



## Laying hens selected for low mortality: Behaviour in tests of fearfulness, anxiety and cognition

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### ABSTRACT

Feather pecking and cannibalism in chickens can lead to injury or to death of the victims, and is thus a serious welfare and economic issue in modern poultry farming. A sib selection scheme has been initiated to genetically select a low mortality line (LML), which shows decreased mortality due to cannibalism compared to a control line (CL). To determine whether undesired behavioural effects such as impaired cognition, increased fearfulness, or increased anxiety might have inadvertently been co-selected, we tested hens from the fourth generation of selection ( $n=9$  per selection line) in several behavioural tasks. When tested in a 122 cm × 122 cm open field at age 7 days, the lines showed no differences in locomotion or vocalization. In a T-maze test in which the chickens could navigate to find conspecifics, testing sociality and fearfulness, 12 to 16-day-old CL chickens showed a lack of exploratory behaviour; they did not leave the start box. In contrast, most LML chickens negotiated the maze, and approximately half of them found and stayed close to their conspecifics. This difference points toward higher levels of fearfulness in the CL than LML. In a voluntary approach test assessing fearfulness for humans, conducted when the chicks were 26 days old, the LML approached a familiar human faster, thus displaying lower levels of fearfulness. The same birds were tested in a holeboard test at an age of 25–65 days, the first time this test of spatial memory has been used in an avian species. Our results demonstrated high levels of working memory performance and low levels of reference memory performance in both lines, with no differences between the lines. Overall, the present results indicate that unwanted behavioural effects were not co-selected with selection on low mortality, and support the feasibility of the use of the LML in farming practice.

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### 1. Introduction

Feather pecking and cannibalism in farm-kept chickens are damaging behaviours both in terms of animal welfare and economic loss, and a major challenge in modern poul-

try farming. Feather pecking is bird-to-bird pecking which can either result in damage to or removal of feathers from the victim (severe feather pecking) or leave the feathers in place (gentle feather pecking) (Savory, 1995). If feathers are removed, bald patches of skin may also be pecked, which can in turn result in cannibalism. Severe feather pecking and cannibalism can lead to pain, damage to or, in extreme cases, death of the recipient chicken. Death by cannibalism in beak trimmed laying hens was reported at 7%, and in untrimmed laying hens of nearly 18%, in a 3-year study of floor-housed birds (Flock et al., 2005).

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Consumer demand and recent changes in legislation have caused a shift from caged housing in very small groups, specifically in battery cages for laying hens, to non-cage housing such as floor or free range systems. Although non-cage systems clearly provide more opportunity for hens to display natural behaviours and to move more freely, there is also more danger of feather pecking damaging the flock (Savory, 2004). A recent study observed severe feather pecking in 85% of 61 free-range and organic flocks visited at 40 weeks of age (Lambton et al., 2010). Physical mutilations to prevent damage caused by feather pecking, such as beak trimming, do reduce or prevent feather damage quite effectively (Henderson et al., 2009), but have raised concern in terms of welfare of the animals undergoing such alterations (Kuenzel, 2007). Beak trimming is thus currently being increasingly regulated or phased out in many European countries, with full bans in place in Norway, Sweden, and Finland, and heavy regulation in Austria, Belgium, Denmark, Germany, the Netherlands, Switzerland and the UK (Van Horne and Achterbosch, 2008). Non-surgical means, such as alterations in housing or genetic selection, of prevention of feather pecking and the resulting negative impacts on welfare and economic issues are necessary.

Genetic selection in farm animals has been proposed as a potential means for reducing unwanted behaviours while maintaining a high performance level within a population (D'Eath et al., 2010; Rodenburg et al., 2010; Beaumont et al., 2010; Hester, 2005). Recently, a selection experiment was initiated using a sib selection scheme, selecting for low mortality in group-housed laying hens (Ellen et al., 2007). This resulted in a low mortality line (LML) and an unselected control line (CL). Given that feather pecking and cannibalism are major causes of mortality in laying hens, the LML was expected to show lower levels of feather pecking and cannibalism. Previous studies confirmed this hypothesis (Rodenburg et al., 2009a). Furthermore, the LML also differs from the CL on a number of behavioural and physiological measures, including altered whole-blood serotonin levels, plasma corticosterone levels and open field behaviour (Rodenburg et al., 2009a,b; Bolhuis et al., 2009).

Selection for desirable characteristics in a breed of animal may produce unwanted side effects if undesired characteristics are inadvertently co-selected (Rauw et al., 1998). Cognitive ability has recently been proposed to play an important role in farm animal welfare (Broom, 2010). The importance of maintaining cognitive function is due to a general wish to protect the integrity of animals, but also practical considerations. Farm animals need adequate cognitive skills to adequately function, i.e. recognize group mates and their place in the group hierarchy in group housing, to find and operate feeding systems that require operant responses, and to cope with changing environments in farming situations. A laying hen will be moved several times in early life, from a hatchery to a brooder to a laying station, and the hen needs adequate cognitive flexibility to cope with these changes. Fear, anxiety and reactivity to humans can also strongly affect an animal's ability to thrive in a farm setting. Alterations in fear, anxiety or cognition, characteristics which could potentially be

co-selected in any selection line, could be detrimental to laying hen welfare.

In order to further elucidate the effects of selection on behavioural measures and to test replicability of previous results, hens from the LML and CL were tested for anxiety in an open field test (Buitenhuis et al., 2004; Jones et al., 2002; Kembro et al., 2008; Satterlee and Marin, 2006; Uitdehaag et al., 2008a), for fear of humans in a voluntary approach test (Waiblinger et al., 2006) and for sociality toward conspecifics in a T-maze task (Jones et al., 1999; Marin et al., 2001). To expand the repertoire of testing available for screening chicken cognition, we tested working and reference memory in a complex spatial holeboard discrimination task (van der Staay, 1999) newly adapted for use in chickens.

## 2. Materials and methods

The study was reviewed and approved by the local ethics committee of Utrecht University, The Netherlands, and was conducted in accordance with the recommendations of the EU directive 86/609/EEC. All effort was taken to minimize the number of animals used and their suffering.

### 2.1. Animals

Chickens of the fourth generation selected for low mortality according to a sib selection approach were used (Ellen et al., 2007; Rodenburg et al., 2009b). The low mortality line was selected on low mortality in the group-housed sisters of the selection candidate and on individual performance criteria. The control line was selected using individual performance criteria only (for details see Rodenburg et al., 2010).

Thirty fertilized eggs from the low mortality line (LML) and 30 fertilized eggs from the control line (CL) were obtained from Institut de Sélection Animale, the layer breeding division of Hendrix Genetics (Boxmeer, The Netherlands). The eggs were incubated and hatched at the Faculty of Veterinary Medicine of Utrecht University; 40 of these 60 eggs hatched. After hatching, the day-old chicks were moved to the Utrecht University farm animal facility.

After determining sex, only female chicks ( $n = 10$  CL,  $n = 9$  LML) were kept for the experiments, the male chicks were humanely euthanized. One of the chicks of the CL died soon after hatching for reasons unknown. Consequently, 9 CL and 9 LML chickens were tested.

Chicks were vaccinated against New Castles Disease and Avian Influenza at 22 days.

### 2.2. Animal housing

The chicks were housed, separated by line, in two adjacent, identical pens measuring 112 cm × 112 cm × 70 cm (length × width × height). The floor of the pen was covered with wood shavings. On the right side of the pen the chicks had access to a perch (62 cm long, 10 cm above the floor). Food (standard layer chick feed from 3 till 12 weeks; supplier: De Heus Voeders, Ede, The Netherlands) and drinking water were available *ad libitum*. For the first 4 weeks, a round food trough (diameter 30 cm) was used. After 4

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