories are expected to increase fitness (Sih et al. 2004; Smith and Blumstein 2008; Homberger et al. 2013). Based on our results, our farm-strain partridges could therefore be expected to suffer higher mortality rates than their wild counterparts after release (Rantanen et al. 2010a; Rymešová et al. 2012, 2013; Homberger et al. 2014). Interestingly, Homberger et al. (2021) found that bolder birds (i.e., those with higher activity and lower tonic immobility responses) survived longer after release despite negative associations postulated and repeatedly reported between proactivity, bold behaviour, survival and longevity (Dammhahn et al., 2018; Smith & Blumstein 2007). Under fluctuating environmental conditions or changing needs at different stages of the life cycle, the advantages and disadvantages of a particular behaviour type may change or even be reversed (Sih et al., 2004). Individuals can acquire their own behavioural strategies and adjust behaviour according to environmental context, making it difficult to predict their probability of survival based solely on captive experiments.

We also detected an interaction between strain and food type, since farm-strain birds fed conventional grains showed lower capacity to detect (i.e., lower FID) and escape a predator in the field (i.e., running instead of flying). Many studies have described sublethal adverse effects of pesticides on behaviour, caused by neurotoxicity (Mitra et al. 2011; Moreau et al. 2022, submitted). For example, organomercury fungicides act mainly through the central nervous system, resulting in disruption to risk-taking behaviour (Kobiela et al. 2015); abamectin insecticides may inhibit GABA receptors and cause environment perception disorder (de Faria et al. 2018); neonicotinoids impact acetylcholine esterase activity, leading to a decrease in foraging activity and ultimately to starvation and body weight loss (Eng et al. 2017, 2019). Although clopyralid is considered relatively non-toxic to birds (EFSA 2018),

some long-term studies have reported weight loss and damage to stomach, liver and kidney at 15 mg/kg per day for mammals, specifically rats and rabbits (Hayes et al. 1984; EFSA 2018). Our current results suggest that chronic ingestion of clopyralid residues may induce neurotoxicity and alter anti-predator responses of farm-strain partridges, whereas wild-strain partridges seemed to be unaffected by conventional grain ingestion.

5. CONCLUSION

Currently, most commercial game farms breed partridges from farm-strain birds and feed them commercial poultry food (Sokos et al. 2009; Homberger et al. 2014). Our results suggest that game farms produce birds that have lower fitness (i.e., less likely to survive and/or reproduce) when released into the wild compared with wild strains, and this is aggravated by food type, (i.e. when fed conventional grains containing pesticide residues). Our results therefore suggest that feeding captive-bred individuals from game farms with organic grains may, to some extent, compensate for this disadvantage, and may therefore contribute to improving post-release survival rates. This strategy may be preferred by partridge farmers who may be more inclined to change the food they provide their partridges rather than completely changing their breeding strategy.

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600 **Table 1 Effects of strain (wild vs. farm), sex and food type (organic vs. conventional) on the physiological condition of grey partridges**
601 **(haematocrit and body mass index) at the beginning and end of the food experiment (week 0 before food type and week 12), and**
602 **differences at 12 weeks based on the combined effect of food and strain.** Wild birds fed organic grains served as a reference in models. $\beta \pm$
603 SE values were extracted from minimal models including only significant factors. Farm, farm-strain; Conv., conventional. Significant effects are
604 in bold.

Response variables	Beginning of the experiment (week 0)					End of the experiment (week 12)				Difference between week 12 and week 0			
	Fixed factors	F	df	p	$\beta \pm$ SE	F	df	p	$\beta \pm$ SE	F	df	p	$\beta \pm$ SE
Haematocrit					Intercept = 44.42 \pm 0.66				Intercept = 46.08 \pm 0.91				Intercept = 2.30 \pm 0.84, $p < 0.01$
	Strain	6.99	1	<0.01	Farm = -2.06 \pm 0.78	6.34	1	<0.01	Farm = -1.63 \pm 1.15	0.01	1	0.98	
	Sex	0.25	1	0.62		6.21	1	<0.01	Males = 2.05 \pm 0.82	10.23	1	<0.01	Males = 2.52 \pm 0.76
	Food	1.54	1	0.22		0.91	1	0.35					

	Strain	8.10	1	<0.01	Farm Conv.	0.27	2	0.61		2.45	2	0.09	
	x Food				= -4.19 ± 1.47								
Body mass index					Intercept =				Intercept =				Intercept = -6.03 ± 6.46
					375.70 ± 5.81				376.26 ± 8.73				
	Strain	14.44	1	<0.001	Farm = 25.52 ± 6.80	0.24	1	0.63		26.30	1	<0.001	Farm = -31.61 ± 8.15
	Sex	20.83	1	<0.001	Male = -31.19 ± 6.83	19.44	1	<0.001	Male = -34.05 ± 7.88	0.53	1	0.53	
	Food	1.37	1	0.25		0.42	1	0.52					
	Strain	2.36	1	0.13		2.50	2	0.12		0.19	2	0.83	
	x Food												

Table 2 Effects of strain (wild vs. farm), sex and food type (organic vs. conventional) on partridge behaviour during cage and open-field tests. Linear models were conducted for FID and square root transformed activity, exploration, and duration of flight. Logistic regression was conducted for escape strategy. Wild birds fed organic grains served as a reference in models. $\beta \pm SE$ values were extracted from minimal models including only significant factors. Significant effects are in bold.

Tests	Response	Fixed factors	F	df	p	$\beta \pm \text{SE}$
Cage test	FID (n = 73)	Strain	1.09	1	0.30	Intercept = 57.73 ± 4.71
		Food	1.98	1	0.16	
		Sex	0.24	1	0.62	
		Strain x Food	8.97	2	<0.01	Farm-strain conventional =
	Escape strategy (n = 73)					Intercept = -0.65 ± 0.54
		Strain	0.71	1	0.39	
		Food	1.11		0.29	
		Sex	0.68	1	0.40	
		Strain \times Food	4.17	2	<0.05	Farm-strain conventional =
	Open-field test	Exploration (square root, n = 73)				
Strain			0.08	1	0.78	
Food			2.16	1	0.15	
Sex			0.89	1	0.34	
Strain \times Food		0.11	2	0.11		
Activity (square root, n = 73)		Strain	14.26	1	<0.001	Farm-strain = -3.35 ± 0.88
		Food	3.52	1	0.06	
	Sex	0.22	1	0.90		

		Strain x Food	0.30	2	0.58	
						Intercept = 3.04 ± 0.81
		Strain	8.19	1	<0.01	Farm-strain = - 1.80 ± 0.70
Escape strategy		Food	1.15		0.28	
(n = 73)		Sex	0.70	1	0.40	
		Strain × Food	1.84	2	0.17	
Duration of						Intercept = 3.17 ± 0.20
flight		Strain	12.85	1	<0.001	Farm-strain = -0.94 ± 0.26
(for birds that		Food	7.30	1	<0.01	Conventional = -0.67 ± 0.25
flew, n = 57)		Sex	2.07	1	0.16	
		Strain × Food	5.07	2	0.03	Farm-strain conventional =

611

612

613 **Table 3 Summary of escape strategy (flying versus running) during cage and open-field**
614 **tests with the percentage of partridges who tended to escape by flying rather than**
615 **running depending on strain (wild vs. farm) and food type (organic vs. conventional**
616 **grains).**

	Wild strain		Farm strain	
	Organic	Conventional	Organic	Conventional
Partridges flying during cage test	39%	50%	72%	37%
Partridges flying during open-field test	89%	94%	78%	53%

617

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620

621 **Figures captions**

622 **Fig. 1** Schematic representation of the experimental design, involving consecutives (A)
623 outside FID test (B) inside open-field behavioural tests on game farm or wild grey partridges
624 fed organic or conventional grains.

625

626 **Fig. 2** Temporal variation of (a) haematocrit and (b) body mass index over time, from the
627 beginning (week 0) to the end (week 12) of the food type experiment, with different strains (wild
628 vs. farm) and food types (organic vs. conventional grains). The horizontal line corresponds to the
629 median, the top and bottom of boxes are the first and third quartiles, and whiskers show the lower
630 and higher values included in the 95% confidence interval. Black points correspond to the mean,
631 open dots correspond to outliers. Stars correspond to significant effects of post-hoc tests and
632 Bonferroni correction for multiple comparisons when the interaction between food type and strain
633 was significant (i.e., $p < 0.05$).

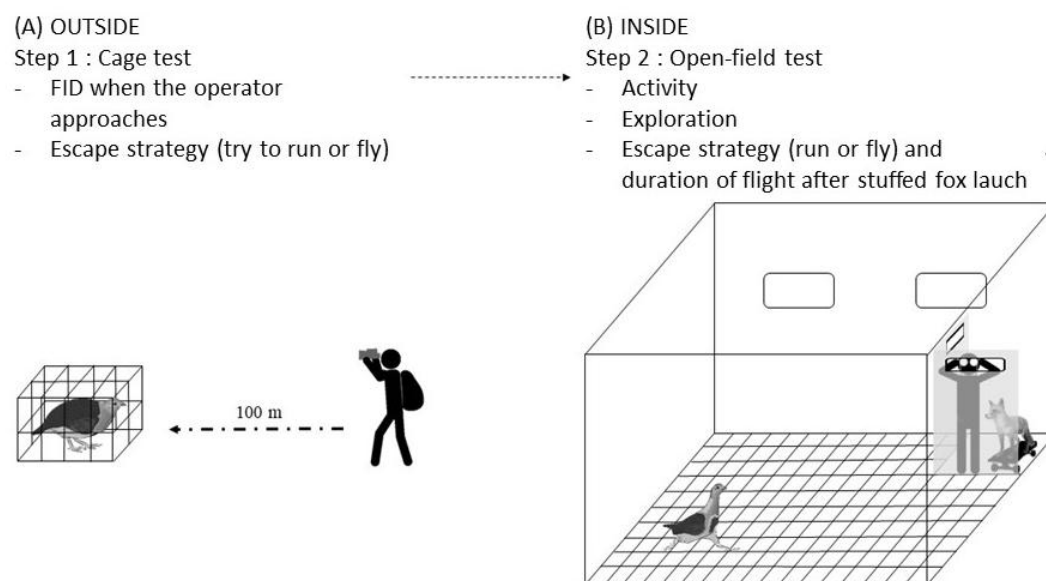
634

635 **Fig. 3** Variation in (a) flight initiation distance, (b) activity and (c) duration of flight according to
636 strain (wild vs. farm) and food type (organic vs. conventional grains). The horizontal line
637 corresponds to the median, the top and bottom of boxes are the first and third quartiles, and
638 whiskers show the lower and higher values included in the 95% confidence interval. Black points
639 correspond to the mean, open dots correspond to outliers.

640

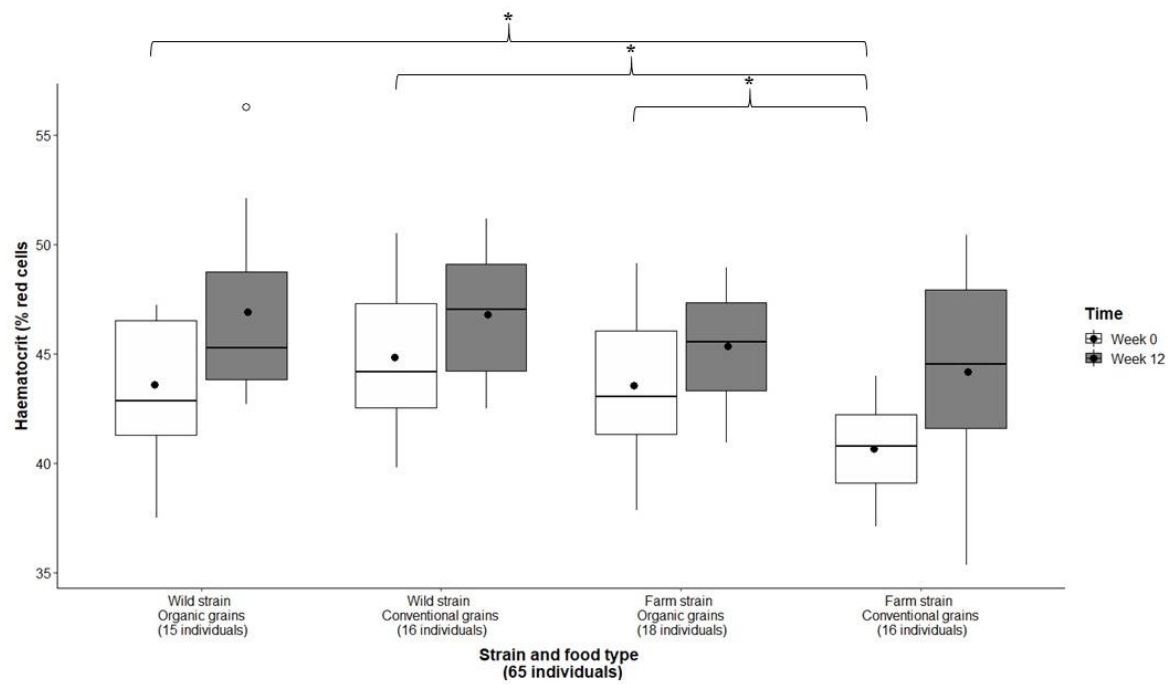
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642 Fig 1



643

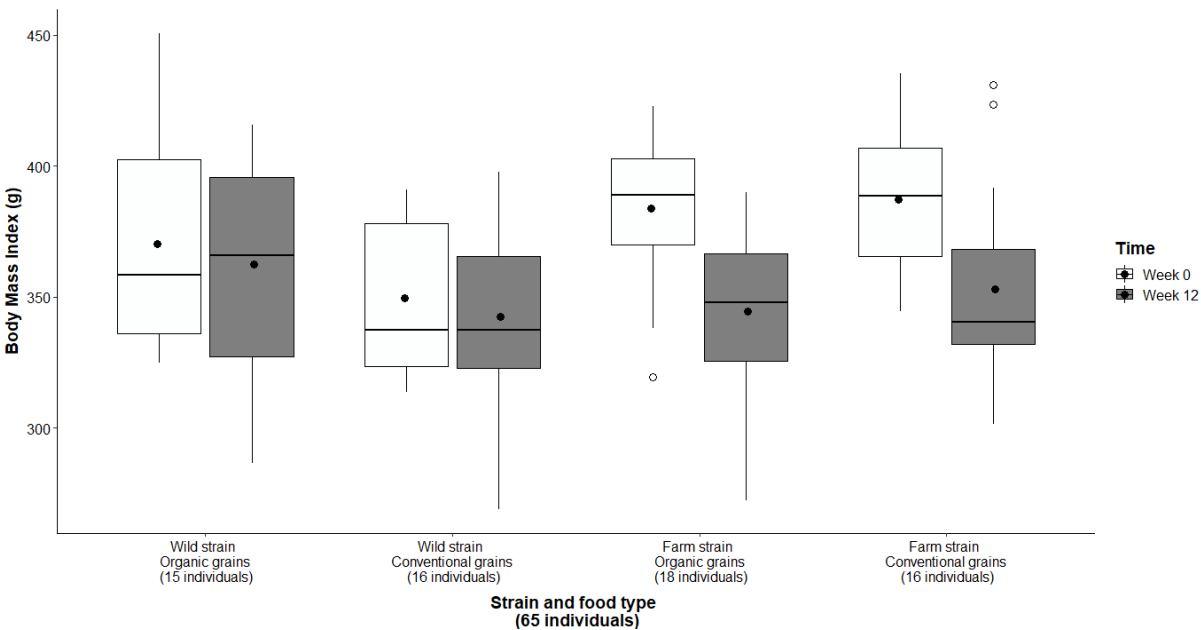
644 Fig 2.a



645

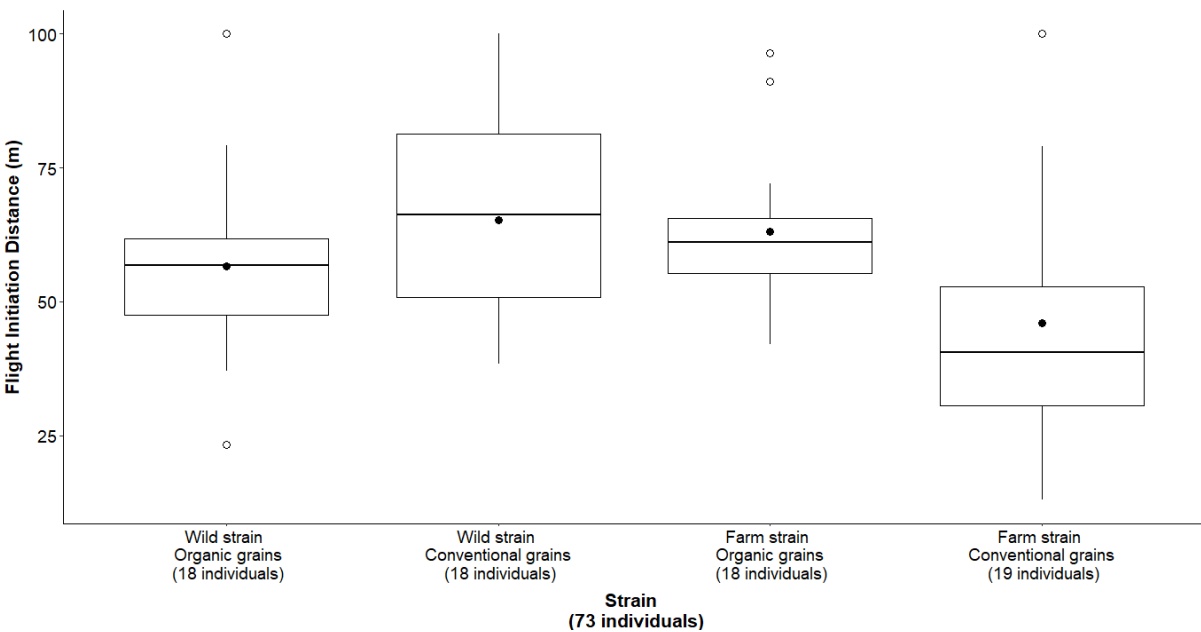
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647 Fig 2.b



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649 Fig 3.a



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Fig 3.b

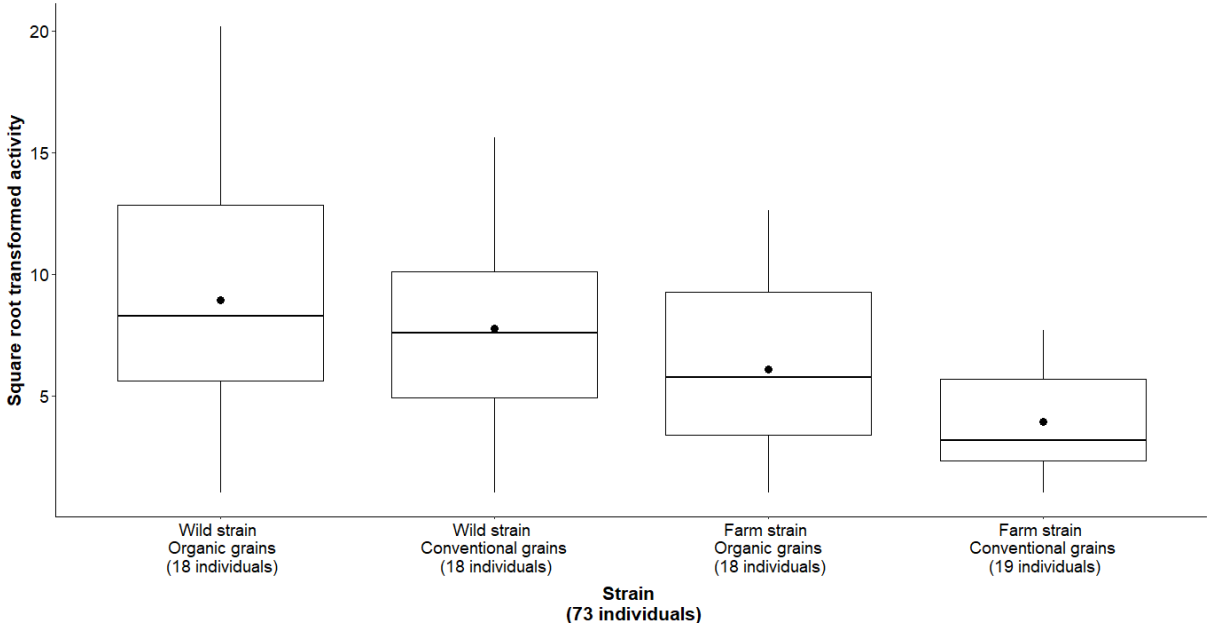


Fig 3.c

