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Preparing for life

Effects of environmental choice and change during rearing on adaptability in laying hens

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Cover: “Don’t forget about the me before today”
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Abstract

The importance of the early environment for later animal behaviour, health and welfare is well known. Hens (*Gallus gallus domesticus*) kept for egg production are exposed to various challenges and opportunities during their life time, such as relocation from a rearing to a laying facility or access to free range. Promoting greater adaptability in young laying hens could lead to improved welfare, especially considering the increasing complexity of commercial loose housing and aviary systems. However, early environmental inputs that could improve laying hen adaptability have not been clearly identified. This thesis investigated the impact of two environmental inputs (“choice” and “change”) during rearing, based upon well-established theories on the effects of controllability and predictability on coping ability. Experiments using two relevant environmental resources, litter and perches, showed that young laying hens with regular changes of litter and perch type were less fearful when placed in a novel environment. Young laying hens given the option to choose between different litter and perch types during rearing, showed increased exploration in a novel environment, were better able to locate a hidden feed reward and showed improved stress coping and immunocompetence. Both short- and long-term effects were seen among hens reared with different levels of environmental choice and change, depending on the stage of rearing in which hens were exposed to the environments and when the evaluations were conducted. In particular, providing greater possibilities for environmental choice could be a biologically relevant approach for a rearing environment that goes beyond simply providing basic resources and can enhance laying hen adaptability and welfare.

Keywords: early life development, postnatal, chicks, litter, perches, behaviour, coping, exploration, immune defence, animal welfare

Redo för livet - Kan vi genom att öka valbarhet och förändring av den tidiga miljön påverka värphönors anpassningsbarhet?

Sammanfattning

Vetskapen om en signifikant koppling mellan den tidiga miljön och senare beteende, hälsa och välmående finns för många djurarter, även för vår domesticerade värphöna (*Gallus gallus domesticus*). En höna i en produktionsmiljö behöver både kunna hantera utmaningar och ta till vara på möjligheter, exempelvis i flytten mellan uppväxt- och värpstall eller när de får tillgång till utevistelse. De tidiga miljöinslag som skulle kunna bidra till en mer anpassningsbar höna är inte väl undersökta, men skulle både kunna öka djurvälfärden och underlätta för lantbrukarna. Vi undersökte effekten av två typer av miljöinslag under uppväxten, ”val” och ”förändring”, baserat på redan välgrundade teorier kring stresshantering med hjälp av två relevanta miljöresurser, strö och sittpinnar. Resultaten visade att unga höns som haft kontinuerligt utbyte av det strö och sittpinne som fanns i sin uppväxtmiljö (högre förändring) tycktes visa mindre initial rädsla i en ny miljö. Unga höns med olika typer av strö och sittpinnar i sin uppväxtmiljö (ökade möjligheter till val) hade högre utforskningsbeteende i en ny miljö, högre användande av nya resurser men även en förbättrad fysiologisk förmåga att hantera möjliga stressorer inklusive främmande patogener. Vi såg påverkan av den tidiga miljön både kort och långsiktigt, men effekterna var beroende av den del av uppväxten där vi gav vår behandling och när vi undersökte effekterna. Att öka möjligheterna till val i sin uppväxtmiljö, kring relevanta resurser, kan vara ett biologiskt relevant sätt att erbjuda en rikare miljö som kan främja hönors anpassningsbarhet och välfärd.

Nyckelord: uppväxtmiljö, tidig utvecklingsfas, kycklingar, strömaterial, sittpinnar, beteende, stress, utforskande, immunförsvar, djurvälfärd

Dedication

To Ronja

“Some people talk to animals. Not many listen though. That’s the problem.”

A.A Milne, Winnie-the-Pooh

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Skånberg, L., Bramgaard Kjærsgaard Nielsen, C & Keeling, L.J. (2021). Litter and perch type matter already from the start: exploring preferences and perch balance in laying hen chicks. *Poultry Science* 100 (2), 431-440.

II. Skånberg, L., Newberry, R.C., Estevez, I & Keeling, L.J. Prepared for the unexpected: Environmental change or choice during early rearing improves behavioural adaptability in laying hen chicks. (Submitted).

III. Nazar, F.N*., Skånberg, L*., McCrea, K & Keeling, L.J. (2022). Increasing environmental complexity by providing different types of litter and perches during early rearing boosts coping abilities in domestic fowl chicks. *Animals* 12 (15), 1969.

IV. Skånberg, L., Holt, R.V., McCrea, K.M., Newberry, R.C., Estevez, I & Keeling, L.J. Making the most of life – Effect of early environmental choice on the ability of laying hens to take opportunities. (Manuscript).

*Shared first authorship

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The contribution of Lena Skånberg to the papers included in this thesis was as follows:

I. Contributed to designing the study, performed the experiments and collected the data together with the co-authors. Conducted the analysis, interpreted the data and produced the figures and tables with input from the co-authors. Wrote the manuscript with input from the co-authors.

II. Contributed to designing the study, performed the experiments and collected the data. Conducted the analysis, interpreted the data and produced the figures and tables with input from the co-authors. Wrote the manuscript with input from the co-authors.

III. Contributed to designing the study. Performed the experiments and collected the data together with the co-authors. Conducted the analysis, interpreted the data and produced the figures and tables together with the co-authors. Wrote the manuscript together with the other main author, with input from the other-co-authors.

IV. Contributed to designing the study, performed the experiments and collected the data together with the co-authors. Conducted the analysis, interpreted the data and produced the figures and tables with input from the co-authors. Wrote the manuscript with input from the co-authors.

Abbreviations

EU	European Union
HPA	hypothalamus-pituitary-adrenal
e.g.	*exempli gratia*/for example
H/L	heterophil/lymphocyte
TI	tonic immobility
CRF	corticotrophine release factor
ACTH	adrenocorticotropic hormone
IFN-γ	interferon gamma
SRBC	sheep red blood cells
nAb ag	natural antibodies against
PHA-P	phytohaemagglutinin-P
i.e.	*id est*/in other words
etc.	*et cetera*

1. Introduction

The early rearing environment is important for the subsequent behaviour, health and welfare of domestic animals. Laying hens (*Gallus gallus domesticus*) are exposed to some key challenges and opportunities during their life time, such as relocation from a rearing facility to a laying hen house or access to an outdoor environment as adults. Greater adaptability of commercial laying hens could be essential for their welfare, especially with the wider use of loose housing systems. It may be possible to adjust the rearing environment to promote laying hen adaptability and better prepare them for the opportunities and challenges to come. Improving laying hen adaptability can be a way to improve bird welfare, but can also have a positive impact on the industry. Today, the most common types among domestic fowl are chickens reared for meat and laying hens reared and kept for egg production. However, laying hens are kept longer and therefore require a higher level of adaptability (Widowski & Torrey 2017). There are around 7.5 billion laying hens in the world (Schuck-Paim et al. 2021), with 6.4 million housed in Sweden in June 2021 (Swedish Board of Agriculture, 2022).

1.1 Selection for reduced adaptability?

> *Definition of adaptability*
> The term "adaptability" refers to "the degree to which an organism, population or species can remain or become adapted to a wider range of environments by physiological or genetic means" (Barker 2009). The term has also been used to describe the capacity of laying hens to adapt to the challenges and opportunities associated with changes in housing or management routines (Widowski & Torrey 2017). In this thesis, adaptability is defined as *the degree to which an individual can remain or become adapted to environmental change by behavioural or physiological means.*

The red jungle fowl (*Gallus gallus*), the wild ancestor of the domestic fowl (*Gallus gallus domesticus*), was domesticated around 8000 years ago (West & Zhou 1988). Since the beginning of the domestication process, many morphological and behavioural changes have taken place in the domestic fowl, *e.g*. they can vary widely in colour and size. However, domestication and artificial selection for higher productivity among farm animals seems to have resulted in reduced coping ability (Rauw et al. 2017), which could impair adaptability. One definition of coping is "behavioural and physiological efforts to master the impact of aversive stimuli or stressors" (Koolhaas et al. 1999). For example, domestic fowl initially show a lower behavioural and physiological response to human restraint, indicating less fear and stress, but the red jungle fowl show a faster return to basal levels after this procedure (Ericsson et al. 2014). This indicates that jungle fowl are better able to cope with the handling situation (Koolhaas et al. 1999). Similarly, domestic fowl seem to be more affected by unpredictable events, such as varying light schedules in their home environment, responding *e.g.* by showing more passive behaviour in a food test (Lindqvist & Jensen 2009), indicating suppressed adaptability. Compared with red jungle fowl, domestic fowl also display impaired spatial abilities and are less willing to explore and feed from a hidden food source (Lindqvist et al. 2002; Lindqvist & Jensen 2009), further confirming that they are less able to adapt to environmental change. Foraging motivation may be crucial for adaptability when young or adult birds are allowed to explore novel feed sources in new surroundings (Martin & Fitzgerald 2005; Miller et al. 2015).

It is possible that during the domestication process, there may have been accidental selection for traits leading to reduced adaptability in laying hens. However, animal phenotype is not dependent solely on genotype, since the environment also plays a large role for gene expression. In fact, it has been shown that life-time experiences of individuals in previous generations can have a long-lasting effect on their offspring, through epigenetic changes (De Haas et al. 2021). For example, loose housing of parental animals has been found to lead to improved coping responses in their offspring, compared with cage housing (Peixoto et al. 2021). Thus, multiple generations of cage housing for parental animals could have a long-term negative effect on adaptability. Important questions for animal welfare are whether modern laying hens need higher adaptability and whether this apparent predisposition to suppressed adaptability can be reversed.

1.2 Commercial practice

In Sweden, parental stocks lay eggs that are incubated and hatched at a hatching company. These day-old birds are then sorted, vaccinated and transported to rearing farms, where they stay until 15-16 weeks of age, when they are moved to laying farms (Wallström, 2022; Svenska Ägg, 2015). The first eggs are laid from 18 weeks of age (Wallström, 2022). To facilitate an easier transition between the rearing farm and laying hen farm, Swedish animal welfare regulations (SJVFS 2022:5 [L 111]) require laying hens to be reared in a system that is similar to the laying hen environment. In the general advice provided with the regulations, it is specified that birds should be reared in cages if later housed in cages, or reared in loose housing systems if later housed in loose housing systems. This follows recommendations from reviews conducted on welfare risks (Janczak & Riber 2015). Loose housing systems, such as single- and multi-tier systems for laying hens, are increasing in popularity around the world (Schuck-Paim et al. 2021). These systems are believed to provide birds with better possibilities to perform species-specific behaviour, such as perching and foraging, and also allow birds to move over a larger space compared with the restrictions associated with the formerly dominant cage housing systems. Of all eggs produced in Sweden between 2018 and 2019, 92% came from hens kept in loose housing systems, with or without outdoor access, of which the majority were multi-tier systems (Global Laying Hen Statistics, 2022). Multi-tier systems allow perching on higher levels, which is believed to be preferred by laying hens (Brendler & Schrader 2016), but also enable farmers to keep more birds on the same area. One could suggest that multi-tier systems demand even greater adaptability in the birds, because of the more complex space, larger social groups and degree of novelty after moving from a rearing facility. Even if rearing and layer environments are matched as fully as possible, the move still involves a substantial environmental change that demands suitable levels of adaptability in the hens. Environmental change, such as relocation and subsequent group mixing, can lead to a higher risk of agonistic interactions in laying hens, leading to injuries in the head and neck region (Cloutier & Newberry 2002; Carvalho et al. 2018). Larger size of a novel environment, such a larger loose housing area in the laying house, seems to be linked to higher fearfulness and also impaired feather condition (Alm et al. 2014). According to reports by the Swedish Egg Association, farmers would prefer birds with higher adaptability, since they have found that their birds are generally sensitive to changes and sudden environmental stimuli (Wallström, 2022).

1.2.1 Welfare concerns

Despite the move away from cage housing to loose housing, there are still many welfare concerns with laying hens today and some problems seem to be even greater in loose housing systems, such as mortality (Weeks et al. 2016; Schuck-Paim et al. 2021) and keel bone fractures (Petrik et al. 2014).

Laying hens generally finish in production at between 70 and 90 weeks of age (Svenska ägg, 2015), since after this point it is not profitable to keep them in production (Wallström, 2022). However, meta-studies by *e.g.* Weeks et al. (2016) and Schuck-Paim et al. (2021) have shown that cumulative mortality can be high at much younger ages, most likely because of disease, injuries or other health issues (Rodenburg et al. 2008). The study by Weeks et al. (2016) found significant differences in cumulative mortality levels among different housing systems based on data from 3851 flocks aged between 60 and 80 weeks obtained from several countries (United Kingdom, Netherlands, Sweden). The lowest mortality level was found in cage systems, while the highest was found among single-tier systems, with or without access to the outdoors (Weeks et al., 2016). The meta-study by Schuck-Paim et al. (2021) showed that mortality in loose housing systems has begun to decrease in recent years, possibly due to greater farmer experience of these systems. However, loose housing systems still have higher mortality risks than conventional cages (Schuck-Paim et al., 2021), indicating that birds may not be fully prepared for these more complex housing systems.

An increasing welfare concern in egg production in many European Union (EU) countries is keel bone fractures, the underlying factors for which are not yet known (Riber et al. 2018). Fractures can vary in degree, but cause pain and affect bird movement (Nasr et al. 2013; Riber et al. 2018). Loose housing systems are associated with almost double the risk of keel bone fractures compared with cage systems, as seen *e.g.* in Canada (48% and 25% incidence in loose and cage systems, respectively) (Petrik et al. 2014). A study investigating 47 loose housing laying hen facilities in Belgium and the Netherlands found 82.5% incidence of keel bone fractures at around 60 weeks of age (Heerkens et al. 2016). Multi-tier systems seem to have a higher risk, based on a study in Denmark showing that the incidence of keel bone fractures at 62 weeks of age was 11.6% in multi-tier systems and 4.9% in single-tier systems (Riber & Hinrichsen 2016). This may be because multi-tier systems lead to higher risks of collisions or falls from a greater height (Toscano et al. 2013). It suggests that birds are not sufficiently prepared for these more complex systems (Harlander-Matauschek et al. 2015).

Another major welfare concern in modern egg production is severe feather pecking, involving pulling feathers and pain in the receiver, which has been observed in all types of housing system (Cronin & Glatz 2020). Apart from the pain aspect, feather pecking can also lead to impaired plumage condition and mortality (Rodenburg et al. 2013). In an attempt to reduce severe feather pecking, beak trimming of young chicks is standard procedure in many countries (Riber & Hinrichsen 2017). However, the procedure is associated with both acute and chronic pain and can itself be seen as a welfare concern (Hughes & Gentle 1995). In some countries, such as Sweden, beak trimming is banned (SFS 2018:1192), while many other EU countries are discussing a ban (Riber & Hinrichsen 2017). Severe feather pecking is believed to be a multi-factor problem (Cronin & Glatz 2020) and links have been found to fearfulness, activity and coping style (van der Eijk et al. 2018), foraging behaviour (Dixon et al. 2008) and prenatal or early postnatal experiences (De Haas et al. 2021). This suggests a possible connection to the rearing period.

1.3 Developmental plasticity

Definition of developmental plasticity
Developmental plasticity has been defined as "the extent to which the current phenotype of an agent varies as a function of external experiences, stimuli or environmental conditions that occurred in the past (*e.g*. learning, ontogenetic plasticity, life-cycle staging)", where ontogenetic plasticity includes the effects of rearing condition on adult behaviour (Stamps 2016).

Behavioural patterns can be programmed early in life and welfare scores in the rearing flock are often correlated with those in the adult flock (Janczak & Riber 2015). For example, severe feather pecking occurrence in a young flock is highly correlated with feather pecking occurrence in the adult flock (Bestman et al. 2009; Gilani et al. 2013). Birds reared with a mother hen, which could be seen as the natural baseline, show reduced mortality and less severe feather pecking as adults compared with birds reared with only a heat lamp (Riber et al., 2007). In a semi-wild environment, red jungle fowl chicks remain dependent on their mothers for warmth, foraging and anti-predator behaviour until eight weeks after hatch (Collias & Jennrich 1994). Mother hens are not included in the commercial production environment due to associated contamination risks. However, their absence could be an argument for concentrating more strongly on other environmental inputs provided in the early housing facility. The early environment could provide an important opportunity for influencing adaptability, since birds already from inside the egg can be influenced by outer stimuli such as sounds and lights resulting in long-term alterations in chick brain morphology, stress response mechanisms and immune functions (Dixon et al. 2016). The hypothalamus-pituitary-adrenal (HPA) axis, which is highly involved in coping responses, is already fully functional at the first day after hatch (Ericsson & Jensen 2016). It is believed to be especially sensitive in young individuals with a developing brain and can thus influence adult coping abilities (Gunnar & Quevedo 2007; Ericsson & Jensen 2016). At hatch, it is crucial for a young chick to interact with, and learn from its surroundings. A variety of physiological systems are key during development and the immune system is not an exception. The developing immune system of a chick may be particularly sensitive to environmental inputs, as birds lack the "pathogen boost" that mammalian species acquire during birth (Ding et al.

2017). For example, early experiences such as cold stress (18-20 ºC for six hours on day 2 after hatch) can lead to long-term suppressive effects on laying hens' stress coping ability as well as immune capacity (Campderrich et al. 2019). This example further illustrates a clear link between stress and immune responses, which has been referred to as the immune-neuroendocrine system (Nazar and Estevez, 2022).

1.3.1 Adaptive developmental plasticity theories

Developmental plasticity is believed to be adaptive, an adaptation to improve fitness by enabling a better fit in the adult environment (Nettle & Bateson 2015). There are different theories about the adaptive function (Nettle & Bateson 2015). Informational adaptive developmental plasticity theory and predictive adaptive response theory both suggest that matching the early and adult environments is crucial for fitness and health of the adult (Gluckman et al. 2005; Bateson et al. 2014). Somatic state-based adaptive developmental plasticity theory suggests that certain inputs in the early environment could result in generally improved fitness and health in individuals (Nettle & Bateson 2015). One example related to the informational theory in laying hens is that a non-matched early and later environment (cage *vs* aviary) can lead to welfare risks (see review by (Janczak & Riber 2015). For instance, higher mortality rates are seen among aviary-reared birds when moved to a cage at the point of lay (Tahamtani et al. 2014). However, rearing in loose-house systems can reduce keel bone fractures in later cage-housed laying hens (Casey-Trott et al. 2017), supporting somatic state-based theory by indicating that an environment allowing a more functional development could be just as important. Nettle and Bateson (2015) suggested that informational adaptive developmental plasticity could have less value in more long-living species, due to larger risks of environmental fluctuation.

1.4 Behavioural needs

There is general consensus that animals from a vast range of taxa are sentient beings, and thus it is important for their welfare to have an understanding of what animals want and need (Dawkins 2006). Applying a more holistic view and paying attention not only to physical health indicators, but also to animal behaviour, can provide greater possibilities to improve animal welfare (Dawkins 1999). It has been suggested that all species-specific behaviour should be considered a behavioural need and that it can be context-dependent (Jensen & Toates 1993). One review of laying hen welfare defined behavioural needs as "those (instinctive) behaviours that are performed even in the absence of an optimum environment or resource" and, based on this definition, concluded that foraging and dust bathing are behavioural needs, while perching is a behavioural priority for laying hens (Weeks & Nicol 2006).

Studies of time budgets in semi-wild red jungle fowl have shown that these birds spent 60% of their daylight hours foraging, expressed as ground scratching and pecking (Dawkins 1989). Foraging behaviour starts to develop already at the time of hatch and commonly increases during the first few weeks of life (Vestergaard & Baranyiová 1996). It has been suggested that severe feather pecking, although triggered by impaired coping ability, is in fact a redirected foraging behaviour connected to lack of sufficient foraging material (Blokhuis & Haar 1992; Gilani et al. 2013; Rodenburg et al. 2013). Dust bathing is a less frequent behaviour, but is highly important for feather maintenance, which is crucial for bird welfare (van Liere & Bokma 1987). Dust bathing behaviour is performed by chicks already during the first days after hatch (Riedstra & Groothuis 2002).

Laying hen chicks use perches already from the second week after hatch (Heikkilä et al. 2006; Kozak et al. 2016) and has been seen to be used by all hens in a flock during night-time (Olsson & Keeling 2000). Perch access is thought to be important for thermoregulation (Pickel et al. 2011), may be of importance for anti-predator behaviour in hens (Newberry et al. 2001), reduced fear and aggression in a laying hen flock (Donaldson & O'Connell 2012).

1.4.1 Litter and perches during rearing

Litter and perches have been shown to be linked to the welfare issues raised in previous sections, especially presence of litter and perches during the early rearing phase. For example, access to litter during the first weeks after hatch, compared with no access, leads to decreased mortality in adults (Aerni et al. 2005), reduced severe feather pecking and improved feather condition (Johnsen et al. 1998). Access to perches during the first weeks after hatch can improve the ability of the birds to move in a three-dimensional space (Gunnarsson et al. 2000) and decreases the risk of cannibalism, another welfare issue, possibly by a reduced number of floor eggs, which places hens in an exposed and vulnerable position (Gunnarsson et al. 1999). According to EU Council Directive 1999/74/EC, all hens must have a nest, perching space, litter to satisfy their ethological needs, and unrestricted access to a feed trough. However, laying hen chicks or pullets are not mentioned specifically, leaving the Directive relatively open to interpretation in terms of the rearing environment. Swedish regulations (SJVFS 2022:5 [L111]) specify that laying hen chicks should have perch and litter access at all times and that the litter should satisfy behavioural needs for foraging and dust-bathing. However, the actual litter type is not specified and research is lacking regarding preferences in the home environment and suitable litter types for these behaviours in laying hen chicks. It is possible that not all litter types will satisfy these behavioural needs in chicks, as seen in short-term preferences (Sanotra et al. 1995; Vestergaard & Baranyiová 1996; Shields et al. 2004). Furthermore, not all perch designs may be suitable for young chicks (EFSA 2015).

1.5 Measuring adaptability

Adaptability derives from a series of adaptation mechanisms occurring at the molecular, cellular, systemic and behavioural levels, integrating genetics and environmental inputs (Wolf & Linden 2012). As mentioned, in this thesis adaptability was defined as "the degree to which an individual can remain or become adapted to environmental change by behavioural or physiological means", adjusted after Barker (2000). For an individual, greater adaptability would then require a more holistic view, especially since it demands greater ability to cope with the challenges and exploit the opportunities that can arise with environmental change. Behaviourally, improved adaptability could be expected to lead to more movement or exploration of a novel environment and higher usage of novel resources. Physiologically, this would be apparent as improved coping ability including an immune system better at coping with a potential pathogen.

1.5.1 Behavioural measurements

Hens' ability to move in novel environments, locate novel resources and learn new routines can be evaluated using several different behavioural tests. Movement in a novel environment may reflect a combination of fearfulness and exploration (Forkman et al. 2007; Favati et al. 2016). Movement in novel environments has been investigated previously using levels of activity in an novel arena (Zidar et al. 2017; Hedlund et al. 2019), or in an open field test (Jones & Waddington 1992; Nordquist et al. 2011; de Haas et al. 2017b; Campbell et al. 2019a) or usage of an outdoor arena (Grigor et al. 1995; Campbell et al. 2016). Response to novelty has also been investigated in terms of distance to a novel object (de Haas et al. 2013; Alm et al. 2014). Some of the cited studies interpreted a higher degree of movement or approach as reduced fearfulness, while others interpreted it to also imply a higher exploration. Individual's fearfulness is also commonly investigated in a standardised tonic immobility (TI) test (Forkman et al. 2007). Adapting to a novel environment with novel routines also requires additional abilities such as spatial awareness (Janczak & Riber 2015) and learning (Wechsler & Lea 2007). A detour test can be used to determine spatial abilities (Regolin et al. 1995a), while behavioural tests investigating learning abilities need to have elements of repetition.

1.5.2 Physiological measurements

> *HPA axis activation*
>
> Internal or external threats can lead to release of corticotrophine release factor (CRF) in the hypothalamus, which together with arginine vasotocin leads to release of adrenocorticotropic hormone (ACTH) from the pituitary gland. This stimulates synthesis and release of glucocorticoids (corticosterone) from the adrenal cortex into the blood stream. Adrenaline can also stimulate ACTH release, leading to HPA axis activation.

Improved physiological adaptability would require an individual to cope with potential stressors arising from a novel environment or novelty including potential pathogens (such as viruses and bacteria).

Stress response

As in other vertebrates, experience of a stressor activates the HPA axis (see box) in birds (De Kloet et al. 2005). Responses to physiological stress can be categorised into "immediate", with direct effects on behaviour, catecholamines and HPA-axis response, and "slow", leading to behavioural adaptation, recovery and homeostasis (De Kloet & Derijk 2004). The "slow" stress response therefore reflects how well an individual is coping with environmental change. The "immediate" or acute stress hormone response can in many ways be important for survival, for instance by enabling a faster flight response, while chronic stress, characterised by an unsuccessful "slow" stress response, can lead to prolonged activation of the HPA axis (De Kloet & Derijk 2004). Generally, circulating corticosterone results in negative feedback to the hypothalamus, inhibiting release of corticotrophine release factor (CRF) and production of adrenocorticotropic hormone (ACTH). Chronic stress will elevate basal levels of corticosterone, which in turn can lead to alterations in the abundance of glucocorticoid receptors in the hypothalamus leading to a suppressed feedback loop of the HPA axis (Wang et al. 2013). Basal levels of corticosterone can thus reveal how well an individual is coping in an environment. However, corticosterone levels in the blood can be altered within a few minutes in response to a stressful stimulus (Ericsson & Jensen 2016), and are thereby very easily influenced by the sampling procedure itself, making basal corticosterone levels difficult to measure. Heterophil/lymphocyte (H/L) ratio has been suggested as a suitable alternative and more stable measure when investigating chronic stress states, as long as the stress is mild or moderate (Maxwell 1993). H/L ratio has been successful to reflect chronic stress states in laying hens (Campo et al. 2005; Nazar & Marin 2011).

Immunocompetence

A hen's immune capacity can be very tightly linked to the HPA axis (Shini et al. 2008; Nazar & Estevez, 2022). Secretion of glucocorticoids and catecholamines arising from an activated stress response system has been shown to have a close connection to modulations of the immune system (Shini et al. 2009). For example, treating laying hens with ACTH can reduce the abundance of antibodies (Mumma et al. 2006). Acute stress can enhance the innate and adaptive immune system in a way that makes it better prepared for potential challenges to come, while chronic stress is almost always immunosuppressive and leads to immunopathology and health risks (Dhabhar & McEwen 1997; Dhabhar 2009). Chronic stress can also result in over-activation of the immune system and worsen allergies and autoimmune and inflammatory diseases (Dhabhar 2009). The immune system is complex and its different parts interconnect with each other, so multiple measurements are often needed in order to successfully interpret an individual's immune status (Lee 2006). Previous studies in laying hens have investigated the lymphoproliferative response to phytohaemagglutinin-P (PHA-P), such as (Nazar & Marin 2011; Campderrich et al. 2019) which reflects the potential to mount an inflammatory response (Vinkler et al. 2010) and also the acquired antibody response to injected SRBC as a humoral acquired immune response (Nazar & Marin 2011; Campderrich et al. 2019).

1.6 Early enriched or complex environments

> *Definition of environmental enrichment*
> Environmental enrichment is defined as "a modification of the environment of captive animals, thereby increasing the animal's behavioral possibilities and leading to improvements of the biological function", which should "increase the animal's ability to handle behavioral and physiological challenges" (Newberry 1995).

In current efforts to develop an optimal rearing environment that could better facilitate animal welfare and promote traits related to adaptability, there is inconsistency in the use of terms defining environmental characteristics. For example, an "enriched" or "more complex" environment can often describe the same type of environments tested in different studies, but these terms can also be used to describe very different environments. In another example, the term "environmental enrichment" is often used for all possible environmental inputs, even if the input may not lead to improved biological functioning according to the definition (see box). Furthermore, the different types of enrichments ('foraging opportunities', 'structural complexity, 'sensory/novelty' and 'social stimulation') stated by Newberry (1995) are not often compared, even if they can have a very different impact on an animal (Miller & Mench 2005, 2006). In animal research, an "enriched" or "more complex" environment is commonly constructed by adding different materials or structures. In poultry, this could involve providing biologically relevant resources such as litter (Johnsen et al. 1998; Brantsæter et al. 2017) or perches and shelter (Zidar et al. 2018; Campderrich et al. 2019), or providing non-biologically relevant resources such as plastic bottles and CDs (Heikkilä et al. 2006). These environments could be defined as more "complex in space".

The term "enriched" or "complex" has also been used for environments with short-term experiences, such as an environment with provision of different novel objects, different sounds or visual stimuli (Campbell et al. 2018). These environments could be defined as "more complex over time".

The effect of these types of environments (complex in space and over time) are commonly compared with an environment that does not have these resources or additions, *i.e*. a more barren environment.

1.6.1 Rearing environments more complex in space

Behaviourally, providing litter and perches during rearing can enable foraging, dust bathing and perching, defined as behavioural needs and priorities for laying hens (Weeks & Nicol 2006). Provision of litter during the first four weeks has also been seen to reduce fearfulness among the birds, based on a tonic immobility test (Johnsen et al. 1998). Providing additional structures during rearing has been shown to improve hens' learning ability in a spatial task (Krause et al. 2006; Tahamtani et al. 2018). Physiologically, providing litter, shelter and perches compared with only providing litter can lower the H/L ratio in birds and increase resting behaviour, which could indicate improved coping abilities with respect to potential stressors in their home environment (Campderrich et al. 2019). Positive effects have also been seen on the immune system, *e.g*. rearing in floor pens with peat, wood shavings and perches, compared with cage rearing, can improve recovery after infectious challenges (Walstra et al. 2010). Similar results have been seen in many different species, such as in salmonid fish, where "enriched" housing (by comparing gravel to a flat tank floor) can result in improved parasite resistance (Räihä et al. 2019). Results in previous studies suggest further that provision of additional resources in the early environment can even buffer the stress response to adverse experiences, including its suppressive effects on the immune system (Nazar & Marin 2011; Campderrich et al. 2019).

There is thus evidence that manipulation of the early environment, such as providing biologically relevant resources, can enhance the long-term adaptability of individuals. Greater environmental variation that is biologically relevant can lead to greater possibilities for environmental choice, which could encourage environmental engagement such as exploration (Fife-Cook & Franks 2019; Špinka 2019). A higher degree of environmental engagement is associated with higher animal agency, which enhances the motivation to explore and gather information from the environment and can therefore be considered central to the concept of adaptability (Špinka 2019). A perception of choice is also tightly linked to experienced controllability (Leotti et al. 2010), which is one of the key factors involved in the ability to cope with potential stressful stimuli (Koolhaas et al. 2011). ‘Level of environmental choice’ could perhaps be a more relevant way to describe and define an environment, since this could be one underlying factor for the positive effects seen for the “enriched” or “complex” environments in the studies cited above.

1.6.2 Rearing environments more complex over time

It has been suggested that early mild adversity could lead to higher resilience later in life and the development of a more effective coping style (Daskalakis et al. 2013; Fokos et al. 2017). Environments with short-term experiences, through provision of different novel objects, different sounds or visual stimuli (Campbell et al. 2018; Bari et al. 2020) or brief relocation of an animal (Tang et al. 2006), could be expected to induce a stress response, at least initially. Koolhaas et al. (2017) raised the importance of matching the early adversity and the later potential adversity or stressor for it to have beneficial long-term effects. For example, rats with an early experience of a long-term schedule of various stressors connected to environmental change, such as social isolation and crowding showed shorter latency to approach a novel object after 70 days of age (Chaby et al. 2013), suggesting adaptation to novelty. Another example in rats is that early brief visits to novel environments can improve spatial working memory and reduce corticosterone response to sudden stimuli (Tang et al. 2006). Similarly, early experience of human handling in young chicks may initially be associated with a mild stress response, but then can reduce long-term fearfulness. One study showed that daily gentle human handling (lifting, holding and 10 s of stroking) twice per day during the first month after hatch resulted in less freezing towards a novel object and in an open field test, which was interpreted as reduced general non-specific fear (Jones & Waddington 1992). Another study showed that a handling procedure during one week or during all of the first three weeks reduced human avoidance and tonic immobility duration compared with chicks not handled during this phase (Jones & Waddington 1993).

Providing short-term experiences often involves some level of environmental change or unpredictability at an early age and can be of relevance if promoting adaptability. Predictability is the other key aspect in the ability to cope with potential stressful stimuli (Koolhaas et al. 2011). It has been suggested that some level of unpredictability in the environment of captive animals could be advantageous to prevent states of boredom (Meagher 2019). Unpredictability in the environment is commonly investigated by providing “environmental change” (Favre et al. 2015; Campbell et al. 2018; van Horik et al. 2019) and could be a better way to define these type of environments. Early exposure of young birds to “environmental change” could potentially enhance their adaptability and thus better prepare them for later environmental change.

1.7 Research needs

It has been shown that early environments defined as “enriched” or “complex” can improve various traits connected to improved adaptability. However, there are is a need for validation and for mapping the precise mechanisms behind these effects (Campbell et al. 2019b). Given the possible connections to ‘environmental choice’ and ‘environmental change’, it is important to evaluate their influence, separately and combined, in the rearing environment on laying hen adaptability. Evaluations were performed in this thesis by constructing different levels of environmental choice and change, using two resources (litter and perches) identified in the literature as being highly important for young hens (Janczak & Riber 2015). A standard rearing environment commonly only provides one type of litter and perch type, giving few possibilities for environmental choice and low levels of environmental change. However, young laying hens may not be able to distinguish different litter and perch types as greater possibilities of environmental choice or may not recognise a change of litter and perch type as an environmental change. Furthermore, a higher level of environmental choice or change may have the greatest effects in a particular rearing phase. In multi-tier systems, young laying hens may be locked in the aviary rows during the first weeks after hatch to ensure that they feed and experience the higher tier levels. This is a common procedure in Sweden (Wallström, 2022) and in other European countries where multi-tier systems are increasing (De Haas et al. 2014; Brantsæter et al. 2017). If there are positive long-term effects of increased environmental choice or change during this early rearing period, it could be a very practical and feasible measure to implement in practice. Research on optimising the rearing period for more robust hens destined for a complex laying housing system has been called for (Leenstra et al. 2016; Ferry Leenstra et al. 2014).

This thesis sought to identify the characteristics of an early “enriched” or “complex” environment with the greatest positive impact on laying hen adaptability. It also sought to contribute in developing terms that can describe these environmental characteristics in a way that moves beyond simply providing the lowest level of necessary resources in the environment of captive animals.

2. Aim

The overall aim of this thesis was to investigate whether providing greater possibilities for environmental choice or higher levels of environmental change during rearing improves adaptability in laying hens. A series of experiments investigating short-term effects were conducted to refine the rearing treatments, before investigating possible long-term effects.

Specific objectives of the Experiments I-IV (Papers I-IV) were as follows:

I. To determine whether young laying hens during early rearing show different types of behaviour to different types of litter and perches presented in their home environment; and to assess whether litter and perch types could be used to manipulate the level of environmental choice or/and change in subsequent experiments comparing different rearing treatments.

II. To examine whether providing greater possibilities for environmental choice (by presenting several litter and perch types) or/and higher level of environmental change (by regularly changing litter and perch types) during early rearing can improve young laying hens' short-term behavioural adaptability compared with a standard rearing environment (only one litter and perch type).

III. To assess whether the two environmental treatments in Experiment II that resulted in the greatest differences in young laying hens' behavioural adaptability also lead to differences in young laying hens' short-term physiological adaptability.

IV. To assess whether the two environmental treatments in Experiment II that resulted in the greatest differences in young laying hens' behavioural adaptability also have long-term effects on hens' behavioural adaptability; and to compare the effects when these treatments are provided during the early or late rearing period.

3. Material and methods

This chapter summarises the locations, pen designs and measurements made in the different experiments. Effects of environmental choice were investigated in all experiments, while effects of environmental change were investigated in Experiment II and IV. The methods used in each experiment are described in more detail in Papers I-IV.

3.1 Location of the experiments

All experiments were conducted at the Swedish Livestock Research Centre (SLU Lövsta lantbruksforskning), in the laying hen experimental facility (Figure 1). The experiments were conducted in three different stalls within the facility (Figure 2), depending on group size and bird age. For each experiment, birds were hatched, sorted and vaccinated at a commercial hatchery before being transported and placed in their rearing pen at the experimental facility. All birds in each experiment came from the same hatchery and were one day old at arrival. A common commercial white layer hybrid was used in all studies (Lohmann Selected Leghorn Classic (Lohmann breeders) in Experiment I and Bovans Robust (Swedfarm) in Experiments II-IV). Lövsta staff took care of the birds and checked them daily. Birds were given standard starter or layer feed, while light intensity, light cycles and temperature in the rooms were adjusted according to the breeders' recommendations. Different birds were used in the different experiments and all birds were rehomed to private backyard farms after each experiment.

Figure 1. The laying hen facility at Lövsta Research Centre outside Uppsala.

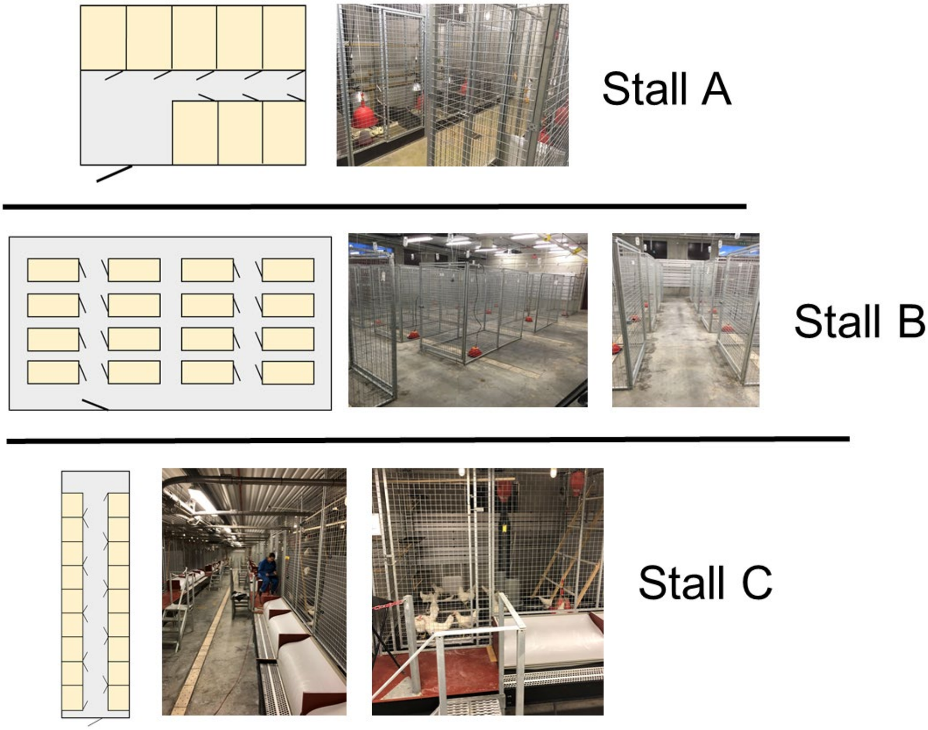

Figure 2. Overview of pen design and images of Stall A (Experiment I, novel pen test in Experiment II), Stall B (Experiment II and III, rearing period in Experiment IV) and Stall C (laying period in Experiment IV).

3.2 Rearing pen designs

In the experiments, the levels of environmental choice and change were manipulated by using different types of litter and perches, resources known to be important for young chicks (Janczak & Riber 2015).

3.2.1 Providing environmental choice

Experiment I

In **Experiment I** (Paper I**)** 93 chicks were housed in six pens (1.5 x 1.15 m, 15-16 chicks per pen) in Stall A during the first four weeks after hatch. All chicks had three litter trays and perches (Figure 3). Chicks' usage of six different types of litter (wood shavings, peat, sand, crushed straw pellets, straw and hemp shavings) and perch types (a wide and a narrow; rope, flat wood and round wooden perch) during a systematic and balanced changing schedule (3 times per week) were compared (see Paper I for details). This was the only experiment in which increased possibilities of environmental choice (several litter and perch types) were provided to all pens.

Figure 3. A pen in Experiment I.

Experiment II

In **Experiment II** (Paper II), 320 chicks were housed in 16 pens (1.2 x 2.4 m, 20 chicks per pen) in Stall B during the first five weeks after hatch. All pens had four litter trays and four perches, but eight of the pens had four different types of litter and perches (Multi-choice environment), while the other eight pens had the same types of litter and perches in all locations (Single-choice environment) (Figure 4). The litters used were peat, wood shavings, straw and sand. The perch types used were flat wood, round rubber, flat wire and wide rope. To reduce the risk of possible effects due to within-pen location, the litter and perch types were evenly distributed within the Multi-choice pens. To reduce the effects of specific litter and perch types, each type presented in the Multi-choice pens was also presented in two Single-choice pens (Figure 5). See Paper II for a more detailed description. This was also done in Experiments III-IV.

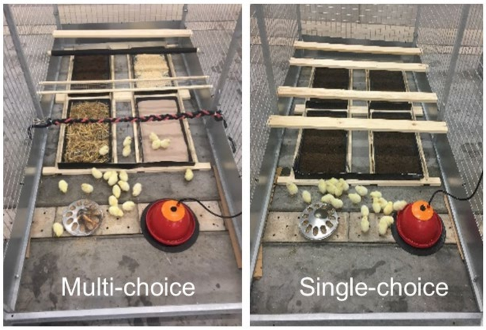

Figure 4. Examples of (Left) a Multi-choice pen and (right) a Single-choice pen used in Experiment II.

Experiment III

In **Experiment III** (Paper III), 104 chicks were housed in eight pens (13 chicks per pen) in Stall B during the first three weeks after hatch. The same pens and the same environmental treatments as in Experiment II were used, apart from addition of a heat lamp in the middle of the pen and a ground substrate for both the Multi-choice and Single-choice environments (Figure

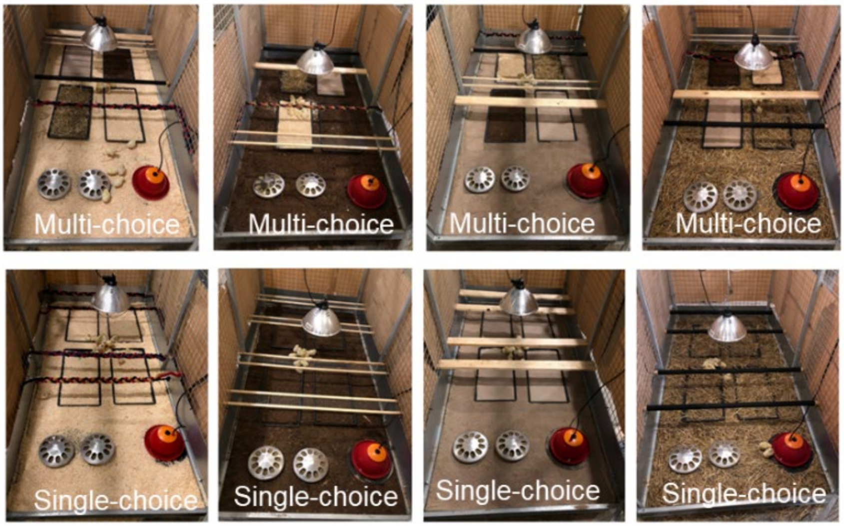

Figure 5. (Upper row) Multi-choice pens and (lower row) Single-choice pens used in Papers III and IV.

5). The type of ground substrate was balanced so it corresponded to the substrate in the Single-choice pens, and different types were tested in different Multi-choice pens. See Paper III for a more detailed description.

Experiment IV

In **Experiment IV** (Paper IV), 364 chicks were housed in 16 pens (22-23 chicks/pen) in Stall B. The same pens and treatments as in Experiment III (Figure 5) were used during the first 15 weeks after hatch. At week 5, the pens were adjusted to bird size by presenting the litter in larger boxes, raising the perches and removing the ground substrate in both the Multi-choice and Single-choice environments (Figure 6). At week 15, all hens were relocated to a pen in the laying hen facility (Stall C, Figure 2).

Figure 6. Examples of (Left) a Multi-choice pen and (right) a Single-choice pen used between weeks 5-15 in Experiment IV.

3.2.2 Involving environmental change

In Experiments I and III, no further environmental factor than 'choice' was used in the pens. In Experiment II and IV, the additional factor 'change' was used to construct different treatments.

Environmental change during rearing

Experiment II investigated the effects of different levels of environmental change, where a change in environment involved swapping litter and perch types in the pen three times per week between day 6 and 21. Half of all

Single-choice and Multi-choice pens were assigned to a changing or non-changing environment, resulting in four different treatments (Figure 7): Non-changing*Single-choice, Non-changing*Multi-choice, Changing*Single-choice and Changing*Multi-choice. The non-changing environments had the same types of litter and perches on the same locations as at day 1 (left quadrants in Figure 7). The environmental change for the changing environments was different for the two different choice environments (right quadrants in Figure 7). For the Changing*Multi-choice treatment, it involved relocation of the litter and perch types within the pen (top right quadrant in Figure 7). For the Changing*Single-choice treatment, it involved changing the type of litter and perch in the pen to another type (bottom right quadrant in Figure 7).

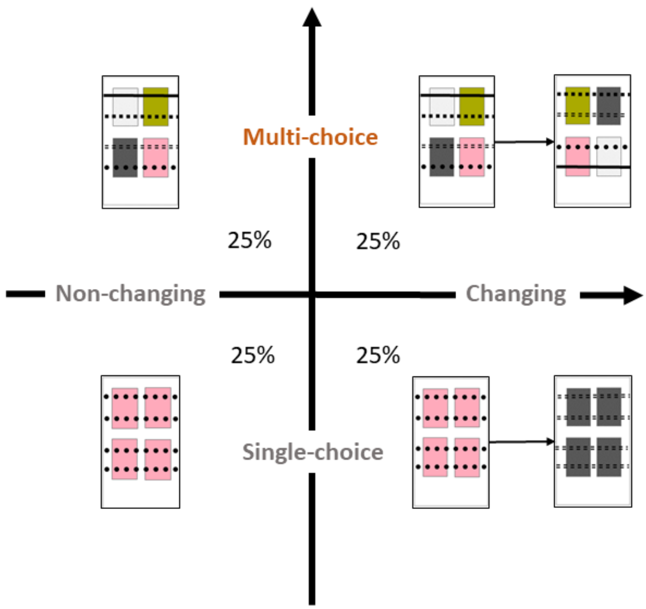

Figure 7. Treatments in Experiment II involving relocation or changing of litter and perch type three times per week in half the rearing pens (right quadrants), while the other half were unchanged (left quadrants). This resulted in four different combinations of different levels of environmental choice and change.

Environmental change at week 5 to investigate effects of rearing period

Experiment IV investigated the effects of swapping environments at different stages during rearing: the first four weeks (Early rearing) and week 5-15 (Late rearing). This was done by swapping environment for half the pens of each choice environment (right quadrants in Figure 8), while the other half had the same choice environment throughout the rearing period (left quadrants in Figure 8). At week 15, all hens were moved to similar laying pens in a laying facility (Stall C).

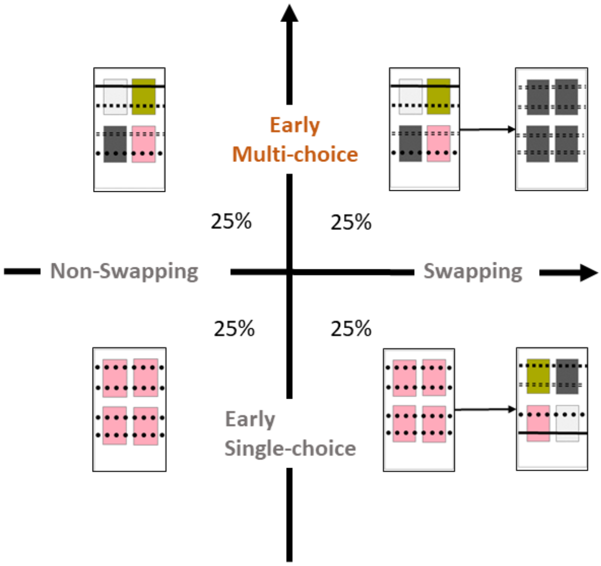

Figure 8. Treatments in Paper IV obtained by swapping half of each choice environment to the other type at week 5, resulting in four different combinations.

3.3 Behavioural observations

Behavioural observations performed in Experiment I-IV included observations in the rearing pens to see how birds were affected by the different treatments and also observations in specific tests to determine the indirect effects of the treatments. Experiment I investigated combinations of behaviour and location, to get a detailed overview of time budgets and behavioural repertoire, depending on the type of litter or perch. Detailed descriptions of ethograms and methods can be found in Paper I-IV.

3.3.1 Fear and exploration tests

In Experiment II, a **multivariate behavioural assay** (Figure 9) was used in week 5 for simultaneous investigation of several characteristics, such as fearfulness, exploration and problem-solving. This method has been used previously on young chicks (Zidar et al. 2018). One chick at a time was placed in a start box (A in Figure 9) from where it could see two companions in a white bird cage (B) in the middle of the open area, through an opening covered with wire. Fearfulness was investigated by measuring 'freezing duration' after being placed in the start box (A). Other measurements made in the multivariate behavioural assay were 'latency to solve the detour problem and enter the open area (C)' (reflecting problem solving and spatial abilities) and 'number of crossed lines in the open area' (reflecting exploration). More details can be found in Paper II.

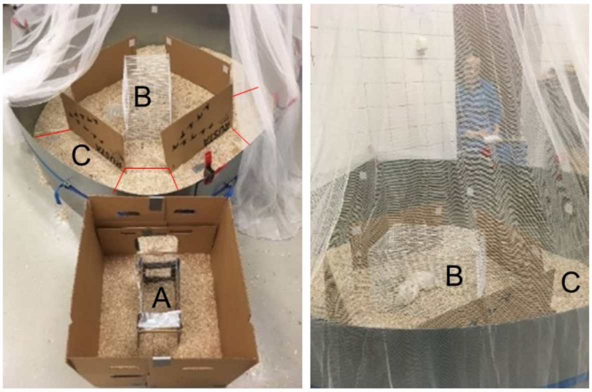

Figure 9. Multivariate behavioural assay used in Paper II, (left) before and (right) during testing.

In Experiment III, a standardised **tonic immobility (TI) test** was used in week 3, as an individual measurement of fear (Forkman et al. 2007). The test is commonly used in poultry research and is described in more detail in Paper III.

3.3.2 Tests involving novel resources

The other behavioural tests performed in this thesis involved provision of novel resources, such as novel perches, litter, mealworms, nests and an outdoor range, as a measure of how the hens exploited opportunities. Use of novel resources can also reflect fear, exploration and spatial abilities.

Figure 10. Pen used in the novel pen test in Experiment II.

In the **novel pen test** in Experiment II, birds were released in groups of 10 into a novel pen with novel resources in week 5 (Figure 10). Exploration was measured as 'average proportion of birds moving per scan' during the first hour. Use of resources was measured as 'average proportion of birds on a novel resource per scan' during the first hour. Further details can be found in Paper II.

The **opportunity tests** were conducted in a test arena or in the home pens during the rearing and laying period (Figure 11). All involved repeated opportunities to locate and consume hidden mealworms, which were initially novel to all birds. In the opportunity tests in the test arena in Experiment IV, birds in week 9-10 spent five minutes, in groups of three or alone, in the test arena with nine hidden mealworms in plastic cups (A in Figure 11), in seven replicate tests. In the opportunity test in the home pen (rearing period) two bowls with hidden mealworms were presented on the ground (A) and on the lap (B) of a test person (C) for 1.5 minutes, with three repetitions, in week 3 in Experiment III and in week 14 in Experiment IV. An opportunity test in the home pen (laying period) was conducted later in Experiment IV, in week 17 in the laying pen. Experiment III investigated improvements with repetitions in terms of 'latency to feed', 'proportion of birds nearby', 'number of pecks' and 'proportion of mealworms eaten'. Experiment IV examined the general success and compared the 'average proportion of mealworms eaten'. Further details can be found in Papers III and IV.

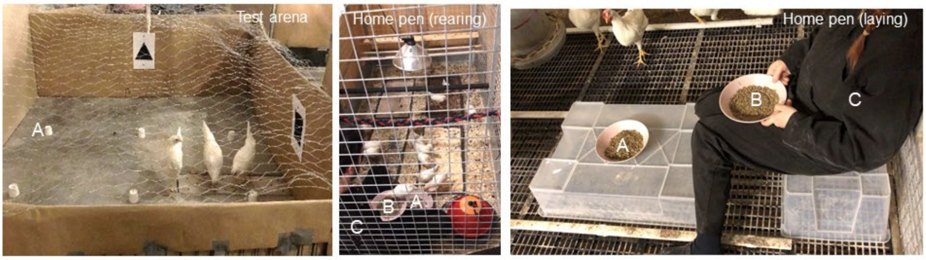

Figure 11. Opportunity tests in (left) a test arena and (centre, right) the home pens during the rearing and laying period.

At week 16, all hens in Experiment IV were **relocated to a laying pen** (Figure 12), where they were video-recorded for one hour. Behavioural observations were made for 'latency to feed' (A in Figure 12), 'latency to be in the litter' (B), 'latency to be on a perch' (C) and 'average proportion of birds in the pen half opposite to the entry (E) per 2-min scan'. During the 72 days of egg production in Experiment IV, egg production and nest usage were also investigated. Birds could lay their eggs in the standard nest boxes on ground level (G), on the floor (litter, B, and slats) or on three elevated platforms (D). Further details can be found in Paper IV.

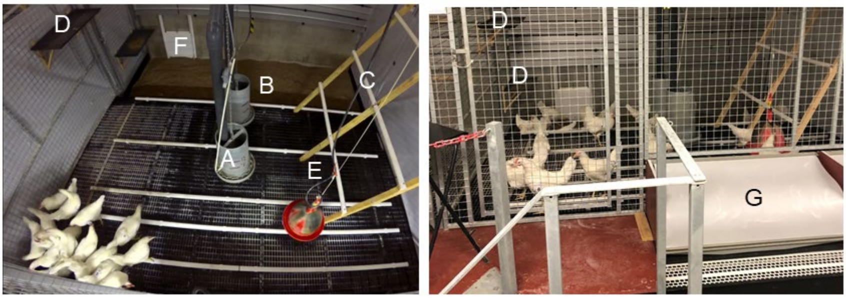

Figure 12. At week 16, birds from same rearing pen were relocated to a laying pen which looked the same for all treatments.

In week 27, all pens were given three days of **access to an outdoor range** (Figure 13), by opening the popholes inside the pen (F in Figure 12). Live behavioural observations were conducted using scan sampling every 30 min when the popholes were open.

Figure 13. In week 27, birds in Experiment IV were given access to an outdoor range attached to each laying pen.

3.4 Physiological measurements

In Experiment III, physiological measurements were performed in week 3, with all parameters measured related to immunology. As when interpreting behavioural measurements, several variables need to be measured in order to interpret an individual's immune status successfully (Lee 2006). Standardised measurements (Nazar & Marin 2011; Nazar et al. 2015; Campderrich et al. 2019) that were familiar to the research group and variables reflecting different parts of the immune system were measured.

3.4.1 *In vivo* measurements

In vivo measurements performed included **lymphoproliferative swelling response to PHA-P**, which involved injection of a non-pathogenic substance (red kidney bean extract) into the wing web (A in Figure 14). The swelling response reflects general pro-inflammatory potential or a bird's potential to mount an inflammatory response (Vinkler et al. 2010). The outcome variable used was 'percentage swelling', calculated as (Basal thickness/Thickness post-24 h) x 100 (Nazar & Marin 2011), where thickness was measured using a digital calliper (B in Figure 14).

Figure 14. (A) Wing injection and (B) swelling measurement procedures in measuring lymphoproliferative swelling response to phytoemaglutinin-p (PHA-P).

3.4.2 *In vitro* measurements

Using blood collected from the brachial wing vein, **interferon gamma (IFN-γ)**, **natural antibodies against sheep red blood cells (nAb ag SRBC)** and **heterophil/lymphocyte (H/L) ratio** were determined. IFN-γ is a pro-inflammatory mediator, while nAb ag SRBC represents a first line of general defence in the humoral immune system.

IFN-γ concentrations were analysed using an ELISA kit (Ray Bio® Chicken IFN-gamma), while natural antibodies (Nab) against SRBC were assessed using a microagglutination assay (Matson et al. 2005) (Figure 15).

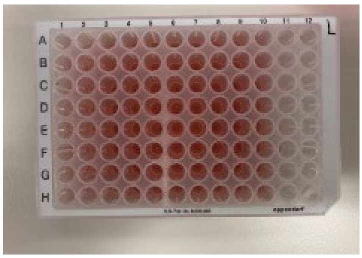

Figure 15. Output in a microagglutination assay.

Blood smears for leukocyte counts were made immediately at blood collection, using one drop from the syringe (without the needle) according to standard practice. They were stained by May Grünwald Giemsa (Figure 16), before calculating 100 white blood cells per smear and then calculating the H/L ratio by dividing the number of heterophils by the number of lymphocytes (Campderrich et al. 2019). Further details can be found in Paper III.

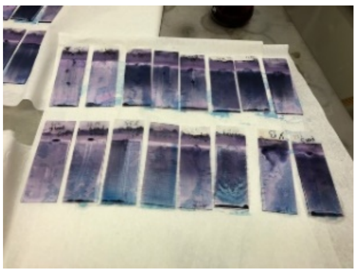

Figure 16. Stained blood smears.

3.5 Statistics

Proportions were used when there were different number of chicks in the pens. Since all proportion data in Experiment I were normally distributed, the proportions were considered as continuous values and linear models were used. For proportion data in Experiment II-IV, generalised linear models were fitted. For data involving several measurements per pen, such as several scans of the same pen or individual data on chicks from the same pen, mixed models were fitted and pen was considered a random effect. For data on continuous variables such as latencies, linear models were fitted. Counts that had high means and showed a normal distribution were also fitted with linear models. Multiple comparisons were penalised using the Tukey method. If the data failed to fulfil the criteria for linear models, a non-parametric test was used on average values per pen. For more detailed descriptions, see the statistical analysis section in Papers I-IV.

4. Main results

4.1 Paper I

Time budget observations in Paper I showed that young laying hens during early rearing (first four weeks after hatch) spent the greatest proportion of daytime observations in the litter (0.5 ± 0.04), followed by on a perch (0.18 ± 0.04) and at the feeder (0.12 ± 0.01). They spent the greatest proportion of daytime observations foraging (0.43 ± 0.04), followed by sleeping or resting (0.25 ± 0.03), preening (0.08 ± 0) and dust-bathing (0.01 ± 0.01). Already from the first week post-hatch, the young hens showed different frequencies of specific behaviours on the different litter and perch types.

4.1.1 Litter use

When observed foraging in litter, the young hens were significantly more likely (p<0.05) to be foraging in wood shavings, hemp shavings and sand, rather than in peat and pellets. When observed sleeping/resting in litter, they were significantly more likely (p<0.05) to be sleeping/resting in straw than in peat, pellets or sand, and significantly more likely (p<0.05) to be sleeping/resting in wood and hemp shavings compared with peat. When observed dust-bathing, 57.7% of all observed bouts were performed in sand, 36.6% in peat, 4.2% in crushed straw pellets and 1.4% in wood shavings, while no dust-bathing bout was seen to be performed in straw or hemp shavings.

4.1.2 Perch use

Perch use was observed from day 5 after hatch, the same day as when perches were raised to 15 cm after being placed on the ground for the first days. The proportion of young hens sleeping or resting on a perch during observations (including dusk period) increased significantly after the first week ($p<0.001$), while the proportion of young hens sleeping and resting in the litter decreased correspondingly ($p<0.001$).

The wide rope perch was significantly more likely ($p<0.05$) to be used the first week than all other perch designs. Different perches were preferred for different behaviours. When observed sleeping/resting on a perch, young hens were more likely ($p<0.05$) to be sleeping/resting on a wide rope compared to a narrow rope or wide wooden perch, while a wide rope was not more preferred than a narrow wooden perch. When landing on a perch design, the young hens were more likely ($p<0.05$) to have a stable landing, rather than showing balancing movements, if landing on the flat wooden perches and on the wide rope. They were equally likely to have a stable or problematic landing on the round wooden perches ($p>0.05$), but more likely to have a problematic landing if attempting to land on the narrow rope ($p<0.05$).

4.2 Paper II

When exposed to two novel environments (the multivariate behavioural assay and the novel pen test) at 5 weeks of age, treatment differences were found in how the young laying hens moved in the novel space. Compared with the standard rearing environment (Non-changing*Single-choice), the following results were obtained:

- A higher level of environmental change (Changing*Single-choice) resulted in shorter freezing duration (t=-2.58, $p=0.024$) when young hens were first placed in the start box of the multivariate behavioural assay. No treatment differences were found in the other part of the assay or in the average movement in the novel pen test ($p>0.05$).
- A higher level of environmental choice (Non-changing*Multi-choice) resulted in a higher average proportion of young hens moving per scan (OR=1.716, $p=0.032$) in the novel pen test, a higher number of crossed lines (t=2.45, $p=0.031$) when birds were given 5 min to be in the open area of the multivariate behavioural assay, and a shorter latency to solve the detour in the multivariate behavioural assay (t=-2.56, $p=0.025$).

- A higher level of both environmental change and choice (Changing*Multi-choice) did not induce any behavioural effects in the two novel environments.

The young hens from the Non-changing*Multi-choice environment showed shorter latency to solve the detour in the multivariate behavioural assay compared to birds from all other treatment combinations ($p<0.05$), while the movement in the open area or in the novel pen were not different to that among birds from the two changing treatments ($p>0.05$).

No treatment differences were found in latency to first use novel resources in the novel pen test ($p>0.05$).

4.3 Paper III

In Paper III, the results were divided into behavioural outcomes in the two behavioural tests and results for the four immunological variables measured.

4.3.1 Behavioural tests

Young laying hens from the Multi-choice environment required more attempts to induce a TI state ($\chi^2=4.57$, $p=0.03$) and had shorter TI duration than chicks from the Single-choice environment ($F=4.67$, $p=0.03$). No treatment differences were found for latency to first head movement or vocalisation ($p>0.05$). Further, young hens in the Multi-choice pens showed greater improvement over repetitions ($p<0.05$) in the opportunity test. These improvements were a shorter latency to start eating in the more challenging bowl placed in the lap of the human test person, a higher proportion of birds being near the bowls and a higher proportion of mealworms eaten, while chicks in the Single-choice pens failed to show similar improvement ($p>0.05$).

4.3.2 Immunological variables

Discriminant analysis based on the variables measured on individual level explained 94.9% of the variation between the groups. The analysis revealed that natural antibody titres against SRBC and H/L ratio were the two most important variables, with discriminant coefficient of 0.98 and 0.20, respectively. Young hens from the Multi-choice environment had higher levels of natural antibody titres ($\chi^2=5.33$, $p=0.02$) and lower H/L ratio

($F_{1,74}$=6.92, p=0.01) than birds from the Single-choice environment. No effects were seen for inflammatory response to PHA-P or for IFN-γ plasma concentration.

4.4 Paper IV

In Paper IV, the results were divided into treatment effects of early rearing (first four weeks after hatch) or late rearing (week 5-15), or effects of the environmental swap at week 5 which was made for half the pens in each choice environment.

4.4.1 Effects of the early rearing period

Compared with laying hens kept in a Single-choice environment during early rearing, those in a Multi-choice environment during this period were faster to use a novel perch ($F_{1,12}$=5.49, p=0.037) when relocated to the laying facility at week 16, irrespective of the late rearing environment.

4.4.2 Effects of the late rearing period

Compared with hens kept in a Single-choice environment during late rearing, those in a Multi-choice environment during this period consumed more mealworms in two opportunity tests, irrespective of the environment in the early rearing period. This was found in the opportunity test conducted in a test arena (χ^2=16.70, p<0.001) in week 9-10 and also in the opportunity test conducted in the home pens (χ^2=6.21, p=0.01) in weeks 14 and 17.

4.4.3 Effects of an environment change at week 5

Laying hens that were changed from a Multi-choice environment to a Single-choice environment at week 5 were less likely to lay their eggs on higher platforms compared with those in all other treatments (p<0.05). The same hens showed the least usage of an outdoor range during the first three days, but this was only significant when compared with the hens that were changed from a Single-choice to a Multi-choice environment at week 5 (t=-2.24, p=0.04).

5. Discussion

This thesis investigated the possibility of enhancing adaptability in laying hens by providing increased environmental choice or change in the rearing environment, using manipulations of the litter and perch types available. This chapter presents a discussion on whether it proved possible to manipulate the degree of environmental choice and change, followed by a discussion on whether it proved possible to enhance laying hen adaptability. The discussion touches on possible pathways or underlying mechanisms for observed treatment effects before discussing practical implications and research contributions.

5.1 Manipulating environmental choice and change

In manipulating environmental choice and change in a way that will be perceived as different for an individual young hen, selecting suitable environmental stimuli to manipulate is of great importance. Young laying hens have been shown to discriminate and choose between different litter types when given short-term access, observed as different latencies to approach, percentage of visits (Shields et al. 2004) or number of pecks (Sanotra et al. 1995). The issue investigated in this thesis was whether the young hens perceived different litter types as sufficiently different to be experienced as increased environmental choice, when presented in their home pens. The different behavioural expressions observed for the types of litter and perches tested in Paper I confirm that differences in litter and perches had an effect on the young hens. This indicates that provision of one or several types of litter and perches could have been perceived as different levels of environmental choice, while a change or relocation of litter and perch types could have been perceived as an environmental change. The different behavioural expressions seen for the different types of litter and perches in Paper I could even be an indication of choice, in that young hens chose a specific type for a specific behaviour or that they chose behaviour depending on the litter or perch type.

5.2 Improving laying hen adaptability

Using an adjusted version of the definition by Barker (2009), in this thesis adaptability was defined as "the degree to which an individual can remain or become adapted to environmental change by behavioural or physiological means". In the experiments in Papers II-IV, it was expected that improved adaptability would be reflected in more movement or exploration of a novel environment and higher usage of novel resources, and also in improved stress coping and immunocompetence. The results indicated that the environmental change and choice treatments tested were able to influence traits related to adaptability in the hens. These effects are discussed below.

5.2.1 Reduced fear response in a novel environment

Environmental change, involving changing the type of litter and perch present in the pen three times per week, reduced initial freezing duration in young laying hens placed in a novel environment in week 5, compared with freezing duration among young hens from the standard rearing environment (Paper II). Reduced freezing duration suggests reduced fearfulness (Jones & Waddington 1992; de Haas et al. 2017a). Exposure to early environmental change or stress in hens (Jones & Waddington 1992; Zidar et al. 2018) as in rats (Chaby et al. 2013) has been shown to result in shorter freezing durations and latencies to leave a familiar start box in individual animals. The environmental change created by changing litter and perch types in this thesis could have resulted in mild stress and this early repeated activation could have boosted the chicks' coping abilities (Daskalakis et al. 2013; Fokos et al. 2017), as reported previously (Goerlich et al. 2012). The reduced initial freezing response could also be a sign of habituation to environmental change (Koch 1999), due to the repeated experience of environmental change, or associative learning connecting environmental change with a positive stimuli (Pontes et al. 2020). The results suggest a positive impact of environmental change or unpredictability on hens' adaptability, at least in the short-term, since hens that are less affected by novelty could be faster to adapt to environmental change.

For birds given four litter and perch types in Paper II, an environmental change meant relocation of the types within the pen and did not lead to the same reductions in freezing duration. Even though the environmental change occurred three times per week in all Changing treatments, the degree of environmental change was different, as relocation could have been perceived as a lower degree of environmental change and also did not involve novelty. Relocation of resources within the rearing pen during rearing may thus have little impact on birds' later ability to handle novelty.

5.2.2 Reduced latency in a detour problem

The initial freezing duration seen in the multivariate behavioural assay did not show a connection to shorter latency to solve the detour or more movement, suggesting that these behaviours were not related. In Paper II, it was found that having four litter and perch types shortened young laying hens' latency to solve a detour problem. The outcome in a detour test is believed to reflect spatial abilities (Regolin et al. 1995b; Regolin & Rose 1999). By using conspecifics as the 'goal' in testing, it was possible to avoid too high influence of birds' fearfulness on the latency outcome (Regolin et al. 1995a). In various animal species from many different taxa, a more complex environment in space and over time, such as additional structures or having social companions, can alter brain morphology and activity among both young and adults (van Praag et al. 2000; Mohammed et al. 2002). A more spatially complex environment can improve outcomes in spatial tasks in hens (Tahamtani et al. 2015; Zidar et al. 2018; Norman et al. 2019) as in other animal species (Salvanes et al. 2013). The hippocampus is believed to be highly involved in spatial navigation in laying hens and in other animal species (Morandi-Raikova & Mayer 2022b). On placing young laying hens in a novel environment already at a young age they show neural activation in the hippocampus (Morandi-Raikova & Mayer 2020). Greater exploration (Morandi-Raikova & Mayer 2022a) as well as a higher degree of spatial complexity increases this neural activity further (Mayer et al. 2018). The results obtained in this thesis suggest that birds given greater environmental choice had improved spatial abilities, potentially seen in hippocampal differences.

However, young hens reared in the environment with regular relocation of the four litter and perch types in the pen were even slower to solve the detour than young hens reared in an environment with no relocation of perch or litter types. Thus the relocation of resources apparently failed to promote spatial abilities. This confirms findings in a previous study, where combining complexity in space and over time in the rearing environment for young hens of the same age resulted in inconsistent results in a spatial task (Campbell et al. 2018).

Providing greater possibilities for non-changing environmental choice had a positive impact on adaptability, at least in the short-term, since spatial ability is advantageous when an animal is relocated to a spatially complex novel environment.

5.2.3 Increased movement in novel environments

It is possible that the same underlying mechanisms acting in the detour test also led to higher movement, both short-term and long-term, in the novel environments tested in Papers II and IV, since improved spatial skills could better enable movement in a novel space and hens reared with greater possibilities for environmental choice were seen to perform better. This indicates that the effect on spatial ability could be both short- and long-term.

Movement in a novel environment has been suggested to be influenced by exploration, activity and emotions (Réale et al. 2007), complicating interpretation of the mechanisms underlying this behaviour. In Paper II, there were no differences in general activity among young hens in their rearing environment, confirming that the differences in movement seen in the novel environments could reflect differences in motivation to explore or in emotional states. Many studies have used degree of movement in a novel arena or degree of approach to a novel object as an indicator of fearfulness, where more movement or reduced approach latency has been interpreted as lower fearfulness (Brantsæter et al. 2016; Hedlund et al. 2019). Young laying hens in the Multi-choice environment in Paper III were seen to be less fearful in the TI test. However, even if a bird has reduced fearfulness in a novel environment, it must still have the motivation to explore in order to move around. This was evident from the fact that difference in freezing duration did not correlate to movement in the open area of the multivariate assay in Paper II. Furthermore, fearfulness measured in a TI test does not always correlate to movement in a novel arena (Hedlund et al. 2019, 2021), in an open field test (Heiblum et al. 1998) or in an outdoor range (Armstrong et al. 2020).

Previous studies have proposed seven basic emotional action systems connected to a negatively valenced or punishing system ('fear', 'rage' and 'panic') or a positively valanced or rewarding system ('seeking', 'lust', 'care' and 'play') (Panksepp 2005, 2016). Seeking is highly involved in exploration and approaching environmental stimuli, and may thus be closely related to adaptability. It is possible that greater opportunities for environmental choice stimulate exploration and seeking by a greater feedback of the action of exploration (Panksepp 2005). This could lead to higher agency and a higher level of information gathering, which could be especially important during ontogeny (Špinka 2019). Furthermore, seeking is connected with reward stimuli in the brain (Alcaro et al. 2007), suggesting that birds with a greater scope for exploration could have a more positive emotional state. It is also possible that a higher predisposition to seeking behaviour can reduce the likelihood of fear, since they are placed on differently valenced systems (Panksepp 2005, 2016).

Birds reared with regular relocation of the four litter and perch types in the pen in Paper II did not show a similar increase in movement in novel environments. Thus the relocation of pen resources could have reduced motivation to explore or could have increased fearfulness (Forkman et al. 2007). That a non-changing spatially complex environment is more preferable than a spatially more complex environment combined with frequent changes (complex in space and time) has been found in studies on rats (Favre et al. 2015). Favre et al. (2015) found that a non-changing rearing environment with running wheel, toys, treats and odours could prevent the development of "autistic-like hyper-emotional" behaviours in rat models, while changing the resources twice weekly prevented these positive effects. The increased preening seen among birds following relocation of the four litter and perch types in the pen in Paper II could indicate stress recovery (Duncan & Wood-Gush 1972; Spruijt et al. 1992), implying that resource relocation was experienced as stressful.

Providing greater possibilities for environmental choice that are not relocated during rearing can lead to improved adaptability in the long-term, due to more exploration in a novel environment enabling faster location and usage of novel resources.

5.2.4 Increased seeking of rewards

Again, it is possible that the same underlying mechanisms influencing outcome in the detour task and increased movement in the novel environments could have influenced the outcome in the opportunity tests in Papers III-IV, since improved spatial skills, lower fear and higher motivation to explore would lead to greater success in locating the hidden food reward. However, this outcome was seen to be influenced by removal of environmental choice at week 5, which decreased success, suggesting that there were other underlying mechanisms in action.

The opportunity test involved repetition, allowing birds to learn and remember the location of the hidden mealworms until the next repetition. Learning has been suggested as one of the most important factors for a farm animal when adapting to changing routines, social mixing and new resources in the production system (Wechsler & Lea 2007). For many animal species, living in a more complex environment has been shown to facilitate learning and associated brain functions (see review by Zentall (2021)). Seeking is reported to be an important underlying factor promoting learning ability (Alcaro et al. 2007), while fearfulness is seen to have inconsistent influences on learning (Krause et al. 2006; de Haas et al. 2017b; a).

Reductions in environmental complexity has been seen to result in a more pessimistic outlook among captive starlings in a cognitive bias task (Bateson & Matheson 2007). It is possible that losing the higher degree of environmental choice in week 5 could have made those young laying hens more pessimistic and thereby being less likely to seek a reward, as seen in the opportunity test (Paper IV). Optimistic responses in a judgement bias test have been found to be related to higher dopamine turnover rates in the mesencephalon in chicks (Zidar et al. 2018), a neurotransmitter that is highly involved in stimulus-reward-learning (Flagel et al. 2011) suggesting a possible link to the outcome in the opportunity tests. Furthermore, a more optimistic cognitive bias is thought to characterise an individual that is more open to environmental change (Faustino et al. 2015) and would thus be preferable in promoting laying hen adaptability.

Providing greater possibilities of environmental choice had a positive impact on behavioural adaptability, at least in the short term, since birds with improved learning capacity and birds more prone to seek a reward will have more success in learning new routines and locating novel opportunities.

5.2.5 Higher usage of the layer environment

Paper IV showed that laying hens which lost the Multi-choice environment at week 5 were less flexible in their nest usage and were less likely to lay eggs on elevated platforms compared with hens from the other treatments. This could be seen as advantageous from a commercial production perspective, since more eggs from these hens were laid in the lower nest boxes. However, it could be viewed as non-advantageous from an evolutionary perspective if all birds 'lay their eggs in the same basket' because of the higher predation risk. Feral hens have been shown to be diverse in their choice of nest and lay their eggs in different nests at different heights, with hens laying eggs on a higher level proving to be more successful (Wood-Gush & Duncan 1976). There were no treatment effects on the proportions of floor eggs in this thesis, which is important since floor eggs are associated with an increased risk of cannibalism (Gunnarsson et al. 1999) and are also dirty or unsellable. Less diverse nest selection could indicate reduced behavioural flexibility, which may be linked to exploration (Réale et al. 2007; Zidar et al. 2019) and possibly also to reduced optimism, as already mentioned. Reduced optimism or seeking could explain the low range use in Paper IV, as hens from only one pen were seen outside.

The hens given a Multi-choice environment at week 5 made the most visits, as birds from all four pens were seen outside, suggesting higher optimism or seeking. It has been suggested that more curious hens would be more likely to use an outdoor range (Kolakshyapati et al. 2020). Usage of an outdoor range is important from a production perspective, since the positive welfare impact of the range, such as reduced risk of feather pecking outbreaks, depends on hens using the range (Nicol et al. 2003). Previous studies have found generally low usage of the outdoor range among laying hens (Pettersson et al. 2016), indicating a need to enhance their abilities or motivation to go outdoors.

Rearing in a spatially more complex environment can have a buffering effect of *e.g.* development of stereotypies in a subsequent more barren environment (Gross et al. 2012), suggesting buffering effects if later experiencing a loss of increased environmental choice. It is possible the environmental loss to which the young hens were exposed in this thesis occurred at too young an age (5 weeks), since semi-wild red jungle fowl are still dependent on their mother until eight weeks after hatch (Collias & Jennrich 1994). It is likely that young laying hens are particularly sensitive and attached to their surrounding environment during these first eight weeks. The results obtained in this thesis suggest that young laying hens of dependent age (<8 weeks) should not be exposed to reductions in environmental choice, but that increasing environmental choice could have positive effects on behavioural adaptability in the long term.

5.2.6 Physical abilities

Having different litter and perch types during the first four weeks after hatch improved later perch use, expressed as shorter latency to use novel perches in the laying house. This was seen despite an eventual reduction of environmental choice at week 5 and indicates a short window for acquiring perch skills. This supports previous findings that access to perches during the first weeks after hatch can improve laying hens' abilities to move in a three-dimensional space long-term, possibly by improved muscle and skeletal development (Hester et al. 2013; Yan et al. 2014) or cognitive spatial skills (Gunnarsson et al. 2000) or both (Norman et al. 2019). The results in this thesis confirmed that it was possible to further stimulate perch use skills, most likely through presenting different perch types. In Paper I, different perch types clearly led to different behavioural responses in the young hens, indicating that the range of perches provided could have led to a higher degree of perch training when landing and moving on different types of structures, demanding different grips and body movements in order not to fall off. Providing different perch types during the first four weeks could improve hens' adaptability in the long term, since hens that are faster at moving in a novel three-dimensional space would have an advantage when moved to a novel complex housing system.

5.2.7 Improved stress coping and immunocompetence

Increased environmental choice, through providing four different litter and perch types in this thesis, resulted in a lower H/L ratio among young laying hens in week 3 after hatch compared with birds reared with only one type. Increased H/L ratio is connected to repeated exposure to corticosterone (Shini et al. 2008, 2009) and thereby chronic stress. Lower H/L ratios indicate reduced chronic stress levels and that these young hens are better able to cope with potential stressors involved in their everyday life. The lower H/L ratio seen among the birds in Paper III could indicate that greater opportunities of environmental choice led to greater experienced controllability (Koolhaas et al. 2011) and is similar to other findings in young laying hens (Campderrich et al. 2019) as well as in young fish, such as the Chinook Salmon (*Oncorhynchus tshawytscha*) (Cogliati et al. 2019). All birds in Paper III were exposed to some level of unpredictability, such as cleaning events, light schedule changes with age *etc*. which could induce stress responses (Lindqvist & Jensen, 2009). Increased possibilities for choice in the environment could have led to higher experienced controllability of the environment (Leotti et al. 2010) and therefore improved ability to cope with these unpredictable challenges as suggested by (Meagher 2019).

Increased environmental choice through providing four different litter and perch types resulted in higher levels of nAb ag SRBC among the young hens in week 3 after hatch compared with the hens reared with only one type. Higher levels of natural antibodies can enable a quicker response to a great variety of pathogen exposures (Reyneveld et al. 2020) and a greater ability to maintain immune homeostasis (Coutinho et al. 1995). A higher prevalence of natural antibodies has been linked to a lower mortality risk in laying hens (Star et al. 2007; Haunshi et al. 2019). A higher level of environmental choice prepared laying hens better for a foreign pathogen and could have large positive effects on laying hen welfare. The findings are in agreement to findings in pigs reared with extra ground substrates (Reimert et al. 2014) and is similar to the higher acquired antibody titer seen among floor-reared hens with litter and perches compared to barren cage-reared hens (Walstra et al. 2010). Paper III showed that young hens from both the Multi-choice and Single-choice environments were equally capable of dealing with pathogens that require an inflammatory response for their elimination, since both environments resulted in a wing-web inflammatory swelling response. This confirms that having a perch and litter *per se* could have been sufficient to stimulate this part of the immune system.

The results obtained in Paper III indicate that improved stress coping, seen as lower stress levels, and higher immunocompetence are linked, as reported in previous studies (Mumma et al. 2006; Dhabhar 2009), although others have found contradictory results (Moe et al. 2010). However, it is important to bear in mind that stress can influence the immune system in different ways, *e.g*. it can inhibit certain parts while stimulating others (Dhabhar 2009).

It is possible that the gut-brain-axis was one possible mechanism behind the differences seen in behaviour, stress and immunology (Nazar & Estevez). While some gut microbes are inherited from the maternal hen through the egg, the gut microbiota in newly hatched chicks is sensitive and highly influenced by early environmental input (Ding et al. 2017; Kubasova et al. 2019). It is possible that the various litter types in the Multi-choice environment enabled a more diverse gut microbiota composition, as the young hens ingested the substrates while foraging. This would have contributed to greater resistance to pathogens (Varmuzova et al. 2016) as well as improved stress coping and cognition (Cryan & Dinan 2012; Nazar & Estevez, 2022).

Regardless of the mechanisms behind the results obtained in Experiment III, providing four different litter and perch types could improve physiological adaptability, at least in the short term, in young laying hens.

5.3 Implications

The findings presented in this thesis make a strong argument for not simply settling for provision of ground substrate and perches in the rearing environment, as specified in the legislation (SJVFS 2022:5 [L111]), and instead presenting several different types. Offering several litter and perch types during the whole rearing period for young laying hens can be a feasible way to promote adaptability in the long term. The actual litter and perch types used do not necessarily have to be the same as those tested in Papers I-IV, but should differ sufficiently in terms of characteristics in order for hens to perceive them as different.

The results presented here indicate that offering several litter and perch types during the whole rearing period would be beneficial for both behavioural and physiological adaptability, most likely by promoting spatial abilities, exploration, physical abilities and improved stress coping and immunocompetence. This would have a large impact on hens' abilities to locate and exploit novel resources when exposed to environmental changes. It would also increase the capacity of the hens to cope with potential stressors in their home environment, such as disturbances or routine changes. Furthermore, the improved immunocompetence implies that hens would be better able to handle foreign pathogens. Together, all these effects would reduce hens' sensitivity to environmental changes, sudden stimuli or pathogen exposure and better meet requests from Swedish farmers (Wallström, 2022). It could also be a way to reduce the high level of mortality seen among the hens in loose housing systems (Schuck-Paim et al. 2021).

This thesis proposes that an environment allowing a more functional development may be even more important than the degree of matching between early and later environment for laying hens. Such an approach is in accordance with the somatic state-based adaptive developmental plasticity theory (Nettle & Bateson 2015) and takes into account that matching is unlikely to be perfect and during their time in production, hens will inevitably be exposed to novel situations.

The foraging environment of the wild ancestor of domestic hens has a great variety in structure, sensory input, pathogen exposure *etc*., so it is complex both in space and over time. The characteristics of this natural environment where red jungle fowl evolved would enable development of the higher adaptability needed to survive in the wild. While domestic fowl may not have the same high potential for adaptability as their wild relative, it may be relevant to enable them to reach their level of potential, so that they can function well in the commercial production environment.

In this thesis, I propose that the terms 'environmental choice' and 'environmental change' could be a suitable way to define environments or environmental inputs given their different influences on the laying hens in the short and long term.

The results indicated that increased spatial complexity by providing a greater possibility for environmental choice, had the largest positive effect on adaptability in laying hens, and should be investigated further.

5.4 Limitations and future work

This thesis was based on four controlled experiments. The environmental treatments used were balanced and based on the same theory, enabling development of the research idea and investigation of different aspects in a more holistic approach. The same experimental design was used in all experiments, making it possible to compare and draw conclusions. However, it would be interesting to investigate the effect of varying environmental change and choice of another resource, such as light, feed or temperature.

Environmental change and choice were manipulated using the same two resources (litter and perches), enabling the effects of environmental change and choice to be distinguished. However, this also resulted in a different level of environmental change, *i.e.* an exchange of the litter and perch type (for Single-choice pens) or relocation of the four types within the pen (for Multi-choice pens). If litter and perch types had been changed in a similar way in the Multi-choice pens as in the Single-choice pens, by exchanging all four types to four new types, it would have resulted in a higher level of change compared with the single exchange in the Single-choice pens. Further, introducing litter and perch type in only one of the treatments could have resulted in a possible bias in the effects of these types.

All pens had the same amount of litter and perch space, making it possible to explore effects of environmental choice and change within these resources. However, since the bird space per litter and perch type was reduced in the Multi-choice pens, it presumably led to a higher risk of competition even if there was no obvious evidence of this. During early rearing all birds could fit in the same litter tray, but this would have been a problem as they grew larger.

The studies were conducted under experimental conditions and only up to 28 weeks. The effects of the treatments should be followed up in a longer-term study in a commercial setting investigating behaviour and health by range use, egg production, feather condition, keel bone damage and mortality.

The same common white layer hybrid was used in Papers II-IV, making it easier to compare the results. In future work it would be interesting to investigate how the treatments influence another hybrid type, *e.g.* a brown hybrid, as it is generally regarded as more "proactive" than the white hybrid (Fraisse & Cockrem 2006; Pusch et al. 2018).

If resources and time had not been limited, several more variables could have been investigated to better understand how the environments influenced the laying hens, *e.g.* in terms of alterations in different brain regions, muscle size and epigenetics. Analysis of several long-term physiological effects examined in Paper IV, such as H/L ratio, nAb ag SRBC, PHA-P swelling response, telomere length and gut microbiota, is ongoing.

6. Conclusions

- Young laying hens used different types of litter and perches for different types of behaviour from the first week after hatch. These resources are suitable to use when manipulating the level of environmental choice or/and change during rearing.

- Increasing the level of environmental change during rearing by changing litter and perch types, reduced initial freezing duration in young hens encountering a novel environment. However, environmental change had less impact on short-term behavioural adaptability than environmental choice.

- Increasing the possibilities for environmental choice during rearing by providing several litter and perch types, promoted hens' movement in a novel environment and increased use of novel resources. The results indicate improved physical skills, greater exploration and better spatial abilities, which all would be advantageous when hens are exposed to environmental transitions and could also indicate a more positive emotional state.

- Increasing the possibilities for environmental choice during rearing improved stress and immunological coping, at least in the short term, resulting in young hens that could better adapt physiologically to potential challenges.

- Reducing the possibilities for environmental choice for young hens in a dependent age (<8 weeks) had negative impacts on behavioural adaptability in the long term, while increases had positive effects.

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Popular science summary

There are around 7.5 billion laying hens in the world and 8.6 million in Sweden. Laying hens are believed to be especially sensitive to disturbances and different pathogens, and are therefore kept in relatively sterile and protected environments already from hatch. Laying hens and broilers are the only farm animals that grow up entirely without parental contact, which can place high demands on the early environment when behaviour patterns and the immune system are forming.

While cage housing is decreasing, loose housing systems are increasing in popularity world-wide and are thought to promote laying hen welfare due to the greater possibility of free movement. However, loose housing systems generally have higher mortality and injury risks, possibly because they are associated with greater challenges for the hens, such as contamination risks, larger group size and a more complex space. Another challenge for laying hens is the move between rearing and laying facility that commonly occurs in practice around 15 weeks after hatch.

To better prepare young laying hens for these later challenges and for the possibilities that loose housing systems offer could be advantageous for laying hen welfare and for commercial producers. It is well-known that there are connections between early experiences and later behaviour and health, but the underlying mechanisms and the specific inputs from the early environment that are most pivotal for later adaptability are not known.

This thesis investigated whether enhanced environmental choice or change in the early environment could promote the abilities of young hens to adapt to a new environment, use new resources and cope with challenges such as potential stressors and pathogens. The research concept was based on well-established theories that controllability and predictability can determine how an individual copes with a potential stressor.

The litter and perch types provided in the rearing environment were manipulated to construct different types of treatments, all with the same amount of litter and number of perches (which are important environmental resources for young laying hens). A standard rearing environment only presents one type of litter and perch type during the first 15 weeks after hatch, which can be interpreted as an environment with limited possibilities for choice and change. This is very different to the forest environment where the wild relatives of domestic fowl evolved. Four different experiments were conducted, with the environmental treatments modified over time. The first experiment showed that young laying hens already from the first week after hatch, prefer different types of litter and perches for different behaviours. The second experiment showed an effect of environmental choice and change on young laying hens' ability to adapt in a novel environment and use new resources. A standard rearing environment (one litter and perch type) was compared with rearing with greater possibilities for environmental choice (four litter and perch types) or higher level of environmental change (change of litter and perch types three times per week) during the early rearing period (first four weeks after hatch). Two behavioural tests in a novel environment in week 5 showed that greater possibilities for environmental choice can promote exploration and spatial abilities, while higher level of environmental change can reduce initial fear response in a novel environment. The two most different treatments were the standard rearing environment and an environment with greater possibilities for environmental choice, so these were explored further in the third and fourth experiments.

The third experiment revealed differences in how young hens coped with challenges such as potential stressors in daily life and potential pathogens. Compared with the standard rearing environment, an early rearing period with increased possibilities for environmental choice promoted stress coping and an immune defence system that was more ready to cope with a potential pathogen. The fourth experiment compared long-term (28 weeks) behavioural effects of these same two treatments regarding the abilities of the hens to exploit opportunities after being moved to a laying hen house and when given access to an outdoor range. It also investigated the treatment effect depending on rearing period. It is common practice in commercial production for young laying hens to be kept in the aviary rows during the first weeks after hatch, to better habituate them to the higher tiers. A long-term effect of an additional environmental input during the first weeks would thus be easy to exploit in practice. The effect of rearing period was investigated by letting half of each environmental treatment change to the other treatment at week 5. Compared with the standard rearing environment, greater possibilities for environmental choice during the first four weeks after hatch led to faster use of novel perches when the hens were moved to a laying house at week 16, irrespective of late rearing environment (week 5 to 15). Hens given greater possibilities for environmental choice during any part of rearing were faster to explore the novel laying pen. Hens with greater possibilities for environmental choice during late rearing were more prone to search, locate and consume a novel feed resource in two behavioural tests conducted between week 9 and 17, irrespective of early rearing environment. The hens that were exposed to a reduction in the possibilities for environmental choice at week 5 showed the lowest usage of a novel outdoor range in week 27 and less flexible nest usage. The hens that were exposed to an increase in the possibilities for environmental choice at week 5 showed the highest usage of the outdoor range in week 27. The results from this thesis indicates that providing an environment with different litter and perch types during rearing could increase the possibilities for young hens to fulfil different behavioural needs and could also improve their short-term and long-term adaptability. Increasing the possibilities for environmental choice could be a biologically relevant way to provide a good environment that goes beyond simply providing basic resources.

Populärvetenskaplig sammanfattning

Det finns ungefär 7,5 miljarder värphöns i världen, varav 8,6 miljoner lever i Sverige. Värphöns anses vara särskilt känsliga för störningar och smittor och hålls därmed i relativt sterila och skyddade miljöer redan från kläckning. Värphöns och slaktkycklingar är det enda lantbruksdjur som växer upp helt utan kontakt från ett föräldradjur, något som kan tänkas ställa ännu högre krav på den tidiga miljön när allt från motorik och beteendemönster till immunförsvar tar sin form. Runtomkring i världen ökar frigående system såsom flervåningssystem, där värphöns kan gå fritt med eller utan utevistelse, medan burhållning minskar. Trots att frigående system tros kunna ge höns en större frihet och ökad välfärd ser man högre dödlighet och skador bland dessa system. Det kan bero på att frigående system kommer med större utmaningar för hönan, t.ex. högre risk för smitta, stora djurgrupper och en mer komplicerad rymd. En ytterligare utmaning för värphöns är förflyttningen mellan uppfödningsstall och värpstall som vanligtvis sker vid 15 veckors ålder när de närmar sig sin första äggläggning. Att bättre förbereda en höna för senare utmaningar men också för de möjligheter som frigående system erbjuder skulle kunna ha stor betydelse för deras välfärd men också för lantbrukarna. Trots att sambanden mellan tidiga erfarenheter och beteende, hälsa och välfärd hos den vuxna värphönan är väl kända är ännu inte de avgörande miljöinslagen kartlagda. Denna avhandling undersöker om ökade möjligheter till ”val” och högre grad av ”förändring” i den tidiga miljön kan främja värphöns förmåga att anpassa sig till en ny miljö, använda nya resurser men även förmåga att hantera yttre utmaningar såsom möjliga stressorer och patogener. Forskningsidén är baserad på redan välgrundade teorier om att kontrollerbarhet och förutsägbarhet av en potentiell stressor kan vara avgörande för hur en individ kan hantera denna. Uppväxtmiljön manipulerades med hjälp av strö och sittpinnar, som är resurser av stor vikt för unga höns (Janczak & Riber, 2015), för att konstruera olika typer av miljöbehandlingar. En vanlig uppväxtmiljö i värphönsindustrin omfattar oftast endast en och samma typ av strö och sittpinne under de första 15 veckorna, vilket kan tolkas som en miljö med begränsade valmöjligheter och låg förändring. Denna omgivning kan därtill tyckas vara väldigt olik den skogsmiljö som deras vilda släktingar växer upp i. Avhandlingens första av totalt fyra studier visade att värphönskycklingar redan från första veckan kan särskilja mellan olika typer av strömaterial och sittpinnar och sågs föredra olika typer för olika beteenden. I andra studien undersöktes påverkan på unga höns förmåga att anpassa sig till en ny miljö och använda nya resurser. En ”vanlig uppväxtmiljö” (en typ av strö och sittpinne) jämfördes med en uppväxtmiljö med högre ”valmöjligheter” (fyra typer av strö och sittpinnar) eller en uppväxtmiljö med ”förändring” (byte av strö- och sittpinnetyp tre gånger per vecka) under den tidiga uppväxten (de första fyra veckorna). Två beteendetester i en främmande miljö som genomfördes under femte veckan visade att högre ”valmöjligheter” i uppväxtmiljön kan främja utforskningsbeteenden i en ny miljö samt spatiala förmågor, medan högre ”förändring” i uppväxtmiljön kan reducera initial rädsla i en ny miljö. De två behandlingar som var mest olika varandra var en ”vanlig uppväxtmiljö” och en miljö med högre ”valmöjligheter” och undersöktes därför vidare i den tredje och fjärde studien. Den tredje studien undersökte hur unga höns i dessa två olika uppväxtmiljöer efter de tre första veckorna, fysiologiskt hanterade yttre utmaningar. Jämfört med den ”vanliga uppväxtmiljön”, kunde uppväxtmiljön med högre ”valmöjligheter” främja hönsens stresshantering och ett immunförvar som var bättre på att hantera främmande patogener såsom virus eller bakterier. Den fjärde och längre studien, undersökte beteendemässiga effekter av samma två uppväxtmiljöer kring vuxna höns förmåga att ta tillvara på möjligheter, såsom vid en flytt till ett värpstall och tillgång till en utevistelse. Härtill undersöktes effekten av dessa två miljöer vid två olika perioder under uppväxten. Praxis vid uppfödning i flervåningssystem är att hålla unga höns inlåsta i våningarna under de första veckorna för att anpassa dem bättre till de högre höjderna. Om man kan se en långsiktig skillnad av ett särskilt miljöinslag under denna period skulle detta kunna vara enkelt att implementera i praktiken. Detta undersöktes genom att låta hälften av grupperna från varje uppväxtmiljö byta till den andra uppväxtmiljön vecka 5. Jämfört med den ”vanliga uppväxtmiljön”, så kunde uppväxtmiljön med högre ”valmöjligheter” under de första fyra veckorna öka hönsens användning av nya sittpinnar efter en flytt till ett värpstall, oavsett senare uppväxtmiljö (mellan vecka 5-15). De höns som upplevt en miljö med högre valmöjlighet under någon del av uppväxten, var dock snabbare att utforska den nya värpstallsmiljön på golvnivå. En miljö med högre ”valmöjligheter” under den senare uppväxtmiljön gjorde att höns var mer benägna att söka, hitta och konsumera en ny födoresurs i två beteendetester kring denna tid, oavsett tidigare uppväxtmiljö. De höns som bytte från en miljö med högre valmöjligheter till en vanlig uppväxtmiljö vecka 5 uppvisade minst användning av en utevistelse vid vecka 27, samt ett mindre flexibelt användande av reden då de var mer sannolika att endast lägga sina ägg i det nedre redena, medan de andra hönsen även lade sina ägg på högre plattformar. De höns som bytte från en vanlig uppväxtmiljö till en miljö med högre valmöjligheter vecka 5 uppvisade mest användning av en utevistelse vid vecka 27. Att erbjuda en uppväxtmiljö med flera typer av strö och sittpinnar skulle kunna öka möjligheten för unga höns att tillgodose deras olika beteendebehov, men det skulle också kunna främja värphöns anpassningsförmåga både på kort och lång sikt. Att öka valmöjligheterna i en uppväxtmiljö kan vara ett biologiskt relevant sätt att erbjuda en rikare miljö som främjar hönsens välfärd.

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Litter and perch type matter already from the start: exploring preferences and perch balance in laying hen chicks

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ABSTRACT Early chick environment, such as provision of litter and perches, can be a predictor of laying hen welfare. Inadequate or nonpreferred litter and perch types could have similar negative effects as those seen when not providing these resources at an early stage, such as increased feather pecking and cannibalism in adult flocks. However, suitable litter and perch types for chicks are not well explored. In the present project, 6 different types of litter (crushed straw pellets, hemp shavings, peat, sand, straw, wood shavings) and 6 different types of perches (narrow or wide forms of rope, flat or round wood) were presented in a controlled way (3 at a time) to chicks in 6 pens. Usage was compared in 93 chicks of Lohmann Selected Leghorn Classic divided across the pens, during their first 3 wk after hatch. Different litter types were seen to be preferred for different behaviors. The majority of dustbathing bouts occurred in sand and peat. Chicks foraged more in wood shavings, hemp shavings, and sand than in peat and pellets ($P < 0.05$). Perch width and shape were found to affect both usage and perch balance, measured as the probability of successful or problematic landings. The wide rope was generally used more during the first week ($P < 0.05$) and was used more for sleeping or resting ($P < 0.05$) than the other wide perch types. Furthermore, birds were more likely to land on the wide rope or on flat perches successfully than they were to have a problematic landing ($P < 0.05$). That birds were more likely to be observed preening on flat perches than on the potentially shaky rope perches could further reflect a sense of security. Our results suggest that presenting several litter types could better fulfill chicks' behavioral needs and that flat perches or a wide rope (4.5-cm diameter) could be appropriate perch types for laying hen chicks and thereby promote early perch use and training.

Key words: laying hen chick, behavioral preference, litter, perch balance, perch design

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INTRODUCTION

An appropriate rearing environment is essential for laying hen welfare (Janczak and Riber, 2015). Welfare scores for young flocks can be directly linked to welfare scores for the adult flock. One example is the connection found between higher levels of severe feather pecking in young birds and an increased risk of feather damage as adults (de Haas et al., 2014a). Several factors in the environment for chicks have been identified as potential predictors for the prevalence of various welfare problems in the adult flock. Some risk factors frequently identified are limited access to litter (Gilani et al., 2013) and perches (Gunnarsson et al., 1999). As per EU directives (1999/74/EC), laying hens must be provided access to litter and perches, whereas there is no equivalent requirement for birds during rearing.

Rearing chicks without continuous litter provision could have long-term negative welfare effects (Johnsen et al., 1998; Aerni et al., 2005). Adult birds that were reared without access to litter during the first 4 wk have been shown to have poorer plumage scores, lower egg production, and higher mortality and feather pecking rates along with higher levels of fear (Johnsen et al., 1998; Aerni et al., 2005). Rearing flocks with a 1-week disruption of litter provision (around 4 wk of age) has been found to result in increased levels of severe feather pecking, higher fear levels, and poorer plumage scores at 10 wk of age (de Haas et al., 2014b). However, letting paper and feed function as a litter substrate, a practice commonly used during the first weeks in multilevel rearing systems, may not be enough to have a positive impact on feather pecking levels (de Haas et al., 2014b). This emphasizes the importance of choosing an adequate type of litter in the rearing environment. Litter

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is considered important for essential laying hen behaviors such as foraging (Blokhuis, 1991) and dustbathing (Larsen et al., 2000; Wichman and Keeling, 2009).

Foraging behavior, such as ground scratching and pecking, starts to develop and increase during the first week after hatch (Vestergaard and Baranyiova, 1996). It has been recommended to stimulate foraging behavior early by providing sufficient litter in the rearing environment because of its potentially suppressive effect on the development of feather pecking (Gilani et al., 2013; Rodenburg et al., 2013). Severe outbreaks of feather pecking have been suggested to be a redirected form of foraging behavior because rearing birds on litter, compared with wire mesh, was found to increase foraging and to decrease feather pecking and pecking at objects during rearing (Blokhuis and van der Haar, 1989). However, research comparing foraging behavior in different litter types in chicks is limited.

Dustbathing is an essential behavior for feather maintenance by removing excessive fat lipids (van Liere and Bokma, 1987; Olsson and Keeling, 2005). The behavior is already evident during a chick's first few days of life (Larsen et al., 2000), and preferences for certain litter types have been identified in adult hens (de Jong et al., 2007) and in young individuals (Sanotra et al., 1995; Vestergaard and Baranyiova, 1996; Shields et al., 2004). Having insufficient dustbathing material could, apart from worsen feather condition (van Liere and Bokma, 1987), induce frustration (Wichman and Keeling, 2009) and increase feather pecking (Larsen et al., 2000). Existing research suggests sand (Sanotra et al., 1995; Shields et al., 2004) and peat (Vestergaard and Baranyiova, 1996; de Jong et al., 2007) to be suitable dustbathing substrates.

Regarding perch use, chicks start using perches when they are 1 wk of age (Heikkilä et al., 2006; Riber et al., 2007). Early perch use has been seen to promote both skeletal development (Yan et al., 2014) and muscle growth (Hester et al., 2013). Chicks without early access to perches may develop impaired cognitive spatial skills (Gunnarsson et al., 2000) and have a higher risk to lay floor eggs and perform cloacal cannibalism as adults (Gunnarsson et al., 1999). It can be hypothesized that the provision of an inadequate perch type could have similar negative effects. There are several different aspects to consider when identifying suitable perch types, such as material, color, shape, width, and height (EFSA, 2005; EFSA 2015). From the animal's perspective, the type of perch could affect balance, thermoregulation, the type of grip, and preference and thereby influence perch use (Pickel et al., 2010, 2011; EFSA, 2005; EFSA 2015). There is limited research exploring which perch material, shape, and width could be appropriate for chicks or pullets (EFSA, 2005; EFSA 2015).

More research has been requested with regard to identifying appropriate facilities for foraging and dustbathing (EFSA, 2005; EFSA 2015) along with adequate perch types (EFSA, 2005; EFSA 2015). Previous preference studies for litter types have used experimental test setups, such as preference tests by pushing weighted doors (de Jong et al., 2007), relocating chicks to a novel test cage (Sanotra et al., 1995; Shields et al., 2004), or allowing brief access (maximum: 35 min) to a box with substrates while otherwise being housed on wire (Vestergaard and Baranyiova, 1996), all of which gives birds access to only one substrate at a time. To our knowledge, no previous study has allowed chicks continuous free access to different litter types in their home pen. Investigating more long-term preferences (i.e., in a more straightforward way, and not potentially affected by litter deprivation, training, or neophobia) could give results that better reflect chicks' behavior in commercial practice. Regarding perches, also to our knowledge, there has been no study that compares chicks' usage of different perch types. Research on perch types conducted in adult hens has been summarized by EFSA (2005, 2015).

The aim of this study was to investigate chicks' preference and usage of litter and perch types when presented with a choice of different types in their home environment. Identifying and using appropriate litter and perch types for chicks in the rearing environment could lead to greater success in the adult environment.

MATERIALS AND METHODS

The study was approved by the Animal Research Ethics Committee in Uppsala (Dnr 5.8.18-11,549/2017).

Birds and Housing

For the present project, 93 female chicks of the hybrid Lohmann Selected Leghorn Classic, from the same commercial hatchery, were transported 383 km on their first day after hatch to the Swedish Livestock Research Center in Uppsala, Sweden. On arrival, chicks were divided across 6 pens in the same stall: 3 pens with 15 chicks and 3 pens with 16 chicks. The pens (1.15 × 1.5 m) contained feed and water ad libitum (Figure 1). The stall had an initial temperature of 30°C. Heating lamps were placed over the middle of the pens during the chicks' first 5 d. Temperature was then successively decreased to 26°C by day 23. The stall had a fixed night schedule of 6 h of dark (4–10 pm) from day 4. Chicks were given 1 h of dark on day 1 and 2 h of dark on day 2–3 to promote feeding. Before and after each dark period, the room had a twilight period of 30 min when light intensity was continuously reduced or increased. All pens were supervised at least 2 times per day.

Experimental Setup

The 6 tested litter and perch types are presented in Figure 2. Litter types were presented in trays (70 × 35 × 3 cm) at locations A–C (Figure 1) (one type on each tray). Trays were kept in place by wooden frames in the pens, to which were also mounted 10-cm vertical plastic barriers to prevent litter types becoming mixed. All perches in all pens had the same height. During the first 5 d, all perches were on the ground, to ensure that

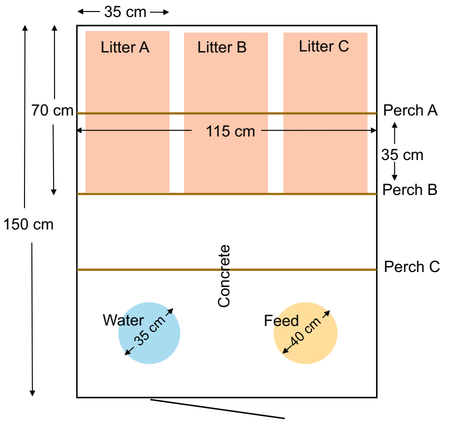

Figure 1. Each pen had 3 different litter types and 3 different perch types at a time, presented at locations A, B, and C. All litter and perch types were presented to all pens and at all locations during the 3-week-long test period.

the chicks came in contact with them. On day 5, we raised the perches to be 15 cm from the ground, which was just enough to allow chicks to pass underneath. When enough birds had started using the perches at a height (at least 3 birds in each pen), all perches were raised a further 5 cm. Perches were raised additionally on day 9, 12, 14, 16, 19, and 21, thereby ending at a height of 45 cm when chicks were 3 wk of age.

Because only 3 litter and 3 perch types could be presented at a time in a pen, the locations of the types were changed within and between pens over the study period so that chicks in all pens came in contact with the different types in a standardized way. This was carried out as per a prepared schedule (Table 1) at midday on Mondays, Wednesdays, and Fridays. By the end of the 3-week period, all litter and perch types had been compared with all others, and each litter type and each perch type had been presented at each of the 3 locations (A, B, and C, Figure 1).

Behavioral Observations

Live behavioral observations were divided into 2 parts: "time budget observations," to determine how chicks were distributing themselves between the different types of each resource and what behavior they were performing in each type, and "perch balance observations," wherein we observed chicks' abilities to jump up onto and maintain their balance on the different perch types (Table 2). An equal number of observations were made in the morning before the change of litter and perch (i.e., when the birds had access to the resource for at least 36 h) and in the afternoon (within 2 h of the resource change). This was to control for eventual behavioral responses connected to novelty.

For "time budget" observations, the location and behavior of each chick was observed by instantaneous scan sampling each pen. See Table 2 for definitions of the different behaviors. We conducted 10 scans per observation day (Monday, Wednesday, and Friday) during 3 wk in each pen during the daytime (5 scans between 9–11 am and 5 scans between 1–3 pm), as well as one observation during the twilight period on these days. At least 15 min passed between each successive scan of a pen to ensure the observations were independent.

For "perch balance" observations, we conducted one 5-min continuous observation per pen on 8 occasions (equally distributed between am and pm) at 5 ages:

Figure 2. (A) The 6 tested litter types: (a) crushed straw pellets (RS MUSTANG, Enköping, SE), (b) peat (RS MUSTANG, Enköping, SE), (c) straw (Smådjurshalm LUPUS, Granngården Uppsala, SE), (d) wood shavings (Kutterspån GRANNGÅRDEN, Uppsala, SE), (e) hemp shavings (Lättströ GRANNGÅRDEN, Uppsala, SE), and (f) fine-grained sand (0–0.03 mm, RÅDASAND, Djur-Hobby Uppsala, SE). (B) The 6 tested perch types: (a) narrow rope (1.8 cm in diameter), (b) wide rope consisting of 3 narrow ropes braided together (4.5 cm in diameter), (c) narrow flat wood (1.5 × 1.5 cm), (d) wide flat wood (6.7 × 0.8 cm), (e) narrow round wood (1.5 cm in diameter), and (f) wide round wood (3.5 cm in diameter).

Table 1. Standardization procedure.

	Pen 1			Pen 2			Pen 3			Pen 4			Pen 5			Pen 6		
Period	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Day 1–5	1	2	3	2	3	4	4	1	5	3	5	6	5	6	2	6	4	1
Day 5–7	4	5	6	5	6	1	6	3	2	2	1	4	1	4	3	3	2	5
Day 7–9	2	4	3	4	5	2	1	6	5	6	3	1	3	2	6	5	1	4
Day 9–12	4	6	1	1	3	5	6	2	4	2	5	3	5	1	2	3	4	6
Day 12–14	3	1	2	5	6	4	2	3	1	1	4	6	4	5	3	6	2	5
Day 14–16	6	5	4	4	1	2	5	4	3	3	2	1	2	6	5	1	3	6
Day 16–19	5	3	1	6	2	3	2	5	6	1	4	5	3	1	4	4	6	2
Day 19–21	1	2	6	3	4	1	5	1	4	4	6	2	6	3	5	2	5	3
Day 21–23	6	4	5	2	1	6	3	6	1	5	2	3	4	5	2	1	3	4

Each of the 6 litter types was randomly paired with one of the 6 perch types, before the start of the experiment, and each pair was assigned a number between 1 and 6. This was to simplify the standardization procedure and decrease risks of errors when changing litter and perch combinations in practice. The table shows which pair of litter and perch type (1–6) was presented at which litter location (A–C) and perch location (A–C, Figure 1) and in which pens (pen 1–6) on which days. Each litter and perch type was presented at all 3 locations (A–C), in different pens at any given time. After the first change (d 5), all pens had come in contact with all litter and perch types.

day 6, 9, 12, 16, and 21 (between 10 am and 3 pm). Observation days were chosen so that the observations were balanced across different perch heights. See Table 2 for definitions of the different behaviors.

A total of 1,764 scans per litter type and per perch type, as well as a total of 720 min of balance observations per perch type, were performed.

Statistical Analysis

All statistical analyses were performed using R software (version 3.3.2; The R Foundation for Statistical Computing, Vienna, Austria; R Development Core Team, 2016).

Preference or usage of different litter and perch types was investigated using the "time budget observation" scans. For dustbathing, the average number of dustbathing observations in each type of litter is presented because this was a relatively rare behavior. For each of the other behaviors, the average proportion of chicks performing that behavior in each of the different litter types, or on each of the different perch types, is presented. Perch balance was investigated by using the proportions of the total number of landing attempts observed for each pen stratified on landing type (problematic or successful) for each perch type.

All proportions were treated as real values, and linear mixed models were fitted using the restricted maximum likelihood approach using Laplace Approximation and R package lme4 (Comprehensive R Archive Network, Vienna, Austria; Bates et al., 2015).

Litter type preferences were investigated using models with litter types as fixed effects and pen as a random effect. Models investigating perch types used perch width and shape (including a possible interaction) as fixed effects and pen as a random effect. The effect of age (as week) was included in the model for litter or perch type preference if an interaction between age and perch or litter type was found to be significant. An effect of age (as week) was only found for general perch use and sleeping/resting on a perch and was therefore only included in these models. There was no effect of time of observation (hour of the day) on preference, so this factor was left out in the statistical models. For perch

Table 2. Ethogram over the behaviors observed in the 2 types of observations; "time budget" and "perch balance."

Behavior	Definition
Time budget	
Sleeping/resting	Sitting or lying down with eyes closed or open
Preening	Chick directs its beak to its own plumage at one of several body parts (thorax, abdomen, shoulder, interior and exterior wings, rump, back and cloaca) and carries out pecking, nibbling, combing or rotating movements once or rapidly. Definition from the study by Pickel et al. (2010).
Foraging	Pecks directed to the floor/substrate while standing or walking and/or the body is bent forward while the bird makes a backward stroke with one leg. Usually 1–4 strokes with one leg are followed by 1–4 strokes with the other. Definition from the study by Blokhuis and van der Haar (1989).
Dustbathing	While lying or squatting, bird performs dustbathing components (bill raking, vertical wing shakes, side lying, rubbing, scratching, ground pecking, feather ruffling). Definition from the study by Newberry et al. (2007).
Moving/standing	The chick is moving or standing and not doing any of the already defined behaviors.
Perch balance	
Successful landing	The landing from the ground on to the perch is stable and without balance-correcting movements.
Problematic landing	The landing involves the body tilting on its axis while tail feathers are spread and rapidly moved up and down, once or repeatedly. The chick's neck may be simultaneously stretched out. Wings are flapped, once or repeatedly, or the chick leaves the perch, without focusing on a landing point, or falls off to the floor. Definition from the study by Pickel et al. (2010).

For "time budget" observations, the behavior of each chick was always noted in combination with its location (litter A–C, perch A–C, concrete, feeder, water).

balance, the mixed model included perch width, perch shape, and landing type (problematic or successful) as fixed effects (including a possible interaction between them) and pen as a random effect.

Significant fixed effects were investigated using type III ANOVA with Satterthwaite's approximation of degrees of freedom and the lmerTest package (Comprehensive R Archive Network, Vienna, Austria; Kuznetsova et al., 2017). Significant main effects or interactions ($P < 0.05$) were further investigated using pairwise comparisons of the least square means with Satterthwaite's approximation. Pairwise comparisons were adjusted for using Tukey's method.

In the case of heteroscedasticity or a non-normal distribution, which was found for the average number of total dustbathing bouts for each litter type, a Friedman test was used to explore the effect of litter type. Post hoc comparison was made using the Friedman-Nemenyi test with error correction using the Friedman Test Data Analysis Tool for Excel (Microsoft Corporation, WA) in the Real Statistics Resource Pack Software (Release 7.2, Copyright 2013-2020, www.real-statistics.com).

RESULTS

In the following section, the general time budgets of the chicks are presented, followed by results on how the different litter and perch types were used. Finally, we present how the different perch types affected a chick's ability to land in a stable manner.

General Time Budget of the Chicks

On average, chicks spent the greatest proportion of their time on the litter (0.5 ± 0.04), followed by being on a perch (0.18 ± 0.04), on the concrete floor (0.17 ± 0.01), at the feeder (0.12 ± 0.01), and at the drinker (0.02 ± 0). Regarding the proportions of observed behaviors, chicks were most frequently foraging (0.43 ± 0.04), followed by sleeping or resting (0.25 ± 0.03), moving or standing (0.22 ± 0.01), preening (0.08 ± 0), and dustbathing (0.01 ± 0.01). Moving/standing was not considered a motivated behavior category, and it is not highly dependent on litter or perch type, so it was not investigated further.

Litter Type Preferences

A main effect of litter type was found in all the investigated behaviors (Figure 3), foraging in litter ($F_{5,30}$ = 9.86, $P < 0.001$), sleeping or resting in litter ($F_{5,30}$ = 7.24, $P < 0.001$), preening in litter ($F_{5,30}$ = 4.59, $P = 0.003$), and the average number of performed dustbathing bouts in litter (χ^2 = 22.70,

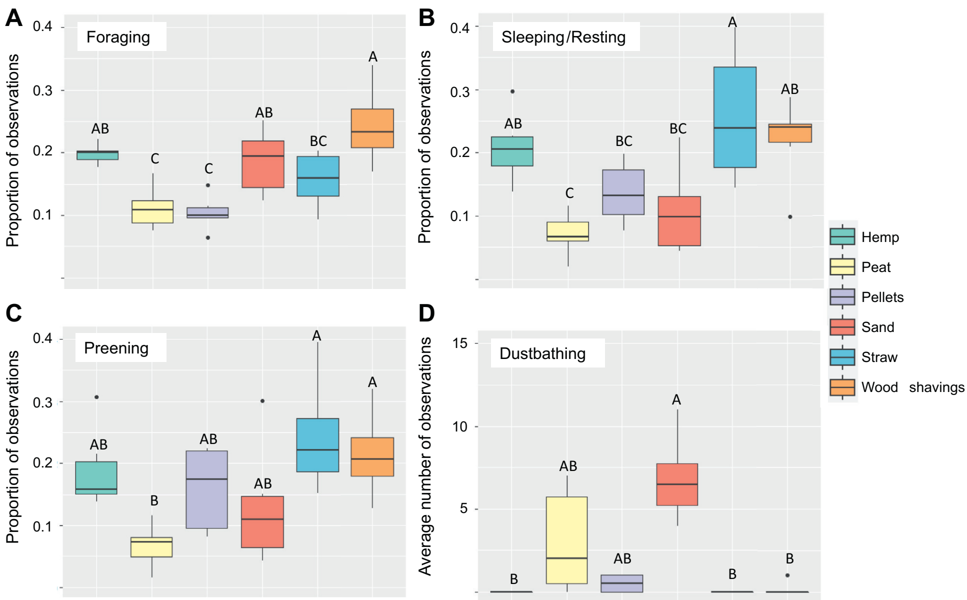

Figure 3. The boxplots show the average proportion of observations when chicks were observed to be (A) foraging, (B) sleeping or resting, or (C) preening in the 6 different types of litter. (D) The average number of observations of dustbathing chicks in the 6 different types of litter. [A,B] Different letters indicate significant ($P \leq 0.05$) differences between litter types. Pairwise comparisons have been corrected for multiple comparisons using Tukey's method.

$P < 0.001$). In pairwise comparisons, there was more foraging in wood shavings than in pellets ($P < 0.001$), peat ($P < 0.001$), and straw ($P = 0.01$; Figure 3A). There were also more observations of chicks foraging in hemp shavings and sand than in pellets ($P = 0.006$, $P = 0.02$) and peat ($P = 0.013$, $P = 0.04$; Figure 3A). Chicks were more likely to be observed sleeping or resting in straw than in peat ($P < 0.001$), sand ($P = 0.006$), and pellets ($P = 0.04$; Figure 3B) and more likely in wood and hemp shavings than in peat ($P = 0.006$, $P = 0.01$; Figure 3B). Furthermore, chicks were more likely to be observed preening in straw and wood shavings than in peat ($P = 0.003$, $P = 0.02$; Figure 3C). Post hoc tests (pairwise signed-rank) show that birds were more likely to be observed dustbathing in sand than in hemp shavings ($P = 0.02$), straw ($P = 0.02$), and wood shavings ($P = 0.04$; Figure 3D), whereas peat showed a tendency of being more preferred than hemp, straw, and wood shavings ($P \leq 0.10$; Figure 3D). Looking at the total number of observations of dustbathing birds, 41 occurred in sand, 26 occurred in peat, 3 occurred in pellets, and 1 occurred in wood shavings. No bird was ever observed to be dustbathing in hemp shavings or straw.

Perch Type Preferences

General perch use showed a significant interaction between perch type and week of age ($F_{4,90} = 6.85$, $P = 0.02$). Pairwise comparisons stratified on weeks showed that the wide rope was used more during the first week than any of the other perch types ($P < 0.05$), whereas no differences between perch types were found in the other weeks (Figure 4A).

The proportion of chicks sleeping or resting on a perch increased after the first week (week 1 = 0.10 ± 0.08, week 2 = 0.67 ± 0.08, week 3 = 0.68 ± 0.07; $F_{2,6.4} = 21.26$, $P = 0.001$), whereas sleeping and resting in litter decreased correspondingly (week 1 = 0.88 ± 0.07, week 2 = 0.33 ± 0.07, week 3 = 0.33 ± 0.07; $F_{2,6.5} = 20.96$, $P = 0.001$). However, there was no interaction between week and perch preference for sleeping or resting on a perch ($P > 0.05$). Instead, a significant interaction between perch width and shape was found for the average proportion of chicks sleeping on a perch ($F_{2,30} = 3.52$, $P = 0.04$; Figure 4B). Pairwise comparisons stratified on perch width showed that chicks slept or rested more on the wide rope than on the wide round ($P = 0.03$) and wide flat perches

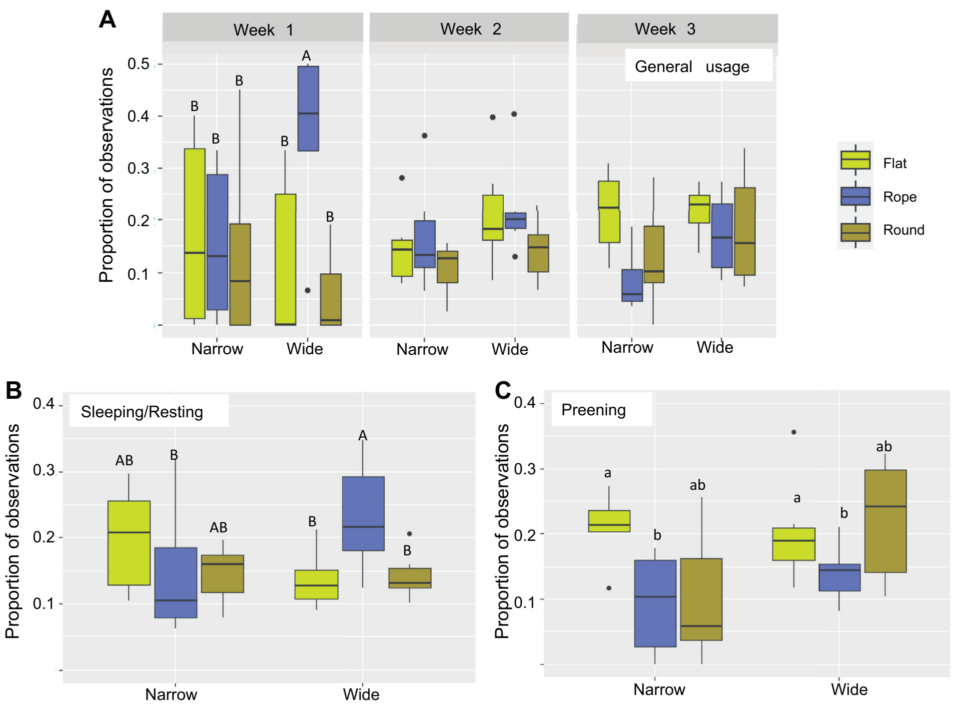

Figure 4. The boxplots show the average proportion of observations on the different types of perches (narrow or wide forms of the flat, rope or round perch shapes) (A) for birds observed on a perch irrespective of their behavior, but divided based on whether they were 1, 2, or 3 wk of age, (B) for birds observed sleeping or resting on a perch, and (C) for birds observed preening on a perch. [A,B] Different capital letters indicate significant ($P \leq 0.05$) differences between perch types (both width and shape). [a,b] Different lower case letters indicate significant ($P \leq 0.05$) general differences between perch shapes. Multiple pairwise comparisons have been controlled for using Tukey's method.

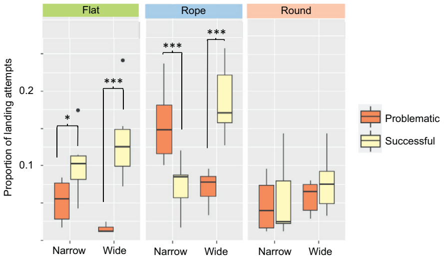

Figure 5. The boxplot shows the average proportion of a total of 472 landing attempts that were problematic or successful, on each of the 6 perch types; 2 widths (narrow and wide) and 3 shapes (flat, rope, and round). Asterisks indicate significant ($P \leq 0.05$) differences between the 2 landing types within a perch type. Pairwise comparisons have been controlled for multiple comparisons using Tukey's method.

(P = 0.02; Figure 4B). Regarding the effect of width within perch shape, chicks slept or rested more on the wide rope than on the narrow rope (P = 0.04; Figure 4B).

Preening observations were found to be affected by perch shape ($F_{2,30}$ = 4.65, P = 0.02; Figure 4C), wherein chicks were more likely to be observed preening on the flat perches than on the rope perches (0.22 ± 0.02 vs. 0.12 ± 0.02; P = 0.005; Figure 4C).

Perch Balance

A total of 472 landing attempts on a perch were observed. Among these attempts, 120 were on the wide rope, 108 were on the narrow rope, 70 were on the wide flat, 68 were on the narrow flat, 65 were on the wide round, and 41 were on the narrow round. Of the total number of attempts, 295 resulted in a stable, "successful landing," whereas in 177 landing attempts, birds showed balance movements or even failed to land on the perch, a "problematic landing."

Regarding average proportion of landing attempts, a significant main effect of shape was found ($F_{2,55}$ = 13.76, $P \leq 0.001$). On average, birds tried to land more often on rope perches than on round ($P \leq 0.001$) and flat perches (P = 0.003; Figure 5). In addition, an interaction between perch type and landing type was found ($F_{2,55}$ = 7.65, $P \leq 0.001$; Figure 5). Further investigations comparing landing type within each perch type showed that a landing attempt was more likely to be successful on the narrow flat (P = 0.05), the wide flat ($P \leq 0.001$), and the wide rope perch ($P \leq 0.001$; Figure 5) than it was to be a problematic landing. On the contrary, a landing attempt on the narrow rope perch was more likely to be problematic than it was to be successful ($P \leq 0.001$). Attempting to land on the 2 round perch types was equally likely to result in a problematic as in a successful landing ($P > 0.05$; Figure 5).

DISCUSSION

Our study has shown that chicks use litter and perch types for different behaviors when given the option to choose. Chicks foraged mainly in wood shavings, hemp, and sand, and they dustbathed almost exclusively in sand and peat. They used the wide rope perch more often in their first week, and over the 3-week observation period, chicks used the wide rope perch most for sleeping or resting, whereas preening was most frequently observed on flat wooden perches. Furthermore, chicks were more likely to land successfully on the wide rope perch and the flat wooden ones. In summary, we conclude that giving chicks a choice of litter, which includes the option of either sand or peat, and a wide flat wooden perch and a wide braided rope perch (or a new perch type with their combined characteristics) would be appropriate to promote early use of these essential resources and thus help reduce some of the welfare problems commonly seen in adult laying hen flocks.

Litter Preference

Chicks were observed to forage in around 50% of our observations, which corresponds to time budgets observed in semiwild relatives to the domestic fowl and red jungle fowl that forage around 60% of their active period (Dawkins, 1989). This suggests that the environment in our study could promote chicks' foraging behavior in similar ways to a natural surrounding. Chicks foraged least in peat and pellets. They foraged most in wood shavings, in hemp shavings, or in fine sand, even if hemp or sand was not more preferred for foraging than straw. These preferences are similar to those of Sanotra et al. (1995) who found that chicks preferred to ground peck in sand, straw, and wood shavings equally. Unlike our findings, Vestergaard and Baranyiova (1996) found lower levels of pecking and scratching in sand compared with peat in 2-week-old chicks. Research on preferences in adult laying hens has found peat to be similarly preferred to wood shavings and sand as a foraging substrate, when exploring usage in a push-door experiment setup (de Jong et al., 2007). It is not possible to say whether these slight differences reflect variation in the type of sand, the age of the birds, or the experimental setup. However, given the correlation between lower frequencies of foraging in the rearing environment and later severe feather pecking (Gilani et al., 2013; Rodenburg et al., 2013), this result suggests a higher likelihood of positive outcomes if presenting wood or hemp shavings, straw, or fine sand in the rearing environment for laying hen chicks because the higher preference could stimulate foraging behavior. However, Newberry et al. (2007) found that young individuals displaying higher levels of foraging behavior within a group were the same individuals showing an increased level of severe feather pecking as adults. This could further illustrate a common background for these behaviors. It could also support the importance of giving birds the opportunity to better fulfill their foraging needs, for example, by presenting litter types of varying characteristics, as birds in the study of Newberry et al. (2007) were only presented with wood shavings.

Our results show that chicks prefer different litter types for dustbathing than for foraging, similar to the results of the study by Sanotra et al., 1995, apart from sand being found to be preferred for both behaviors in our study. Our results indicate a preference for dustbathing in sand and peat and are as per earlier studies in young chicks (Sanotra et al., 1995; Shields et al., 2004). Chicks were only observed to dustbathe on one occasion in wood shavings and never in hemp shavings or straw, leading to the conclusion that these 3 materials are insufficient dustbathing substrates. Other studies have tried to train or promote dustbathing in wood shavings and straw, although sand (peat was not presented as an option) was still the preferred substrate even after the training period (Sanotra et al., 1995). If sand or peat is not provided in the early environment of laying hen chicks, it could, irrespective of later provision of these substrates, result in adults that are dustbathing less often (Johnsen et al., 1998) or performing insufficient dustbathing movements (Olsson et al., 2002), which may impair the hygienic function and thus risk poorer plumage condition (van Liere and Bokma, 1987). Furthermore, low dustbathing activity when young can lead to severe feather pecking activity in adult age (Newberry et al., 2007), which further supports the importance of giving birds the possibility to fulfill behavioral needs also at younger ages.

It has been suggested that litter preference for dustbathing could be affected by substrate particle size and that small particles, such as those found in sand and peat, could be more preferred because of their better ability to get in between the feathers (van Liere and Bokma, 1987; Olsson and Keeling, 2005). The preferred foraging substrates, however, all had different particle sizes, suggesting some other characteristic to be of importance. A common feature could be color as the least preferred foraging substrates were the 2 darkest litter types tested. Chickens can discriminate colors as humans (Olsson et al., 2015), and it could be that particles in the light litter types are more visible to the birds and thereby promote foraging behavior better than the dark litter types.

Straw, a material not seen to be preferred for either foraging or dustbathing in our study or previous studies (Sanotra et al., 1995; Johnsen et al., 1998), was found to be the preferred substrate for resting together with wood and hemp shavings. Chicks were observed to be in straw for 30% of the observations when they were sleeping or resting in litter. Despite the increase in sleeping or resting on perches with age, chicks were observed resting twice as often in litter as on the perches during the first 3 wk and never observed sleeping or resting on the concrete. It is possible that the litter types most preferred for sleeping or resting (i.e., straw, hemp, and wood shavings) could all have better insulating properties, as previously reported for straw (Tuyttens, 2005), than the other substrates. The higher likelihood of observing preening in the same substrates preferred for resting and sleeping may indicate that chicks were relaxed and comfortable in these litter types (Spruijt et al., 1992).

In terms of implementation, certain litter types can have positive or negative effects on ammonia levels and on the concentration and production of dust in an aviary system (Gustafsson and von Wachenfelt, 2004), all of which can have large effects on laying hen health and welfare (David et al., 2015a,b). Peat and straw can keep ammonia levels and total dust concentration low, but can lead to a high production of dust (mg/bird/hour) (Gustafsson and von Wachenfelt, 2004). Wood shavings can keep dust production and concentration low, but can be associated with high ammonia levels. Sand could lead to increased ammonia levels, dust production, and dust concentration and thereby seems unsuitable for production settings in this respect (David et al., 2015a,b).

Perch Preference and Perch Balance

We have shown that perch type, such as width and shape, can affect chicks' perch use, as has been previously shown in adult hens (Pickel et al., 2010; Chen et al., 2014; Scholz et al., 2014, among others).

The EFSA Panel in 2015 recommended, based on previous research on adult hens, that round perches could serve as the perch shape with highest usage and lowest risk of keel bone deformities. In addition, it is common to use round perches in multilevel systems for laying hens (EFSA, 2005; EFSA, 2015). Round perch types were not preferred over any other shape by the birds in our study, and birds were less likely to attempt to land on this perch shape. Instead, the wide rope perch was seen to be preferred, not only as the first perch to be used by young chicks but also for sleeping or resting. This could be explained by the higher likelihood of landing successfully, perhaps leading to greater security and thus further facilitating perch use. The earlier a chick starts using a perch, the more this individual uses perches for nighttime roosting in adult age (Heikkilä et al., 2006). This, in turn, can lead to lower fear levels, reduced aggression, and higher body condition scores in commercial laying hen flocks (Donaldson and O'Connell, 2012), all of which further support the possible positive outcome if presenting wide rope perches in the early rearing environment.

An overall effect of increased balance and use with wider perches, as found by Pickel et al. (2010), was somewhat supported by our results in chicks. The 2 widest perches were the 4.5-cm wide rope and 6.7-cm wide flat perch, both showing high usage and providing good balance (having more successful than problematic landings on them). However, birds showed better balance when landing on the 1.5-cm narrow flat perch (more successful landings than problematic) than when landing on the 3.5-cm wide round perch (as many problematic as successful landings), implying that the round shape negatively affected balance irrespective of width. Pickel et al. (2010) suggest balance movements to be the most sensitive indicator when identifying an appropriate perch type, and our results thereby support the use of flat perches for young chicks. That chicks were stable and thus perhaps felt safe on the flat perches could have explained the increased preening on this perch shape. The slight swinging of the rope perches when the bird was making preening movements could have led to a feeling of instability and thus less preening. The narrow rope perch, being both round and swinging slightly, seemed to result in a high proportion of problematic landings and low perch use in general.

The combination of the braided texture and breadth of the wide rope perch provided both possibilities for grip and a wide surface for the chicks to stand on, suggesting that these could be shape characteristics that promote early perch use. It could also be suggested that the wide rope was most similar to the wooden branches often used as perches under natural conditions. No wounds or damage was found on any bird during our study, but the potential risk of keel bone deformities and foot health for such a perch type is left to be explored in a more long-term study.

Regarding effects of materials, EFSA (2005, 2015) ranked rubber the highest, followed by wood and plastic. Perch shape in our study was dependent on perch material because wood was used for both our flat and round-shaped perch types. However, this was done with the aim of better exploring the effect of shape and width. Wood was chosen because it has been found to be a good material for perches based on usage (Appleby et al., 1992; Pickel et al., 2010; Chen et al., 2014), foot health, plumage condition (Appleby et al., 1992; Valkonen et al., 2005), and slipperiness (Scott and MacAngus, 2004). However, Scott and MacAngus (2004) suggested no difference between materials once perches are covered with manure. Rope may not be an appropriate material for large-scale commercial practice, from a hygiene perspective, but the braided shape that seemed to be preferred by birds in this study could potentially be formed in rubber.

Including the Aspect of Choice in the Rearing Environment

The outcome of comparisons is always dependent, and a study is thereby limited by the number and variability of the types compared. However, the usefulness of results is increased when characteristics that resources have in common, such as particle size, color, shape or width, can be identified. We have shown the possibility and outcome when presenting different litter and perch types in the early rearing environment of laying chicks. That birds were seen to choose between these different types and prefer some over another in general, or for certain behaviors, contributes toward identifying the essential characteristics of the resources. But these results also highlight the potential of giving chicks choices to increase the likelihood of more chicks being able to appropriately perform motivated behaviors, such as ground pecking and perching, something that has been linked to lower levels of problems, such as feather pecking and floor eggs, in laying flocks. Giving choices is also an easy way to present an environment with increased possibilities to experience control, something that has been suggested to be crucial for an individual's well-being (Leotti et al., 2010).

CONCLUSION

The first conclusion is that different behaviors were observed in different types of litter and that wood shavings, hemp shavings, fine sand, and straw are suitable for foraging, whereas fine sand and peat are suitable for dustbathing. Hence, providing more than one type of litter in the rearing environment for laying hen chicks could better fulfill different behavioral needs in commercial settings.

The second conclusion is that both the width and shape of a perch will impact usage and perch balance for young chicks. Flat wooden perches (e.g., 1.5- to 6.7-cm width) or a braided rope shape (e.g., 4.5 cm in diameter) could be adequate perch types for laying hen chicks to promote early perch use, increase general usage, and improve perch balance. All these could be beneficial for bird welfare in commercial aviary systems.

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DISCLOSURES

The authors confirm that there are no conflicts of interest associated with this publication.

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III

1. Introduction

Farm animals are potentially exposed to various challenges and stressors throughout life, such as routine handling, transport, and infections [1–3]. Laying hens are seen as particularly sensitive to these challenges, although there are already many practices in place to protect them, especially during rearing. For example, it is a standard practice to disinfect the building between batches of birds, and many producers knock on the door before going inside a rearing stable to reduce flight responses. However, the scientific literature suggests that coping with some challenges early in life can lead to a more resilient individual. For example, research in rodents suggests that early experience of stress can improve stress coping (i.e., how individuals respond to potential stressors) later in life [4,5]. While minimizing welfare risks is of course important, one can question whether chicks might be over-protected during rearing, leading to sub-optimal development of their ability to cope with the stressors they will most likely experience later in life. In short, can we better prepare chicks by manipulating their early environment?

Experiences early in life are of pivotal importance for the development of a range of abilities. Highly relevant developmental processes occur in the first weeks of life, and this early temporal window is suggested to influence neuroendocrine components, i.e., the hypothalamic–pituitary–adrenal (HPA) axis [6], the immune system, and how these two systems interact [7]. In avian species, hatchlings encounter an entirely new environment, far from the security offered by the in ovo situation. This new environment is full of challenges, ranging from escaping the shell, to exploring this new world and resisting a series of potential pathogens [8]. According to the adaptive development plasticity theory, the environment that individuals encounter in the early stages of their development, and their genetic potential to respond to this environment, will determine their later phenotype and thereby affect their fitness [9]. Within this framework, the particular way each individual responds to challenges can reflect their current coping abilities and, at the same time, provide an indication of their future coping abilities.

The typical rearing environments for modern laying hens are considerably more barren than the complex forest habitat of their ancestors, and it has been suggested that barren environments during early life may incur costs, simply because they lack the stimulation necessary for optimal development [10,11]. There have been several studies showing that rearing environments that allow a greater expression of natural behavior are beneficial for later success in the adult environment [12]. For example, rearing chicks with access to perches during the first weeks of life or rearing them in aviaries compared to cages can have positive and long-term effects on spatial ability and learning [10,13]. Regarding stress coping ability and immune responses, having access to litter and perches during rearing can buffer against different stressors, both in laying hens [14,15] and quail [16]. Such studies indicate that adding resources to a barren environment, thereby making it more complex for young birds in their early life, has both immediate and long-lasting effects on the modulation of their coping phenotypes, as well as on their immunocompetence. Nevertheless, these enriched commercial environments for laying hens are still considerably less complex than the natural environment in which their ancestors evolved.

As illustrated above, most studies investigating the effect of the early environment on poultry welfare, behavior, and physiology focused on a comparison between barren and enriched early environments. It would be, nevertheless, interesting to deepen our understanding of how early environmental complexity can affect birds' development, and specifically their ability to cope with different challenges. Furthermore, as was pointed out by Campbell et al. [12], there is a knowledge gap regarding the link between (and mechanisms behind) complex rearing environments and immunocompetence. Environmental complexity can be increased by offering different resources (which is the usual approach and is mentioned in the studies above), but it can also be increased by offering different forms of the same resource. Given the close connections between choice and controllability [17] and between controllability and coping responses [18], it is possible that it is the experience of making choices that is the underlying reason for the positive effects found from rearing in complex environments.

This study focused on the effects of providing chicks with increased possibilities to express choice as a form of environmental complexity. More specifically, we aimed to elucidate the effects on coping-related traits in laying hen chicks, by offering increased variation within two resources, i.e., perches and litter, known to be important during early rearing (reviewed by Janczak and Riber [19]). By providing litter and perches in both treatments, our single-choice environment corresponded to the complex environments that were used in previous studies [14,20]. Our multi-choice environment then implied a further increase in complexity, by increasing the possibility to express choice within these important resource types. Variables representative of fear, learning, and stress responses, as well as variables for exploring the immune status were selected. We hypothesized that this increased complexity would promote both behavioral and physiological coping abilities in chicks. In practice, this would mean that they are better prepared for challenges, as well as opportunities, in commercial poultry production settings.

2. Material and Methods

2.1. Animals and Husbandry

Day-old laying hen chicks (females) of the white hybrid Bovans Robust were purchased from a Swedish commercial hatchery (Swedfarm AB). After the standard procedure of handling and sorting at the hatchery, chicks were transported 255 km in a temperature-controlled vehicle to the Swedish Livestock Research Centre. On arrival, all 104 birds were weighed and placed in one of eight pens in the same room at an indoor facility. The birds were randomly assigned to each pen, but they were checked so there were no significant differences between pens in the average weight per bird (average weight per bird was 36.2 ± 0.13 g). Each pen was 1.2×2.4 m and housed 13 birds. This low stocking density, compared to commercial conditions, was chosen so that all chicks could potentially use a particular litter box or perch at the same time. The birds were provided with water and feed (commercial standard) ad libitum throughout the entire study, and these were checked and refilled as necessary every morning. The average light intensity in the pens was 20.2 ± 1.67 lux. There was a heat lamp hanging above the middle of the pen during the whole experiment. The average temperature was 43.6 ± 1.65 °C under the heat lamps and 23.6 ± 0.26 °C in the rest of the pen. All birds were marked with numbered leg rings at two weeks of age. At the end of the experiment, when birds were 22-day-old, they were weighed.

2.2. Treatments

Before chicks arrived, pens were assigned to one of two treatments, according to a balanced design. These treatments differed in their level of complexity and this was achieved by varying the types of the key resources: the perches and litter material provided in the pen. The perch types were round rubber, braided rope, flat wood, and flat wire. The litter types were straw, wood shavings, sand, and peat.

In one treatment, all four litter and perch types were offered in each pen (4 pens in total, Figure 1a). This treatment was consequently named the "multi-choice treatment". Test litters were presented in four trays ($71 \times 35 \times 3.5$ cm). To prevent location bias within the pen, litter and perches were placed such that the position of the different types of resources was not repeated between pens. In the other treatment, only one litter type and one perch type were offered in each pen (4 pens in total, Figure 1b). This treatment was subsequently named the "single-choice treatment".

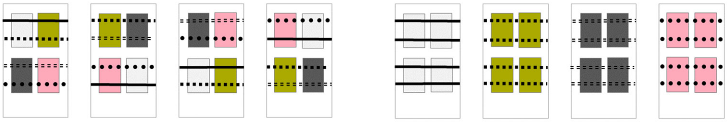


Figure 1. Overview of the treatments: (**a**) multi-choice, with all four types of litter and perches presented in each pen, and (**b**) single-choice, with only one type of litter and perch type per pen. The location of each litter and perch type was balanced across the four multi-choice pens, to control for potential effects of position within the pen on chick usage. Furthermore, each litter and perch type in the multi-choice pens was represented in a single-choice pen. In this way, we could account for any potential effects of specific perch and litter types, since our aim was not to explore the effects of the different litters and perches themselves, but the effects of increased choice per se.

Each of the litter and perch types presented in the multi-choice environments was presented in one single-choice environment. In this way we could account for potential effects of specific perch and litter types, since our aim was not to explore the effects of the different litters and perches themselves, but the effects of increased choice in the offered resources per se. The pairings were as follows: wood shavings–rope, sand–wood, straw–rubber, and peat–wire. Perch height started at 15 cm and was elevated to 45 cm at 14 days of age. Pen walls were covered to minimize visibility between neighboring pens.

2.3. Physiological Measures

Based on previous studies [14,16,21,22], four variables were investigated to assess the status of each individual's immune system: (1) The lymphoproliferative response to phytohaemagglutinin-p (PHA-P), a cellular representative of the immune system reflecting birds' pro-inflammatory potential; (2) Interferon gamma (IFN-ɣ) plasmatic concentration, as a pro-inflammatory mediator; (3) Natural antibodies against sheep red blood cells (SRBC), reflecting general humoral immune capacity; and (4) Heterophil/Lymphocyte (H/L) ratio, a representative of cellular immunity widely used as a hematological indicator of underlying chronic stress responses. The sampling procedure lasted two days and started when chicks were 16 days old. The lymphoproliferative response required an intradermal injection and an in vivo analysis the day after, while the other three variables were analyzed in vitro with blood sampled on one occasion. Blood was sampled 24 h post lymphoproliferative induction. All chicks were sampled on the same day, to account for any potential carry-over effects for the procedure.

For the lymphoproliferative or swelling response to PHA-P, a 0.05 mL solution of PHA-P (*Phaseolus vulgaris* lectin from Sigma Aldrich; Saint Louis, USA) in phosphate buffer saline (PBS), 1 mg/1 mL solution, was injected into the left wing web of each chick, according to previous descriptions [14,22]. After 24 h (±1.5 h), the thickness of the pre-marked injection site was measured and compared with basal thickness, measured just before the injection. The thickness was measured using a digital caliper (Cocraft®) with an accuracy of 0.03 mm. The indicator of swelling was obtained using the following calculation: percentage of swelling = (basal thickness/thickness post 24 h) × 100 [16]. For accuracy and intra-observer reliability, measures were repeated in order to obtain two measures that differed by less than 5%, after which an average of these was used.

For blood sampling, a maximum of 0.75 mL was obtained from the right brachial vein of each chick (opposite wing from the PHA-P response induction). Syringes were prepared with anticoagulant ethylenediaminetetraacetic acid (EDTA). Blood smears were made immediately, using one drop from the syringe according to standard practice, while the remaining blood was placed on ice in a transport box. Blood was then centrifuged at 2000 rpm for 10 min to obtain plasma, which was stored at −20 °C until further analyses. IFN-ɣ was quantified using a validated species-specific ELISA kit (Ray Bio® Chicken IFN-gamma ELISA Kit, ELG-IFNg). The minimum detectable dose was assessed to be 0.06 ng/mL.

Procedures specified by the manufacturer were followed, and the concentrations for all chicks were determined the same day and on the same plate. The intra-assay coefficient of variation (CV) was 5.91%. Natural antibodies (Nab) against SRBC were assessed using a microagglutination assay [23]. Procedures were similar to those conducted for investigating acquired antibody responses for SRBC. An amount of 25 µL complement-inactivated (through a thermal bath at 56 °C) plasma was serially diluted in 25 µL of PBS (1:2, 1:4, 1:8 up to 1:512). Then, 50 µL of a 2% suspension of SRBC in PBS was added to the wells. Microplates were covered with aluminum foil, incubated at 40 °C for 1 h and checked for agglutination every 15 min. Hemagglutination of the test plasma samples was compared to the blanks (PBS only) and negative controls (wells with no SRBC suspension). The same person conducted the analysis of all the samples with an inter-assay CV of 9% and an intra-assay CV of 7%. Antibody titers were reported as the Log_2 of the highest dilution yielding significant agglutination. Blood smears were stained with May Grünwald Giemsa, and differential counts of 100 white cells per smear were made, according to previous practice [14,21]. All counts were made by the same person with an intra-plate CV of 2.1%. The H/L ratio was then calculated by dividing the number of heterophils by the number of lymphocytes. The same person obtained all blood samples, and the same person held chicks for blood withdrawal. Blood collection took less than 90 s for each chick.

2.4. Behavioral Measures

The treatment effects on behavior were evaluated using two behavioral tests when the chicks were three weeks of age: one on an individual level, a tonic immobility (TI) test; and one on a pen level, a repeated opportunity test. This time interval was chosen to give chicks time to recover from the stress associated with the physiological measurements.

The duration of TI response is thought to reflect an individual's level of fearfulness [24] and is frequently used in poultry research. TI was induced by placing a chick on its back and then a hand was lightly held against its chest for 15 s. If the individual moved within three seconds, induction was repeated a maximum of three times. The number of attempts to induce TI, latency to first head movement, latency to first vocalization, and latency to standing up from the tonic position (TI duration) were registered. Individuals who were not induced after three attempts were given a TI duration of one second, while individuals not standing up after five minutes received the maximum score of 300 s. Three different people conducted the test according to standardized procedures, so that all chicks could be tested on the same day within a period of five hours. Treatments were balanced between different test operators. Before the test started, inter- and intra-observer reliability were secured by joint evaluations of test chicks, so that the CV was <10% for all latency measurements.

The repeated opportunity test was constructed to explore the ability of chicks to adapt to routine procedures, i.e., repeated exposure to an initially novel situation, as well as ability to take opportunities, i.e., access to an attractive food source. The test consisted of two phases, with an increased challenge level in the second phase. Each phase consisted of three repetitions, and all were carried out in each pen. In the first phase of the test (repetitions 1–3), the test operator opened the entrance door to the pen and placed an initially novel object (a porcelain bowl) with initially novel feed (ten live mealworms) mixed with an initially novel litter (crushed straw pellets) in the home pen for 90 s. The bowl was placed in the middle of the pen, one arm's length from the entrance, before closing the door. Time and video recording started once the door was closed. In the second phase (repetitions 4–6), the challenge level was increased by the test operator actually entering the pen and sitting down in the corner by the entrance. She presented two feed bowls (the same bowls and content as in the first three repetitions) for 90 s (see Figure 2). One bowl was placed on the ground in front of her, in a similar position to in phase 1, whereas the other bowl was held on her lap. She had her gaze downwards and to the right, avoiding eye contact with the chicks. An assistant closed the pen door while the tester sat down. Time and video recording started once the door was closed. There was at least one hour between each repetition.

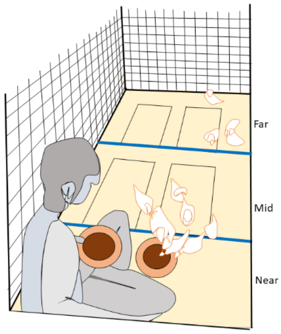


Figure 2. Schematic view of a pen during the repeated opportunity test. Each pen was divided into three areas: "far", "mid", and "near". During the second phase (the last three repetitions), a tester sat down with legs crossed in the pen, placing one feed bowl on the ground and one in her lap, as illustrated.

This test can be considered as a series of challenges that the chicks have repeated opportunities to overcome, in order to access the food reward. Given that the chicks were initially allowed to move to the far end of the pen, the latencies for overcoming consecutive challenges can be placed in the following order: latency to approach the mid and then the near part of the pen (Figure 2), latency to peck in the bowl on the ground (repetitions 1–6), latency to jump up onto the person, and, finally, latency to peck in the bowl on the person's lap (repetitions 4–6). The latency recorded was the time for the first chick in the pen to overcome the challenge. Additionally, the number of chicks in each area of the pen (far, mid, near) every 10 s and the total number of pecks to each bowl were determined. The total number of worms eaten in each bowl was registered by counting the number of worms remaining in the bowl at the end of the test, as an indicator of overall success. The test can therefore reflect coping, as well as exploration and learning. All measures, apart from counting the number of worms, were made by the same observer from the video recordings. Before the analysis, an intra-observer reliability of CV < 10% was secured for all behavior variables.

2.5. Statistical Analysis

Statistical analyses were conducted in R software (version 3.3.2; Development Core Team, 2016). Means, calculated using mixed models, are presented as estimated marginal means, and error values show the standard errors of this estimated mean. Linear mixed models, fit using the restricted maximum likelihood (REML) and lme4-package, were used for variables, showing a normal distribution and homoscedasticity. Significant fixed effects were investigated using Type III ANOVA, with Kenward–Roger approximation of degrees of freedom, and the lmer Test package.

For all mixed models, pen ID was used as a random effect, to account for potential influences between chicks in the same pen and to control for the effect of having different types of litter and perches in each pen (see Figure 1), while treatment ("single-choice" and "multi-choice") was used as a fixed effect. This approach allowed us to move from comparing different types of litter and perches to focusing instead on the statistical effects of different levels of environmental complexity. These models were fit for "swelling response to PHA-P", "weight increase", and "latency to first head movement (TI)". If the random effect was too small, resulting in convergence issues, this type of model was dropped, and a general linear model was fit instead. This was the case for the variables "H/L ratio" and "latency to stand up (TI)". For variables showing non-normality and/or heteroscedasticity, average values per pen were explored for treatment effects using Kruskal–Wallis tests. This was the case for the variables "natural antibody titers", "IFN-ɣ plasmatic concentrations", and "number of attempts (TI)". The results for these variables are presented as means and standard errors.

In the linear mixed models investigating variables from the repeated opportunity test, interactions between "treatment", "phase", and "repetition" were included as fixed effects. Since the aim of this test was to explore treatment differences in the ability to improve with each repetition through learning or adaptation, pairwise comparisons of repetition differences were explored within each phase within treatments, by stratifying repetitions by phase and treatment. This comparison was always made if the effect of "repetition", "phase", or their interaction was marginally significant $p \leq 0.1$ in the ANOVA. The potential risk of false positives was taken into account using the Tukey method. The Tukey method was used to adjust *p*-values and control for multiple comparisons in all linear models. Transformed values are presented as back-transformed, apart from the natural antibody titers.

For integrating information from all the variables with individual chick data, a multivariate approach was used (MVN package). A lineal discriminant analysis was used, with the different treatments as a priori categories. The different rearing environments "multi-choice" vs. "single-choice" were considered as the different classes in this analysis. In this way, within-class distance was minimized and the between-class distance was simultaneously maximized, to achieve the maximum class discrimination. The used variables (standardized before analysis) were "swelling response to PHA-P", "natural antibody titers against SRBC", "H/L ratio", "IFN-ɣ plasmatic concentrations", "latency to stand up", and "number of attempts to induce the TI state" in the TI test. A dispersion graph (biplot) was constructed, to visualize both the experimental individuals and the variables in the same space.

3. Results

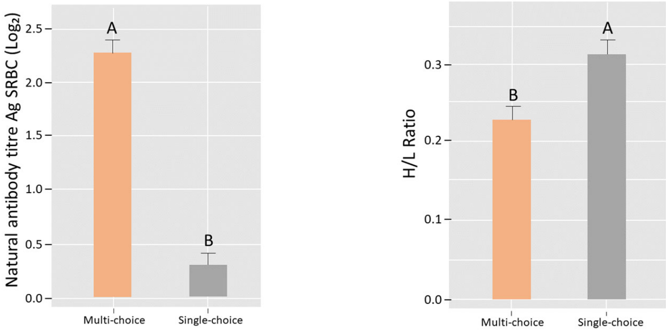
One chick from a "multi-choice" environment was euthanized during the first week of the experiment because of a leg injury. Furthermore, blood withdrawal was not successful for all chicks, resulting in a lower number of individuals used in the immunology-related analyses. Exact numbers for each analysis are given in the legend to the figure. There was no difference between treatments in the overall weight gain of chicks ("multi-choice": 182 ± 3.85 g vs. "single-choice": 184 ± 3.85 g; $F_{1,6} = 0.12$, $p = 0.73$).

3.1. Immunological Treatment Effects

A main effect of treatment was found for natural antibodies against SRBC, where chicks reared in a "multi-choice" environment had higher natural antibody titers than their counterparts reared in "single-choice" environments ($\chi = 5.33$, df = 1, $p = 0.02$; Figure 3a). A treatment effect was also found for H/L ratios, where chicks reared in "multi-choice" environments had lower H/L ratios compared to chicks from "single-choice" environments ($F_{1,74} = 6.92$, $p = 0.01$; Figure 3b). No effect of the treatment was found on the inflammatory response to PHA-P ("multi-choice" = 94.2 ± 3.03; "single-choice" = 95.3 ± 3.03; $F_{1,6} = 0.06$, $p = 0.81$), nor on the IFN-ɣ plasmatic concentration ("multi-choice" = 10.96 ± 1.84; "single-choice" = 11.13 ± 2.18; $\chi^2 = 0.08$, df = 1, $p = 0.77$).

3.2. Tonic Immobility Test

Compared to chicks from "single-choice" environments, chicks from "multi-choice" environments required more attempts to induce the TI state ("multi-choice" = 1.14 ± 0.03; "single-choice" = 1.02 ± 0.02; $\chi^2 = 4.57$, df = 1, $p = 0.03$) and they had a shorter latency to standing up after TI had been induced ("multi-choice" = 68.1 ± 10.6; "single-choice" = 109.5 ± 17.0; $F = _{4.67}$, df = 1, $p = 0.03$). No treatment differences were found regarding latency to first head movement ("multi-choice" = 37.35 ± 9.06; "single-choice" = 37.78 ± 9.16; $F_{1,6} = 0.001$, $p = 0.97$) or first vocalization ("multi-choice" = 39.14 ± 3.86; "single-choice" = 37.39 ± 10.20; $\chi^2 = 0.19$, df = 1, $p = 0.66$).



(**a**) (**b**)

Figure 3. Immunological effects of "multi-choice" and "single-choice" treatments for (**a**) natural antibody titer against sheep red blood cells (SRBC), presented as the Log_2 of the highest dilution yielding significant agglutination (mean and SE; "multi-choice": 39; "single-choice": 40 chicks were analyzed) and (**b**) H/L ratio (estimated marginal mean and SEM; "multi-choice": 36; "single-choice": 39 chicks were analyzed) in blood sampled from 16-day-old domestic fowl layer chicks. Different letters (A,B) indicate significant treatment differences.

3.3. Repeated Opportunity Test

During the first phase of the repeated opportunity test (repetitions 1–3), chicks from both treatments showed significant reductions in their latency to reach the mid ("multi-choice": t = 2.45, df = 30, p = 0.05) or near area of the pen ("multi-choice: t = 2.73, df = 30, p =0.03; "single-choice": t = 3.96, df = 30, p = 0.001) and in their latencies to start pecking in the ground bowl ("multi-choice": t = 4.83, df = 30, $p \leq 0.001$; "single-choice": t = 4.22, df = 30, $p \leq 0.001$; Figure 4). In the second phase (repetitions 4–6), only chicks from the "multi-choice" environment showed improvements in the consecutive challenges shown in Figure 4. Significant reductions in latencies for "multi-choice" chicks in this phase were found for latency to reach the near area (t = 3.96, df = 30, p = 0.001), start pecking in the ground bowl (t = 2.91, df = 30, p = 0.02), jump up on the person (t = 2.76, df = 30, p = 0.04), and to start pecking the top bowl (t = 2.77, df = 30, p = 0.04).

Compared to the first phase, and irrespective of treatment, pens in the second phase of the test (when the experimenter was in the pen), had a lower average proportion of chicks in the near area (0.55 ± 0.04 vs. 0.25 ± 0.04; $F_{1,30}$ = 100.37, $p \leq 0.001$; Figure 5a), a higher average number of pecks (68.4 ± 6.39 vs. 38.2 ± 6.39; $F_{1,30}$ = 30.2, $p \leq 0.001$; Figure 5b) and a lower average proportion of eaten worms (0.58 ± 0.03 vs. 0.82 ± 0.04; $F_{1,27}$ = 29.62, $p \leq 0.001$; Figure 5c). In pairwise comparisons investigating how chicks from each treatment improved with repetition, there was no increase in the proportion of chicks in the area near the novel bowl in the first phase (p > 0.05). However, in the second phase in the "multi-choice" environments, there was a significant increase in the proportion of chicks in the near area between the fourth and sixth repetition (t = −2.47, df = 30, p = 0.049). No equivalent increase was found for chicks from "single-choice" environments (Figure 5a). Chicks from both environments showed an increase in the number of pecks with repetition in both phases ($p \leq 0.05$; Figure 5b) and in the proportion of worms eaten in the first phase ($p \leq 0.05$; Figure 5c). However, in the second phase, there was only a significant increase in the proportion of worms eaten for "multi-choice" environments (t = −2.78, df = 30, p = 0.025; Figure 5c).

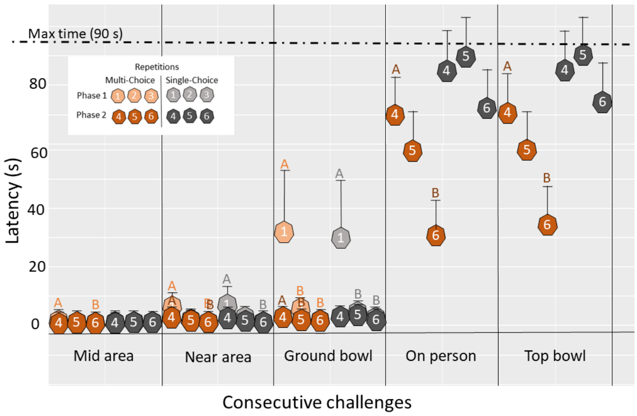
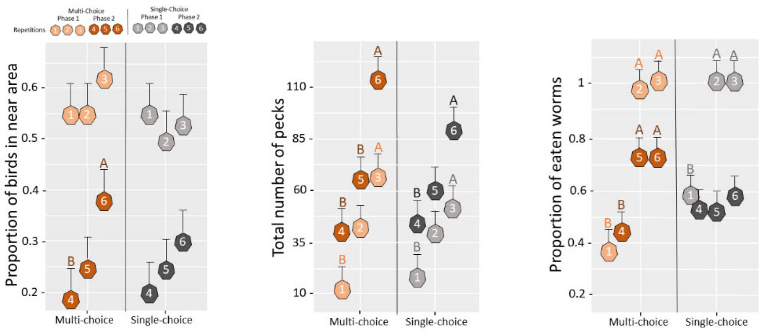


Figure 4. The approach dynamics in the repeated opportunity test. Dot plot (estimated marginal mean and SEM) showing the approach dynamics with each repetition during the repeated opportunity test of chicks from "multi-choice" and "single-choice" treatments. The first phase (repetitions 1–3) involved one food bowl on the ground, while the second phase (repetitions 4–6) involved a person inside the pen, one food bowl on the ground and another in the lap of the person. Latencies to overcome the challenges are shown in seconds, to enter the mid area, to enter the near area, to peck in the ground food bowl, to jump up on the person where the top food bowl was located, and, finally, latency to peck in the top food bowl. Different letters (A,B; note also the different shades within each color) indicate significant differences between repetitions, i.e., improvements with repetition, within each treatment and the different phases of the test.



(**a**) (**b**) (**c**)

Figure 5. The response dynamics in the repeated opportunity test. Dot plot (estimated marginal mean and SEM) showing the response dynamics with each repetition during the repeated opportunity test for chicks from the "multi-choice" and "single-choice" treatments, regarding (**a**) the proportion of chicks located in the near area (the area where the ground food bowl was located for repetitions 1–3 and where both food bowls and the test person were located in repetitions 4–6), (**b**) the total number of pecks to feed bowl(s), and (**c**) the proportion of worms eaten during each repetition. Different letters (A,B; note also the different shades within each color) indicate significant differences between repetitions within treatments and phases.

3.4. Multivariate Treatment Effects

Figure 6 shows a lineal discriminant analysis using those variables measured individually for chicks in this study: inflammatory response against PHA-P, Nab production against SRBC, H/L ratio, IFN-ɣ plasmatic concentrations, weight gain, latency to stand up, and number of attempts to induce the tonic state in a TI test. The two treatments are defined by the distribution of the colored dots in the discriminant space determined by the canonical axes. The figure shows an effective discrimination of the individuals according to their a priori treatment: being reared in "multi-choice" or "single-choice" environments. This discrimination can be clearly observed in canonical axis 1 (94.9% of variability between the groups explained), for which natural antibody titers against SRBC and the heterphil/lymphocyte ratio are the two most important (discriminant coefficients of 0.98 and 0.20, respectively).

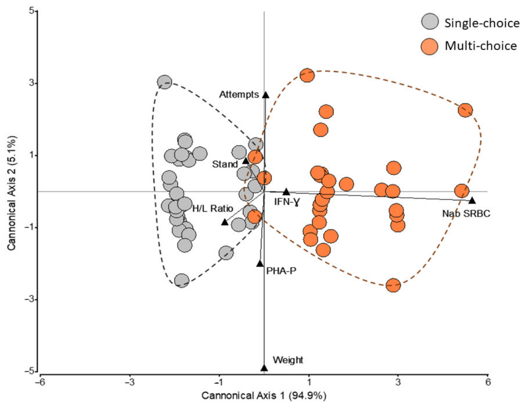


Figure 6. Exploration of the discriminatory capacity of the two treatments. Lineal discriminant analysis, including the following standardized variables (shown in black triangles): inflammatory response against phytohaemagglutinin-p (PHA-P), natural antibody titer against sheep red blood cells (Nab SRBC), heterophil/lymphocyte (H/L) ratio, IFN-ɣ plasmatic concentrations (IFN-ɣ), latency to stand up in a tonic immobility test (Stand), and number of attempts to induce the TI state (Attempts) and weight gain (Weight). Each dot represents a laying hen chick in the study for which the register of all variables was complete. Grey dots represent chicks reared in the "single-choice" environment (37 chicks were analyzed), whereas orange dots represent chicks reared in the "multi-choice" environment (32 chicks were analyzed).

4. Discussion

Chicks reared in an environment where there was a variety of different litters and perch types showed improved immune potential, indicators of diminished fear and stress responses, as well as increased exploratory behavior compared to chicks reared in a similar environment but without variety within these resource types. The results support our hypothesis that the increased complexity, achieved by providing more choice in the environment, altered the phenotype of the chicks, by boosting their coping abilities. In practice, this better preparation for environmental challenges could be a practical way to improve chick welfare. Although cost and bird performance were early key considerations in poultry production, bird health and welfare are now also important considerations [25]. The novelty of this work lies in how we changed the complexity of the environment, which was done by offering chicks the possibility to choose between different perch types and different litter materials, while keeping the total allocation of resources the same. This allowed us to move beyond the effects of providing basic resources. Additionally, we analyzed immediate effects (those found during the first three weeks of life), whereas most previous research in the area of early environmental manipulation in domestic birds falls within one of two categories: (1) prenatal/parental and in ovo manipulations (reviewed by Dixon et al. [8]), or (2) early manipulations with effects analyzed later in life (youth or adulthood) (reviewed by Campbell et al. [12]). Maternal passive immunity protection lasts until about two weeks post hatch [26], so our variables were collected when the chick was learning to rely on its own immune system. We first discuss the results from the immune related variables that were selected as indicators of the chick's ability to resist a potentially pathogenic challenge, using non-pathogenic techniques. We then go on to discuss the behavioral results and how they relate to a chick's ability to learn in new and potentially challenging situations, as well as how success in such a situation may be influenced by fearfulness. Finally, we return to the broader issue of how the early environment can influence the later phenotype and how that knowledge might be advantageous when rearing commercial laying hens.

A difference was found when quantifying natural antibodies, where chicks reared in the multi-choice environments showed higher circulating concentrations. Natural antibodies are present in non-immunized individuals and cover a broad specificity repertoire [27]. They originate from continuous stimulation by exogenous microbes, or correspond to the secretion of naturally occurring auto-reactive B cells, or both [28]. It is likely that the multi-choice environment, especially due to the various litter types, could have had a more diverse microbe community, greater pathogenic load, and a wider pathogenic diversity (as previously proposed for enriched conditions [16,29]), which triggered the higher production of natural antibodies. Natural antibodies are of great importance, because they are key to activating other immunological compartments, such as the complement system and adaptive immune responses [27,30,31]. It was also this variable that had the greatest discrimination power between our treatments. The results suggest that chicks reared in an environment with various litter and perch types had the advantage of a potentially better prepared immune system compared to those with the same allocation of resources but no variation. In the long run, survival would be enhanced, based on studies in hens that proposed a relation between elevated natural antibody concentration and increased probability of surviving the laying period [30]. No treatment effect was found on the in vivo pro-inflammatory potential nor in the IFN-γ concentrations, which implies chicks were equally prepared to deal with a potential pathogen requiring inflammatory milieus for its clearance [21,22]. This information gives a clue, for the first time, about the specificity of the immunological effects of increased environmental complexity. It starts to fill the gap mentioned by Campbell et al. [12], and points towards an enhanced immunological potential related to humoral mediators and the series of responses that are dependent on natural antibodies being activated. This would provide the chicks reared in the multi-choice environments with the advantage of a potentially faster activation of these responses, thus reducing the time and energy allocated to immune coping.

Regarding the behavioral variables, both the tonic immobility and the repeated opportunity test were able to identify specific treatment effects that also supported our hypothesis that offering more choice in the early environment improves the coping abilities of young chicks. Chicks reared in multi-choice environments required more attempts to induce tonic immobility and showed a shorter latency to stand up, suggesting that they were less fearful than chicks from single-choice environments [24]. Previous studies found that increased environmental complexity, by adding enrichments, can result in less fearful chicks [32]. This is advantageous from a welfare perspective, since the production environment usually contains various potential stressors that can lead to fear states. Increased fear has been found to be associated with negative consequences, such as increased feather damage; low body weight, egg weight, and feed intake; and even mortality [33], all of which may indicate impaired adaptability. The repeated opportunity test, specifically constructed and designed for testing our hypothesis, illustrated treatment differences attributable to differences in the ability of chicks to adapt to challenges associated with rewarding opportunities. The lower proportion of chicks in the area close to the feed bowls in the repetitions involving a human supports that the second phase of the test was more challenging (as it was intended to be).

In the first phase, pens from both treatments improved with repetition, shown as reduced latencies to approach and peck at the food bowl and an increased proportion of worms eaten, thereby indicating increased exploration and some level of adaptation. However, in the second and more challenging phase, only chicks reared in multi-choice environments showed an improvement over repetitions in these same variables and so were better able to take the opportunity to access the additional food reward. One possible explanation for the difference in the repeated opportunity test and the greater success of chicks from the multi-choice environments could be that they had experience of approaching and using various forms of resources from day one. We have previously shown, using the same litter and perch types, that chicks prefer certain litter types for certain behaviors and that different perch types affect chicks' ability to land on them [34]. This suggests that the multi-choice environment would have given chicks a more diverse training in the behaviors involved in perch use, such as jumping and balancing, as well as increased and diversified foraging and dustbathing opportunities. It is therefore possible that the multi-choice environments led to an improved exploration and learning ability. Learning ability in farm animals has been shown to affect adaptability to a novel environment [3]. Another possible explanation, now focusing on the lack of success to exploit a new food opportunity in the chicks reared in the single-choice environments, could refer to fear and priming. While repeating a test situation can result in decreased reaction times [35], no such improvements are seen if the repeated stimulus is experienced as too aversive [36]. That chicks from the single-choice environments were more fearful was supported by the previously mentioned results from the tonic immobility test. There is no reason to expect a difference in food motivation between chicks in the different treatments, as food was always freely available in all pens.

One could suggest that the chicks' responses in the repeated opportunity test would be comparable to their response during routine procedures in their home pen, for example when the caretaker entered to check feed and water supplies. That chicks from the single-choice environments had a higher H/L ratio, a physiological indicator of underlying chronic stress responses, implies that the chicks from this treatment were having more difficulties in coping with these everyday situations. This is in keeping with other studies showing that birds from non- or less-enriched environments had increased circulating chronic stress mediators [12]. Laying hen chicks are physiologically ready to process stress at day one [6] and the experience in commercial hatcheries has been shown to be stressful for them [37]. The chicks in our experiment were exposed to the typical husbandry procedures, i.e., incubation, handling, post-hatch feed and water deprivation, and being subsequently transported and placed in the poultry barn. Our results could also be interpreted as suggesting that early and increased environmental choice could help to alleviate the effects associated with these routine but nevertheless challenging events, supporting the results of Campderrich et al. [14].

In the context of the adaptive developmental plasticity theory [9], the increased stimulation available to chicks in an environment offering several variants of litter and perches seems to have had both immediate and potentially long lasting positive effects. All chicks were obtained from the same hatchery and randomly allocated to treatments, thus their prenatal environment could be assumed homogenous [8]. Furthermore, the physiological and behavior tests were carried out on the same days for all the chicks, restricting the interpretation of the results to the effects of the experimental treatments themselves. The influence of each treatment led to a phenotype with particular characteristics, as shown in the discriminant analysis. Chicks from the multi-choice environments also had emergent characteristics as a group, as evidenced by the quicker changes in the behavioral variables (latency to approach and to peck in the feed bowl) and greater success in exploiting novel food sources (proportion of worms eaten) in the repeated opportunity test. The traits that were found to be more pronounced after being reared in the multi-choice environments can define a more "adaptive" phenotype, as they are associated with enhanced coping abilities for a variety of future challenges, while those traits found among chicks from the single-choice environments collectively define a "less adaptive" phenotype. Our results could represent an early expression of the silver-spoon phenomenon [38]. That is to say, "multi-choice chicks" could be considered as having the advantage of growing up in a stimulating environment, with different variants of resources to "pick and choose" between, allowing for optimal development regarding the coping abilities studied. Environmental choice can allow a greater individual fit and, in this way, increase overall welfare [39]. It is also possible that choice on its own is rewarding [17], although it may be necessary to adapt the relative proportions of the different variants of the resources to avoid competition for particularly attractive litter or perch types.

That increased choice is the most likely explanation for the results is strengthened by the fact that, even though the four different pens in the single-choice treatment each contained only one litter and perch type, together they offered the same four litter and perch types as the pens in the multi-choice treatment. If the pens in the single-choice treatment pens had all contained the same litter and perch type, then it would not have been possible to exclude that the beneficial effects of being reared in a multi-choice treatment pen were attributable to some aspect of the novel litter or perch type. Apart from supporting that chicks do seem to choose between variants [34], it is also possible that the different combinations of litter and perch types, even in the single-choice treatment, may have affected the physiological and behavioral development of the chicks. We cannot assess this, since we did not have all possible combinations of the litter and perch types. In future studies, increased replication, especially of the different options within the single-choice treatment, might make it possible to explore how different litter and perch types (alone or in combination) influence the variables measured. It would also be interesting to explore the relative benefits of the number of choice options for a specific resource (in our study the increase was from one to four options).

5. Conclusions

In summary, the results support our hypothesis that increased complexity, achieved by providing young chicks with several variants of resources to choose between in their environment, can potentiate both behavioral and physiological coping abilities. The immunological, stress coping, and behavioral results obtained were indicative of the laying hen chicks being better prepared for immediate, and potentially for future environmental challenges, while at the same time possessing a greater potential to adapt and thus make better use of opportunities.

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Experiences during the rearing period can have a long term effect on animal behaviour and welfare. This thesis investigated the impact of environmental choice and change in the rearing environment on laying hens' adaptability. The results showed that providing the possibility to choose between different litter and perch types during rearing, can be a way to enhance laying hens' later ability to take opportunities as well as ability to cope with challenges.

Lena Skånberg received her doctoral education at the Department of Animal Environment and Health, Swedish University of Agricultural Sciences. Her undergraduate degree was received at Linköping University.

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