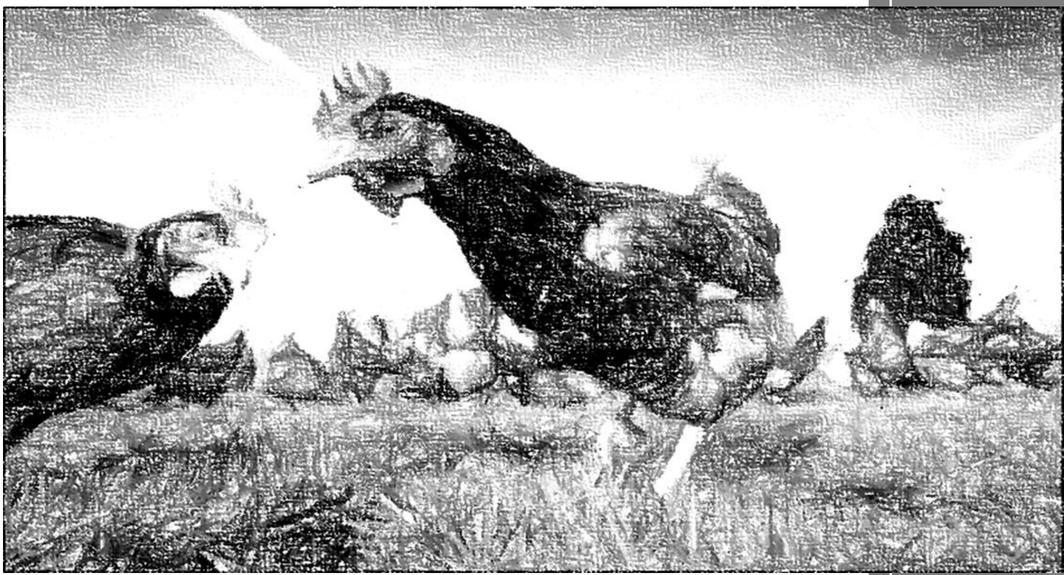
The behaviour and use of space of the domestic fowl in alternative meat and egg production systems



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The behaviour and use of space of the domestic fowl in alternative meat and egg production systems

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Aita eta amarentzat,

beti hor egon zaterelako…

The price to pay for our self-considered omnipotence is a seriously ill society. Religion, State or Power make us forget our intrinsic needs based on our animal nature. We believe that acknowledging our limitations and showing humbleness is a synonym of failure. We are not aware that, in fact, are those limitations the ones that protect us from falling into madness…

A. R. Wilson

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Abstract

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Alternative poultry production is characterized by a large space availability provided to the birds as compared to intensive systems. A better space availability increased the behavioural and movement opportunities and, therefore, alternative poultry production is commonly associated with high animal welfare. However, in practice, it is commonly found that birds do not use the available space in a homogeneous way, and that the use of the outdoor area is lower than expected. In addition, increased freedom of movements and behavioural opportunities may raise the incidence of problematic behaviours such as aggressive interactions. Increasing the complexity of the environment (environmental enrichment) has been proposed to manage uneven spatial distribution and social interactions, but only in few instances implementation has been conducted under commercial conditions. The focus of this work was to investigate the way that egg and meat producing domestic fowl use the available space in alternative commercial production systems and their relation to behaviour and welfare indicators. The first study aimed at determining the use of space patterns in free-range laying hens, analysing their association with welfare outcomes at an individual level. To this aim three flocks located at three free-range farms were studied from 20-69 weeks of age. Behavioural and spatial data were collected over 150 individually tagged hens/farm. In a second study, using video recordings, the behaviour and the inter-individual distances of laying hens prior to an agonistic interaction were analysed to determine the causal

factors triggering the encounter. The third and fourth studies investigated the impact of increasing environmental complexity by providing panels and perches in four commercial free-range slow-growing meat chicken farms. In each of the three houses (with panels, perches or controls), within each farm 50 birds were tagged for individual identification and their location and behaviour registered during a production cycle (82 days). The main findings of these studies indicate that on average 32.58% of the free-range laying hens used the outdoor area regularly, while 49.45% were never observed using it. The hens´ previous experience was identified as a primary factor affecting to the frequency of use of the outdoor area in successive age periods. The frequency of use of the outdoor area and total walked distance inside correlated with welfare indicators such as plumage condition and pododermatitis, respectively. On the other hand, the study on the inter-individual distances and behaviour prior to an aggressive encounter suggest that aggression in laying hens does not depend on the invasion of the critical distance *per se*, but would greatly depend on the activity level and directionality of the individuals which would be perceived as a threat by the aggressor. In free-range slow-growing meat chickens, a higher frequency of locomotive behaviours in the central area within the panel treatment was observed in comparison to controls. The size of the 50 and 100% core areas increased with age but no effect of increasing environmental complexity was detected. The effect of panels and perches in this study had a more noticeable effect on the use of the space patterns inside the house and very limited impact on their behaviour, probably because the number of devices used was limited. In this study, the environmental complexity interventions did not appeared to have a direct impact on welfare indicators assessed.

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General introduction

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1.1 Background

The poultry industry has undergone a massive transformation during the last hundred years. At the onset of the 20th Century poultry was reared in small back yard flocks. It was in the early decades of the 20th century when poultry production started a massive transformation into large scale egg and poultry meat production, becoming an economically important sector in agriculture. Flock sizes and densities increased and poultry production became intensive (Frölich et al., 2012).

In 1964, when the intensification of the poultry industry was fully developed, Ruth Harrison´s book *Animal Machines* shocked the public by describing intensive egg production as a 'factory farming' system. In response to the social alarm generated by the book, the UK Government convened the Brambell Committee to look into the welfare of intensively housed farm animals. In 1965, the committee, chaired by Professor Brambell presented the "Report of the Technical Committee to Enquire into the Welfare of Animals Kept under Intensive Livestock Husbandry Systems" nowadays known as "The Brambell Report". As a direct result of the Brambell Report, Farm Animal Welfare Council (FAWC) was set up. The council established the Five Freedoms (1979) for farm animals with~~The report stated the~~ five basic conditions ~~(the Brambell's Five Freedoms)~~ that should be provided to farm animals for acceptable welfare which included:

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1. *Freedom from hunger and thirst:* by ready access to fresh water and a diet to maintain full health and vigour.
2. *Freedom from discomfort:* by providing an appropriate environment including shelter and a comfortable resting area.

3. *Freedom from pain, injury or disease:* by prevention or rapid diagnosis and treatment.
4. *Freedom to express normal behaviour:* by providing sufficient space, proper facilities and company of the animal's own kind.
5. *Freedom from fear and distress:* by ensuring conditions and treatment which avoid mental suffering.

The ~~Brambell's~~ Five Freedoms is consider the first government document related specifically to the welfare of farm animals and the precursor of the legislative actions that took place in European countries.

1.2 Alternative egg and meat chicken production systems

1.2.1 Egg production

The European Union (EU) Directive 1999/74/EC ´laying down minimum standards for the protection of laying hens´ banned the use of conventional cages in Member States from January 2012. Thus, the Directive limited egg production to furnished cages and alternative systems which includes all other non-cage systems. Most common alternative systems are; single or multiple tier aviary systems and fully slatted floor systems, with or without access to an outdoor area. In addition, the Directive requires that all systems provide nesting and perching space, litter to allow dust bathing, pecking and scratching and unrestricted access to feed. In furnished cages laying hens must have at least 750 cm^2 area/hen, while in alternative systems densities must not surpass 9 bird/m^2, or 6 birds/m^2 for organic production.

Within alternative production the **single tier system** (Fig. 1) is, perhaps, the most similar to the traditional small scale egg production, although bird densities and group sizes can be much larger. In the single tier system, laying hens usually have access to an outdoor area or to a covered area called veranda (or winter garden) and it is usually the chosen system for free-range and organic production. Depending on the genetic strain or on the labelling program, bird density ranges from 6 (organic production) to 9 birds/m^2 of usable area. Flock sizes may also be restricted by specific requirements of the existing labelling programs or by country legislation. In this system aerial perches, usually located over the slats, and a good availability of deep litter allow laying hens to perform a wide variety of behaviours such as perching, dust bathing and foraging behaviours (Frölich et al., 2012).

A variant of this system, the **fully slatted floor system**, is characterized by having the entire surface covered with slatted floor. This characteristic permits a good hygiene and the freedom of movement of single level systems. The option of an adjacent winter garden provides hens with access to a scratching area.

Figure 1. *Single tier system for laying hen used in Eukolabel productions (A. Rodriguez Aurrekoetxea).*

The multiple tier aviary system (Fig. 2) is the most intensive within alternative egg production. There are multiple variants on the design of this type of housing, although in general they consist of multiple tiers (a maximum of three are allowed by the Directive 1999/74/EC) where nest boxes, perches, feeders and drinkers are provided. The floor is covered with litter permitting activities such as explore, scratch, forage or dust bath. In some cases aviaries provide laying hens with access to an outdoor area or to a winter garden (Frölich et al., 2012).

Figure 2. *Aviary system for laying hens (A. Rodriguez Aurrekoetxea).*

1.2.2 Meat chickens

In intensive meat chicken production, broilers (genetic chicken lines selected for fast growth) are reared in deep litter at high densities that within Member States can range between a maximum of 33 to 42 kg/m² (Directive 2007/43/EC). In alternative meat chicken production bird densities are lower and may include changes in the housing and managing conditions that often depend on the demands of specific labelling requirements. In the most extensive, generally slow-growing genetic strains are used, and birds are provided with access to an outdoor area where they have opportunities to scratch, sun bath and forage. According to the Commission Regulation 543/2008/EC, under the 'barn reared' labelling, density must not exceed 25 kg/m² live weight, and birds must be slaughtered with a minimum of 56 days. In 'free-range' production, densities must not exceed 27.5 kg/m² and birds have to have access to a minimum outdoor area of 1 m²/ bird. In 'traditional free-range' densities must not exceed 25 kg/m², birds must be slaughtered with a minimum of 81 days, have daytime access to 2 m²/bird outdoor area, and the birds used in production must be a recognised slow-growing genetic strain. The most extensive system is the 'free-range total freedom' which requires conformity criteria of the 'traditional free-range', but in addition this system requires that birds must have continuous daytime access to an unlimited outdoor area (Commission Regulation 543/2008/EC).

Natural daylight, lighting schedules with an uninterrupted dark period, low input feed and improved litter and air quality, are some of the main aspects that define the alternative production systems for meat chickens (Commission Regulation 543/2008/EC). Contrary to laying hens, current EU legislation does not require meat chickens to be provided with access to perches, in spite of the potential health and welfare benefits demonstrated for broilers (Newberry, 1995; Mench, 1998; Bizeray et al., 2002a, Ventura et al., 2010; Ventura et al., 2012). Currently, the specific characteristics of most alternative meat chicken production systems depend on specific requirements of local or organic labelling such as the *´Label Rouge´* (www.labelrouge.fr) or *´Eusko-Label'* (www.euskolabel.hazi.eus), which usually require the use of slow-growing strains (Fig. 3).

Figure 3. *Alternative slow-growing meat chicken production in the Basque Country (A. Rodriguez Aurrekoetxea).*

1.3 Behaviour and welfare challenges in free-range production

Since the basic requirements for an acceptable animal welfare in farm animals were stated in the Brambell report (1965) many scientists attempted to define animal welfare. Perhaps the most widely used definition is the one provided by Broom (1996), indicating that "*the welfare of an animal is its state as regards its attempts to cope with the environment*". This definition infers that when an animal is confronted with environmental challenges it reacts with adaptive biological mechanisms to maintain an adequate homeostasis (Cannon, 1932). The potential adaptation of an animal to a particular environment is limited by its own physiological capabilities. However, the characteristics of the environment may also facilitate or hinder such response. Non-cage systems, for example, provide laying hens with additional space, increasing their opportunities to move and to express a wider behavioural repertoire, but also with better possibilities to adapt physically and physiologically to the environment (e.g. by moving to a cooler area if ambient temperature is perceived to be high). Nevertheless, alternative production systems are not exempt of problems. Freedom to move in large groups may promote a higher incidence of behavioural problems such as increased aggression, feather pecking, hysteria or uneven use of space, which can be highly problematic.

1.3.1 Social behaviour and use of space in the domestic fowl

The domestic fowl is highly social species that, when in small groups, is organized in a more or less complex hierarchical structure (Schjelderup-Ebbe, 1922). Early work on the social behaviour of the domestic fowl suggested that because birds housed in large loose commercial flocks would be unable to establish a hierarchy, social instability would lead to increased frequency of aggressive interactions, as observed with increasing group size in small experimental groups (Hughes and Wood-Gush, 1977). In addition, it was hypothesised that birds would restrict their movements to smaller house areas in order to avoid aggressive encounters with non-familiar individuals (McBride and Foenander, 1962). Grigor et al., (1995a) explained the result of an experiment on free-range laying hens indicating that birds in the study did not leave the house likely due to fear of encountering unfamiliar individuals. This result was considered to support McBride y Foenander (1962) hypothesis. On the contrary, other studies conducted in laying hens under experimental conditions (Hughes *et al*, 1974; Appleby *et al.*, 1989) and in commercial broiler breeder flocks (Appleby et al., 1985), showed that although some individuals had preferences for particular areas, most used the all the available space.

Many of the available studies on use of space in commercial or semi-commercial conditions analysed mainly the factors affecting to the frequency of use of the outdoor area (Keeling et al., 1988; Hirt et al., 2000; Dawkins et al., 2003; Zeltner and Hirt, 2003; Hegelund et al., 2005, Gebhardt-Henrich et al., 2014; Gilani et al., 2014). However, none of these studies examined in detail the inter-individual differences in the use space patterns or the relation between use of space and welfare indicators. On the other hand, the results of other more detailed studies were conducted in experimental conditions (Hughes *et al*, 1974; Newberry and Hall, 1990; Grigor et al., 1995a,b,c; Newberry, 1999; Cornetto and Estevez, 2001a; Leone et al, 2007), and therefore their results are not easily applicable to commercial farms. Thus, additional studies are still required to determine more precisely the use of space patterns in large commercial flocks of the domestic fowl and in particular for slow-growing meat chickens where the lack of studies is more evident.

Regarding the impact of large group sizes on aggressive interactions, contrary to the expected, aggressive encounters in large flocks of laying hens and meat chickens have been found to be low (Hughes et al., 1997; Nicol et al, 1999; Estevez et al, 1997; Estevez et al, 2003). To explain the reduced frequency of aggressive interactions, Estevez et al., (1997) proposed the social tolerance hypothesis that suggest that it is uneconomical for birds in large groups with unlimited food and water to defend resources from other individuals, when the number of competitors is high, and depletion of resources by others has little cost. Almost in parallel, Pagel and Dawkins (1997) provided a mathematical model explaining that the cost of stablishing a hierachy in large flocks is too high and in such conditions social assessment would be based on badges of status (e.g. body or comb size; Gulh and Ortmann, 1953). However, commercial meat chickens and laying hens flocks tend to be quite homogeneous in age and in phenotipical atributes, and thus, is still not clear what factors may regulate aggressive interactions in large commercial flocks. It is also necessary to consider that alternative production systems offer birds higher mobility opportunities and wider environmental choices that might not be equally available to all birds which may increase the potential risk of enhancing local competition for preferred or limited resources.

An added source of potential problems in meat chickens is the uneven bird distribution, whose consequences could be more relevant in alternative meat poultry due to the lower bird density. Meat chickens tend to seek refuge undeneath the feeders and, especially, around the house walls where they tend to aggregate (Newberry and Hall, 1990; Pamment et al., 1983; Preston and Murphy, 1988), while open areas may be underused. This behaviour in the domestic fowl may result from the interaction between their tendency to stay close to conspecifics (Keeling and Duncan, 1991) and of protecting themselves near the wall (Newberry and Shackleton, 1997). Uneven bird distribution may increase their relative density at specific locations contributing to faster litter quality deterioration. Because meat chickens spend a large proportion of their time resting (Weeks et al., 2000; Cornetto and Estevez, 2001b), the reducction in litter quality increases the risk of foot and breast dermatitis (Cravener et al., 1992; De Jong et al., 2014; McIlroy et al., 1987). Additionally, the limited wall space to accommodate all birds as they grow imply that birds will often walk over others in an attempt to reach a better resting location (Proudfoot and Hulan, 1985; Estevez, 1994). This behaviour may trigger a higher frecuency of disturbances in wall areas, which may translate in higher incidence of wounds and scratches, perhaps leading to a decline in meat quality (Cornetto et al., 2002). These effects are likely to be similar for slow-growing birds normally used in alternative meat chicken production. However, as these birds tend to be more active (Vestergaard and Sanotra, 1999; Kestin et al. 1992), their spatial distribution pattens may differ, as well as their impact on health, welfare and performance.

1.3.2 Use of the outdoor area

Assuring access to an outdoor area is perceived by consumers as a guarantee of good animal welfare as it wider the opportunities to express birds´ normal behaviour such as sun bathing, grass eating or locomotive behaviours (Duncan et al, 1998; Keppler and Folsch, 2000). In free-range egg (Fig. 4 a) and free-range meat production (Fig. 4 b) a minimum of 4 m^2 and 2 m^2 per bird, respectively, must be provided (Commission Regulations; 589/2008, 543/2008). Despite the large space availability, the frequency of use of the outdoor area is generally low, with birds showing a strong tendency to remain inside or within the immediate proximity of the house (Weeks et al. 1994; Keeling et al., 1988; Zeltner and Hirt, 2003). It has been estimated that the use of the outdoor area by laying hens ranges from 8 to 18% (Hegelund et al., 2005; Hegelund et al., 2006; Gilani et al., 2014), with 75% of the birds staying within 20 m from the hen house (Fürmetz et al, 2005). For commercial free-range meat chickens Dawkins et al., (2003) reported a maximum use of 15%.

The apparent higher use of the outdoor area in laying hens might relate to the longer accessibility period, as previous experience it is known to affect the use of the outdoor area in laying hens (Grigor et al., 1995b). In addition to the length of the exposure, factors such as climatic conditions, temperature and light intensity (Hegelund et al, 2002; Richards et al., 2011), group size (Hirt et al., 2000), or the presence of cover (Nicol et al., 2003; Bestman and Wagenaar, 2003) are known to affect to the frequency of use of the outdoor area in laying hens. Similar factors are known to affect to meat chickens´ use of the outdoor area (Dawkins et al., 2003; Nielsen et al., 2003).

a)

b)

Figure 4. *Free-range hens (a) and free range slow-growing chickens (b) in the outdoor area (A. Rodriguez Aurrekoetxea).*

In alternative poultry production, flocks should be managed to maximize the use of the outdoor area as besides the potential welfare benefits to the birds, it is one of the main consumers´ expectations. Additional known benefits of a high use of the outdoor area may include reduced risk of feather pecking, feather damage and cannibalism in laying hens (Green et al., 2000; Bestman and Wagenaar, 2003; Nicol et al., 2003; Mahboub et al., 2004; Lambton et al., 2010). Bone strength is positively associated exercise (Whitehead, 2004), thus, it can be expected that leg strength may also improve with higher use of the outdoor area. In free-range meat chickens because of the limited exposure time to the outdoor area to obtain a good use of the outdoor area may be more of a challenge and the benefits may not be as clear.

1.4 Environmenal enrichment

It is speculated that one of the reasons for low bird activity in commercial meat chickens is that feed supply is freely available at predictable locations, predation risk is minimal, and the simplicity of their environment does not challenge birds to explore, reducing their motivation to move and use the available space (Newberry, 1999). On the other hand, reduced opportunities to explore and forage may lead to behavioural problems such as feather pecking (Dixon et al., 2010) that is relatively common in alternative egg production (Green et al., 2000), but it may also occur occasionally in slow-growing meat chickens (Nielsen et al., 2003).

Increase motivation to explore, forage and exercise can be attained by increasing the complexity of the environment through enrichment, thus minimizing the risk of leg problems due to inactivity or chances of developing feather pecking. Environmental enrichment has been defined as an improvement in the biological functioning of captive animals resulting from modifications to their environment (Newberry, 1995). Environmental enrichment can be attained in many different ways, from simply changing the daily routine or the feeding method, to more complex strategies that provide birds with structures, or new materials to promote a positive behavioural change. However, for the interventions to be effective it is essential that the change in complexity serves to improve the biological functioning of the animals otherwise will have a very limited impact (Newberry, 1995).

Perches are the most widely used forms of increasing the complexity of the environment in poultry. Perches are currently available to laying hens in nearly all housing systems in Europe, but not to meat poultry, even though beneficial effects have been shown for both. For example, access to perches has been shown to reduce the incidence of feather pecking (Huber-Eicher and Audige, 1999) and promote exercise and leg condition in laying hens (Haye and Simons, 1978; Newman and Leeson, 1998; Leyendecker et al., 2005) and in meat chickens (Bizeray et al., 2002a; Ventura et al., 2010). In meat chickens, perches decrease aggression and disturbances (Ventura et al., 2012) and vigorous wing flapping when handled (Newberry and Blair, 1993).

The presence of cover in nature has been shown to provide wild animals with shelter to hide from predators and conspecifics (Elton, 1939), reducing the need for vigilance (Lazarus and Symonds, 1992), and inter-animal communication by minimizing visual contact (Estep and Baker, 1991). The provision of vertical structures that serves as a form of artificial cover has been used effectively in several species of farm animals. Vertical panels have been shown to be effective in reducing the incidence of wounds in pigs (McGlone and Curtis, 1985), feather pecking in turkeys (Sherwin et al., 1999), aggressive behaviour and disturbances in meat chickens and pheasants (Cornetto et al., 2002, Deeming et al., 2011a), while increasing the use of central pen areas in meat chickens (Cornetto and Estevez, 2001a), and resting and preening in laying hens

(Newberry and Shackleton, 1997). Even fertility was improved in broiler breeders and pheasants by the provision of vertical panels (Leone and Estevez, 2008a; Deeming et al. 2011b).

The majority of the studies on environmental complexity in poultry housing with access to an outdoor area have been conducted in laying hens (Zelter and Hirt, 2003; Hegelund et al., 2005; Rault et al., 2013) and some in broilers (Kells et al., 2001), while little is known on their effectiveness in slow-growing meat chickens under commercial conditions (Fig.5). Comparing with broilers, the propensity to perch and activity in slow-growing meat chickens is higher (Lewis et al., 1997; Bookers and Koene, 2003). Therefore, it is expected that the impact of environmental complexity would be more relevant in slow-growing meat chickens.

In laying hens, despite the diversity in the forms of environmental complexity applied, the results evidenced little or no effect in the proportion of birds using the outdoor area, although most found a higher bird presence around the devices (roofed boxes, domed-shaped tents or vertical structures), or a change in the birds spatial distribution (Zelter and Hirt, 2003; Hegelund et al., 2005; Rault et al., 2013). In meat chickens, Dawkins et al. (2003) found that in commercial conditions birds preferred to use the outdoor area when tree cover was available, while Rivera-Ferre et al. (2006), in experimental conditions observed increased exploration of further areas to the chicken house in slow-growing meat chickens provided with huts surrounded by camouflage nets. But trees and bushes grow slowly, require maintenance, and they will attract wild birds that could be predators or disease vectors, while other forms of complex cover may be expensive.

a)

b)

Figure 5. *Slow-growing meat chickens in the treatment with cover panels indoors (a) and perches in the outdoor area (b) (A. Rodriguez Aurrekoetxea).*

Hence, simpler artificial substitutes such as cover panels that in meat chickens had excellent results (Cornetto and Estevez, 2001a; Cornetto and Estevez, 2001b; Cornetto et al., 2002; Leone et al., 2007; Leone and Estevez, 2008a) may be more effective. Usually perches for poultry have been provided indoors, probably because birds tend to perch mostly at night. However, results from studies conducted in experimental settings in meat chickens indicate that perches are also used during the day, and can positively impact on their health and welfare (Bizeray et al., 2002a; Faure and Jones, 1982; Levan et al., 2000; Newberry and Blair, 1993; Ventura et al., 2012). Therefore, both perches and cover panels are natural candidates when considering providing structural devices to increase the environmental complexity for slow-growing meat chickens in the outdoor area.

1.5 Costs and benefits of increasing animal welfare

It has been estimated that the cost of egg production in alternative systems is 45% higher than in conventional cages due to the higher cost of housing, labour, feed intake, hygiene, mortality, predictability of performance, lower stocking density and maintenance of the outdoor area when available (Agra CEAS Consulting Ltd, 2004). However, improvements animal welfare can also be implemented at a very low cost and actually be beneficial from an economic stand point.

In most cases the benefits of environmental interventions may relate to a reduction on the incidence of behavioural or welfare problems (Appleby et al., 1992; Newberry et al, 2001; Bizeray et al., 2002a) that are difficult to quantify in terms of economic impact. One of the few evidences proving realistic estimates the economic impact of low cost environmental interventions was reported by Leone and Estevez (2008a). By providing cover panels to broiler breeder flocks reproductive performance was increased by 4.5 additional chicks produced per hen. It was estimated that if all breeder houses of the company were outfitted with panels the yearly benefits for the company would increase in $3.3 million.

Nevertheless, animal welfare benefits are not always associated with increasing environmental complexity. One of the freedoms included in the Brambell report (1965) is the freedom from fear and distress. The effects of inappropriate fear responses in poultry may result in injuries, death, increased feed consumption, behavioural inhibition, reduced ability to exploit resources, reduced egg production, increased eggshell abnormalities, decreased growth, and delayed sexual maturation (Jones, 2002). It was estimated that the elicitation of fear could cost the UK broiler industry an additional £5 million on the feed bill each year and twice that amount in reduced egg production to the egg industry (Jones, 1996). Other studies reported that fear of humans accounted for 20% of the variation in egg production (Barnett et al., 1992) and for 28% of the variation in feed conversion efficiency in broiler chickens (Jones et al., 1993). These are only some illustrative examples of the potential economic impact of animal welfare. Therefore assuring animal welfare is essential for optimizing bird performance and could take into account the needs of all stakeholders involved. If proactive approaches towards animal welfare were adopted by industry and society, it should be possible to reach a 'win-win' situation, where the industry, the society and the animals will benefit.

1.6 Aim and thesis outline

The overall aim of this work entitle *'The behaviour and use of space of the domestic fowl in alternative meat and egg production systems'* was to obtain a deep understanding on the factors determining use of space patterns and behaviour of the domestic fowl housed in alternative systems for egg and meat production. Specifically, a study was designed to test the potential management and performance benefits that could derive from implementing environmental complexity interventions in slow-growing meat chickens. The results of the studies comprising this work were intended to be used as a basis for developing effective management strategies based on the knowledge of behavioural and welfare needs of the birds.

The specific aims of chapters II-V were:

- To determine the factors that influence use of space of laying hens in free-range commercial conditions, the characteristics of their movements and its relation with welfare indicators (Chapter II).
- To determine the role of the critical distance in laying hens as a primary factor triggering aggressive encounters while exploring the role of the behaviour (Chapter III).
- To determine the potential benefits associated with increased environmental complexity, on use of the indoor and outdoor areas in commercial slow-growing free-range meat chickens, and relate these to welfare indicators and final product quality (Chapter IV).

- To determine the potential benefits of increased environmental complexity both indoors and in the outdoor areas on the behavioural activity of slow-growing free-range meat chickens under commercial conditions (Chapter V).

Chapter 2:

Use of space and its impact on welfare indicators in commercial free-range laying hens

Rodriguez-Aurrekoetxea. A., Estevez, I., 2016. Use of space and its impact on the welfare of laying hens in a commercial free-range system.

In revision, Poult. Sci.

Abstract

The aim of this study was to explore the factors influencing use of space patterns of commercial free-range laying and their relation with welfare indicators. Three free-range laying hen flocks were studied during a production cycle by collecting spatial locations of 150 individually tagged hens/flock. At the end of production welfare and morphometric measures were collected. The results indicate that use of the outdoor area was lower during midday ($P < 0.05$), but remained stable across age periods ($P > 0.05$, mean use 32.60 ± 15.3%). Tagged hens were classified according to their use of the outdoor area (high, medium, low or never) per age period, and showed that 49.5% were never observed using the outdoor area, percentage that was superior to all other categories ($P < 0.05$). In addition, early experience determined the level of use of the outdoor area at later age periods ($P < 0.05$). Most use of space parameters considered did not varied according to age period ($P > 0.05$), only activity center indoors increased ($P < 0.05$), while mean distance from the hen house that tended to increase ($P = 0.053$). However, birds with higher frequency of use of the outdoor area had larger home ranges and activity centers ($r = 0.956$, $P < 0.0001$; $r = 0.964$ $P < 0.0001$, respectively) and showed lower plumage damage ($r = -0.337$, $P < 0.001$). Birds with higher mean distance to the hen house appeared to have a lower incidence of footpad dermatitis ($r = -0.307$, $P < 0.001$). On the contrary, birds showing higher total walked distance indoors showed a higher incidence of footpad dermatitis ($r = 0.329$, $P < 0.01$). These results suggest that early experience was one of the most relevant factors affecting to the use of outdoor area and that those visiting the outdoor area more frequently also used larger areas. In addition, individual spatial patterns had some relevance on the incidence on foot pad dermatitis and plumage condition.

Key words: Laying hens, Free-range, Use of space, Welfare indicators.

Chapter 2: *Use of space in free-range laying hens*

2.1 Introduction

Access to an outdoor area is essential for the welfare of laying hens as it increases their possibilities to express a wider range of normal behaviour patterns (Duncan et al., 1998). Besides this intrinsic benefit, high use of the outdoor area has been associated with additional welfare benefits such as better plumage (Mahboub et al., 2004), reduced keel bone fractures (Richards et al., 2012) or lower risk of feather pecking (Green et al., 2000; Lambton et al, 2010). Even though access to an outdoor area may increase predation (Moberly et al., 2004) and parasitic risk (Permin et al, 1999), it is perceived by consumers as an important factor for the welfare of laying hens (Bennett and Blaney, 2003; Heng et al., 2012), which may ultimately determine their purchasing decisions.

Given the welfare benefits associated with the use of the outdoor area, it would be expected that laying hens would heavily use the outdoor area. However, research results indicate that it use is relatively low, (8 to 18% of the flock; Hegelund et al., 2005, 2006; Gilani et al., 2014), with most birds remaining in the close proximity of the hen house (Fürmetz et al., 2005). Multiple environmental and social factors such as climatic conditions, flock size and age, among others, have been shown to influence the frequency of use of the outdoor area in free-range laying hens. For example, temperatures close to 18ºC, lack of wind, and medium or high atmospheric humidity are known to favour the use of the outdoor area (Hegelund et al., 2005), while rain and wind have negative effects (Richards et al., 2011). The characteristics of the outdoor area, specially the presence of natural or artificial cover, are also important to promote a high and consistent use of the outdoor area (Hegelund et al., 2002; Bestman and Wagenaar, 2003; Nicol et al., 2003; Hegelund et al., 2005; Zeltner and Hirt, 2008; Nagle and Glatz, 2012; Rault et al., 2013). On the other hand, the impact flock size and age appear to be less clear. Thus, while Hegelund et al. (2005) reported decreased use of the outdoor area with age in commercial flocks, Gilani et al., (2014) found the opposite. Use of the outdoor area was found to diminish with increasing flock size in experimental (Hirt et al., 2000) and commercial conditions (Hegelund et al., 2005; Gilani et al., 2014), although Gebhardt-Henrich et al., (2014) found no association. The discrepancy in results may obey to the wide flock size range of the above mentioned studies (from as low as 50 birds up to 6000) that may also generate different flock dynamics with increasing age. Additionally, factors such as pop-hole availability (Gilani et al., 2014) or hen genotype (Mahbouh et al., 2004), which might relate to differences in fear reactions across breeds (Hocking et al., 2004), may influence the use of the outdoor area and, therefore, to separate the effects of all the interplaying factors is difficult.

Most studies on the use of the outdoor area in free-range laying hens are based on the calculation of the proportion of birds from the total flock size (Bubier and Bradshaw, 1998; Hirt et al., 2000; Hegelund et al., 2005; Gilani et al., 2014). Nonetheless, birds within a flock may differ greatly in their use of space patterns. Determining the potential range of inter-individual variation is important from a management stand point. The sparse literature based on individual data collection has shown that 8% of the flock never used the outdoor area, and that different hen subpopulations within a flock used it at different frequencies (Richards et al., 2011; Gebhardt-Henrich et al., 2014). In addition, Gebhardt-Henrich et al., (2014), reported a positive correlation between the time spent outside daily, and the percentage of days the bird was observed using the outdoor area. Besides these results, little additional information is available regarding the characteristics of use of space patterns, the size of the areas used or on how inter-individual variation use of space patterns may impact on their welfare.

Either because access to the outdoor area might not be available at all times, or because birds within a flock chose to stay indoors, they may be exposed to different environmental conditions for long time periods. In addition to the presence of resources indoors (such as perches, nests, litter, feeders and drinkers), differences between the indoor and outdoor areas include differences in relative bird density, space availability and number of birds. Particularly, enclosure size and density are two factors known to have a relevant effect on movement and use of space patterns (Leone and Estevez, 2008b), time spent walking (Hall, 2001) or number of strides per walking bout (Febrer et al., 2006) in broilers. In the case of laying hens, it is poorly understood how birds use the available space indoors, although, similar to the outdoor area, large inter-individual differences in home ranges (Daigle et al., 2014) and distance moved per day (Keppler and Fölsch, 2000) have been reported.

Just as inter-individual differences in the use of the outdoor area could impact welfare status, use of space patterns indoors may relate to some welfare aspects. It is accepted that skeletal quality in laying hens is positively affected by activity (Rowland and Harms, 1972), and in principle there should not be differences as to where the activity is performed. While it remains necessary to expand in the study of the factors affecting ranging behaviour in free-range laying hens under commercial conditions to optimize flock management, it is also essential to understand the characteristics of their use of space indoors.

The aim of this study was to determine the main factors that influence use of space of free-range laying hens in commercial conditions, the characteristics of their movements and their relation with welfare indicators.

2.2 Material and Methods

2.2.1 Farms and animals

The study was conducted in three commercial free-range laying hens farms located in the Basque Country (Spain), from July, 2011 to January, 2013. All farms were single tier 664 m^2 hen houses with a similar design and construction characteristics. A total of 18m of pop-holes divided in at least 16 hatches (Fig. 6) provided access to the 24.000 m^2 outdoor area (minimum of 4 m^2 / hen) that was limited by a wire fence surrounding the area. Hen houses were equipped with an identical number of self-closing nests, automatic pan feeders, nipple drinkers and 3 cm diameter metal perches (15cm/hen) that were placed over the slatted area in the centre of the hen house. Natural ventilation and natural light, supplemented with artificial light to achieve 16L: 8D was used in all farms. Feed (containing a minimum of 60% cereals) was provided *ad libitum* indoors only. Management procedures were identical for all farms, as indicated by the Eusko–Label quality program (www.euskaber.net).

A total of 6,000 16 wk old Isa Brown females were placed in each hen house at a density of 9 hens/m^2, where they were maintained until 69 wks of age (regulated by the Eusko-Label quality labelling). The day of arrival to each farm 150 birds were randomly captured at different locations for tagging. Two 6 cm diameter cream colour, laminated labels coded with numbers (1 to 150) were placed in each wing following the procedure used in previous studies in chickens (Cornetto and Estevez, 2001b; Rodriguez-Aurrekoetxea et al., 2014) and laying hens (Liste et al., 2015). The birds were maintained indoors during 4 wks to accustom them to the nest boxes, perches and facilities in general. After this period the hens had free access to the outdoor area for a minimum of 8 h per day. Prior to the arrival of the birds, numbered sticks were placed as reference points in each hen house and outdoor areas to facilitate mapping the birds´ locations during data collection.

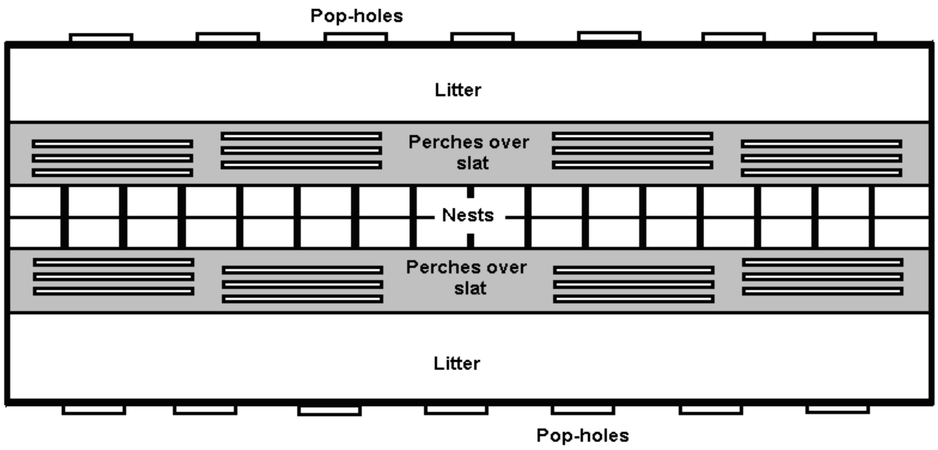


Figure 6. *Schematic drawing of the interior of the hen house.*

2.2.2 Observations

The observations started when the outdoor area was accessible to the hens, and took place one day/week, every other week, from 20 to 69 wks of age. All data were collected by the same person for the entire study. During each sampling day, three observations were performed, alternatively, indoors and in the outdoor area between 10:00 and 19:00. The observations consisted of locating the possitions of as many tagged birds as possible by slowly walking through predefined straight paths that covered the entire indoor or outdoor areas. Two paths, along the slatted and litter areas were conducted alternatively for the indoor observations. In the outdoor area, five paths were completed per observation. The starting point and direction of each path performed was choosen randomly. A 5 minute habituation period was allowed prior to start the observations in order to accustom the birds to the presence of the observer (Marchewka et al., 2013; Rodriguez-Aurrekoetxea et al., 2014). In the case that birds were observed to altered, the observer stayed immobile until the birds returned to their normal behaviour.

During the observations the location of all identified tagged birds positioned at a minimum of two meters in front, or at both sides of the observer were collected while walking along each predefined path. Closer bird locations were not considered in order to minimize the probability of noting birds that were potentially affected by the presence of the observer. Bird locations (registered as XY coordinates) were collected with the Chickitaizer software (*modified from* Sanchez and Estevez, 1998), installed in a portable computer held by the observer. To aid in precisely locating the birds a scaled map of the interior of the hen house and the outdoor area (depending on the farm observed) was superimposed to the computer screen. In addition to the bird location, independent variables such as day, time, temperature and general climatic conditions (sunny, cloudy and rainy) were recorded at the onset of each observation. Ambient temperature was measured using a digital thermometer at the beginning of each observation indoors and in the outdoor area.

Tagged hens were weighed at arrival. At the end of production (69 wks of age) all recaptured tagged hens were weighed again and scored for foot pad dermatitis (FPD), bumble foot, breast blisters, keel bone deformations, comb peck wounds and plumage condition using the Welfare Quality ® scale (Welfare Quality ®, 2009). Daily growth rate was calculated by subtracting the entry to the final body weight divided by the number of days elapsed between both measures. Plumage condition was score on the head, neck, belly, rump and back, with values varying from 0 to 3, with 0 indicating a perfect plumage (*modified from Welfare Quality ®, 2009 and Tauson et al., 1984*). In order to have an overall score, the values for all areas per hen were summed. Tarsal length and width and wing length of recaptured birds were measured twice with a digital caliper (Mitutoyo SC-6, Japan). The average value was used to calculate the relative fluctuating asymetry (RFA), defined as the absolute difference between the right and left leg, or wings, divided by the mean of the left and right measures (Møller et al., 1995).

2.2.3 Use of space calculations

Collected data were used to calculate the proportion of tagged birds (from the total) in the outdoor area per observation. From the outdoor XY locations, the mean, maximun and minimum distance to the hen house for each hen per day were calculated by the pythagorean theorem (euclidean distance ($d(x, y) = \sqrt{((y2 - y1)^2 + (x2 - x1)^2)}$) using the centre of the hen house as the reference point.

From the indoor XY locations, the mean, maximun and minimum travelled distances were calculated as the euclidean distance between two sequential locations. The maximun distance was defined as the furthest distance travelled between two sequential locations for each tagged bird within an observation day, while the minimun distance was the smallest distance between two consecutive locations (Leone and Estevez, 2008b; Rodriguez-Aurrekoetxea et al., 2014). These calculations were based on a minimum of three observations of the same bird within the same day. Unfortunatly, not enough birds were located in the outdoor area to perform the same calculations and statistical analysis.

XY birds´ coordinates were also used to calculate the area of the activity centres (50% core area) and home ranges (90% core area) for each tagged bird within the hen house and in the outdoor area. The activity centres depict the areas of the highest activity, with a 50% probability of finding the bird in the calculate area (Leone et al., 2007). The home range is defined as the ´area traversed by an individual in its normal activities´. Occasional incursions outside the area, perhaps exploratory in nature, should not be considered part of the home range (Burt, 1943), therefore the estimation of the home range was obtained by the calculation of the core area at 90% per birs, which exclude potential incursion outside their normal home range (Estevez et al., 1997; Estevez and Christman, 2006; Leone et al., 2007; Mallapur et al., 2009; Rodriguez-Aurrekoetxea et al., 2014). Activity centres and home ranges represent long term use of space patterns and therefore, they were calculated per age periods. Core areas at 50 and 90% were calculated individually for each tagged bird using nonparamentric Kernel density estimation, which determines the probability of observing a subject at each point in space without making assumptions regarding the distribution of the observation locations (Worton, 1987). Core areas were calculated using the ‘adehabitat’ package for R 2.14., (2008). In addition, the coefficient of variation (CV) of the core areas at 50 and 90% were calculated to estimate the inter-individual variability in space use.

2.2.4. Statistical analysis

To perform the statistical analysis data corresponding to the 49 wks of observations were lumped into three age (AP) and time periods (TP) as follows; AP1, (20 to 36 wks of age), AP2 (37 to 53 wks) and AP3 (54 to 69 wks); and TP1 (10:00 to 13:00), TP2 (13:00 to 16:00) and TP3 (16:00 to 19:00), respectively. Only data corresponding to birds that remained with at least one of the two tags for the entire study period (226 out of 450) were considered for the use of space data analysis. Means per flock for the indoor and outdoor areas were used in all statistical analyses.

In order to determine the effect of early bird experience on use of the outdoor area later on, all individuals holding the identification tags until the end of the study were divided in four categories according to their frequency of use of the outdoor area within each defined age period (AP1 to AP3). The category 'Never' corresponded to birds that were never observed using the outdoor area within each observation period; 'Light' category included birds found in the outdoor area between 1 to 33% of the observations; 'Medium' and 'Heavy' categories included individuals observed in the outdoor area between 34-66% and 67-100%, respectively.

Statistical analyses on the use of the outdoor area and distances were performed by generalized linear mixed model procedures (GLMMs) in SAS V9.3 (SAS Institute, Cary, NC, USA). The models were adjusted to the corresponding type of data distribution (binomial, normal), time period within age period was included as repeated measure and farm as the random factor. Due to the lack of degrees of freedom to consider all independent variables in a unique model a separate analysis was performed to determine the effect of climatic conditions and temperature over the use of the outdoor area. In this analisis temperature was included as a covariate, age period as a repeated measure and farm as random. GLMMs were also used to determine the impact of early experience (AP1) on the use of the outdoor area over subsequent age periods and for the analisys of activity centres and home ranges. In these analyses age period was included as the repeated measure and farm as random factor. The percentage of birds using the outdoor area followed a binomial distribution, while all other parameters were normally distributed. Post-hoc mean differences for all models were analysed with a Kenward-Roger adjustment for the degrees of freedom (Littell et al., 2006).

In order to determine the impact of individual use of space patterns on welfare indicators, the relationships between fluctuating assymetry, body weight, plumage condition, food pad dermatitis, keel bone deformations, comb peck wounds, growth rate with their corresponding means of use of space paremeters were analized using Spearman rank correlations in SAS V9.3 (SAS Institute, Cary, NC, USA). This analisis was perform over birds that were recaptured at the end of the production period. The prevalence of bumble foot, and breast blisters was too low for any possible statistical analysis.

2.2.5. Ethical note

Farms participating in this study followed the guidelines of the Eusko-Label Certification Program of the Kalitatea Foundation of the Basque Government. The study fulfilled the requirements of the European Directive 86/609/ECC regarding the protection of animals used for experimental and other scientific purposes.

2.3 Results

2.3.1 Frequency of use of the outdoor area

Surprisingly, the results of the study detected no effects of age period on the use of the outdoor area (age period, $F_{2,14.62} = 1.64$, P = 0.228; age by time period, $F_{4,14.77} = 1.58$, P = 0.231), with an average use of 32.60 ± 15.30% (mean ± SE) for the study period. However, it was affected by time period ($F_{2,14.5} = 5.71$, P = 0.0148,), with the lowest use observed during midday (Fig. 7). Temperature ($F_{1,34} = 3.11$, P = 0.086), climatic conditions ($F_{1,34} = 1.05$, P = 0.313), age period ($F_{2,34} = 0.22$, P = 0.806) or their interactions (temperature by climatic conditions, $F_{1,34}$ = *1,08*, P = 0.306; temperature by age period, $F_{2,34} = 0.68$, P = 0.511; climatic conditions by age period, $F_{2,34} = 2.72$, P = 0.080) did not have an effect over the percentage of tagged birds observed in the outdoor area.

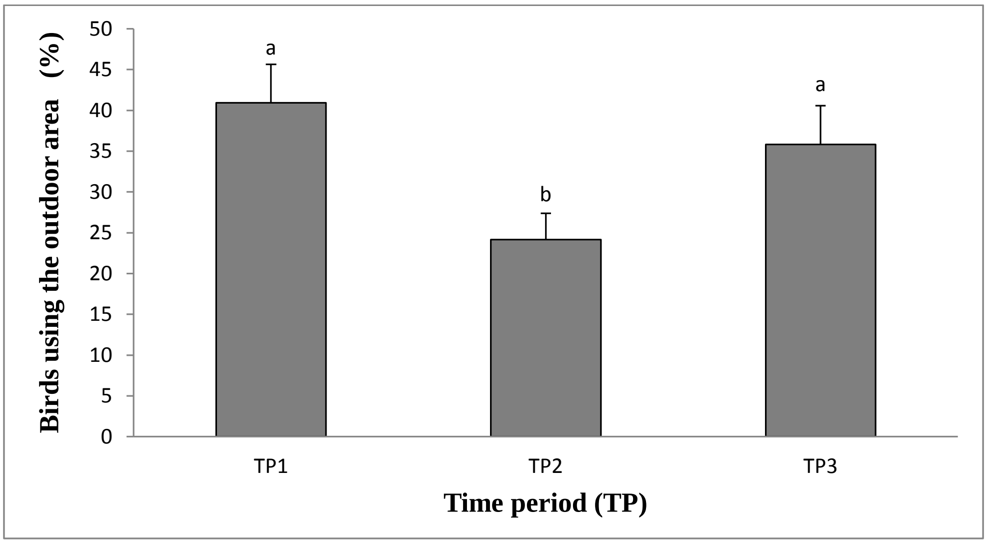


Figure 7. *Effect of time period (TP) on the percentage of birds using the outdoor area (means ± SE). Means sharing any common letters are not statistically different (P > 0.05).*

On the other hand, the results evidenced mayor inter-individual differences in the level of use of the outdoor area ($F_{3,15.53} = 15.49$, $P < 0.0001$; Fig. 8). By tracking the identity of the birds it was shown that 49.5 ± 4.2% (mean ± SE) were never observed using the outdoor area, while for light, medium and heavy users the percentage of birds in each category varied between 13 and 23%, and remained stable across age periods ($F_{6,17.03} = 0.42$, $P > 0.05$). Nonetheless, the use of the outdoor area during AP1 ($F_{3,14.17} = 11.81$, $P = 0.0004$) influenced the level of use detected in AP2 and AP3, while no differences were detected between AP2 and AP3 ($F_{1,14.14} = 0.10$, $P = 0.761$). Thus, birds that never used the outdoor area during AP1 were less likely to use it during AP2 and AP3, as compared to heavy users during AP1, while light and medium users showed intermediate values (Fig. 9).

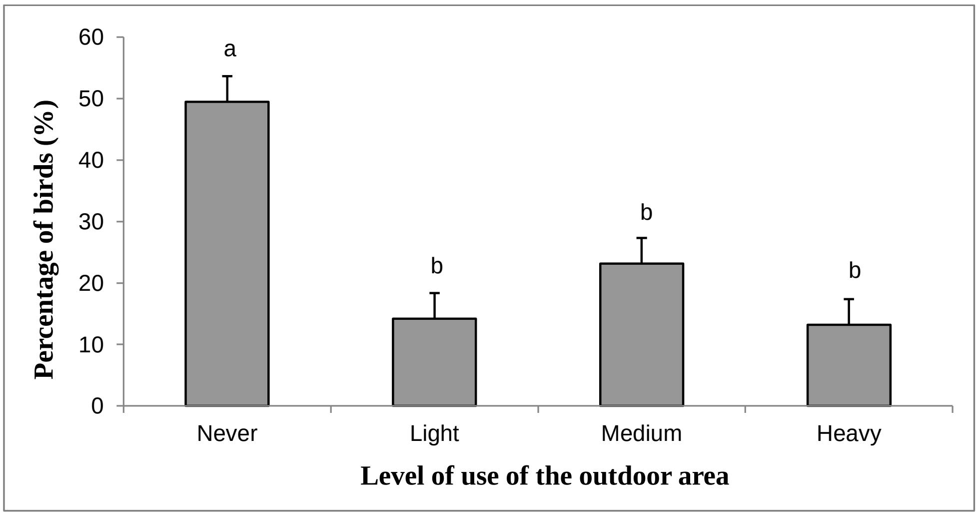


Figure 8. *Percentage of birds using the outdoor area at different levels of use (means ± SE). Means sharing any common letters are not statistically different (P > 0.05).*

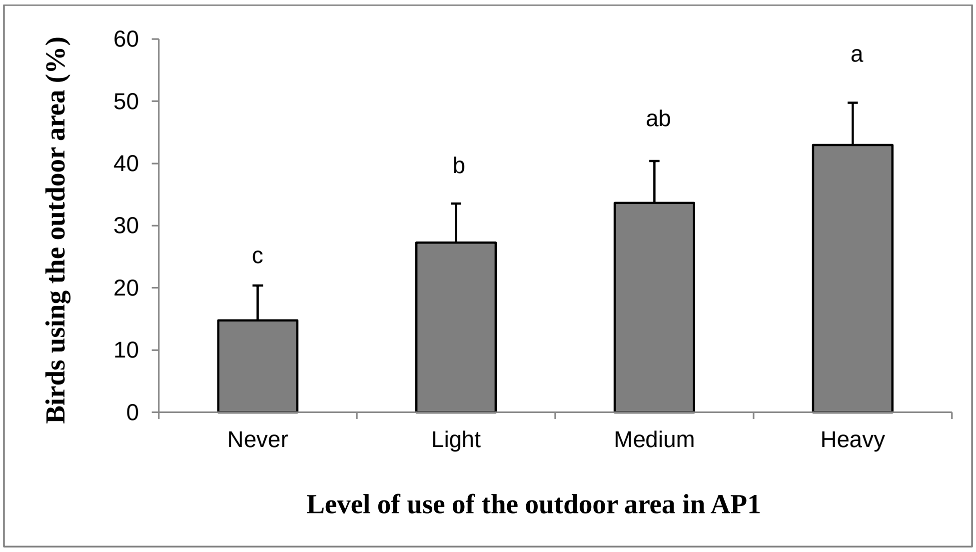


Figure 9. *Level of use of the outdoor area during age period two (AP2) and three (AP3) according to the use of the outdoor area observed during age period one (AP1) (means ± SE). Means sharing any common letters are not statistically different (P > 0.05).*

2.3.2 Spatial measures

Regarding the space use patterns of the tagged birds in the outdoor area, the results indicate that the mean, minimum and maximum distances to the hen house did not varied according to time period ($F_{2,15.02} = 0.75$, P = 0.488, $F_{2,4.26} = 0.15$, P = 0.863 and $F_{2,14.04} = 0.61$, P = 0.553, respectively). While the minimum distance to the hen house was not affected by age period, the mean and maximum distances tended to increase with age period (Table 1). Mean, minimum and maximum distances were not affected by time ($F_{2,15.02} = 0.75$, P = 0.489, $F_{2,4.25} = 0.15$, P = 0.863, $F_{2,15.04} = 0.61$, P = 0.553 respectively), or by the interaction time by age period ($F_{4,15.02} = 1.51$, P = 0.249, $F_{4,12.28} = 2.14$, P = 0.137, $F_{4,15.04} = 2.81$, P = 0.100). The activity centre (50% core area) and home range (90% core area) did not differ in size with age period and their coefficient of variation remained stable (Table 1).

Table 1. *Results of the GLMM of the effect of age period (AP1, AP2 and AP3) for spatial measures (mean ± SE) in the outdoor area. Mean, minimum and maximum distances to the hen house were measured in meters (m). Area of the activity centres and home ranges were measured in square meters (m^2). Coefficients of variation (CV) of the activity centres and the home ranges were presented as percentages (%).*

	AP1	***AP2***	***AP3***	***F-value***	***p***
Mean Dist. to hen house (m)	30.77 ± 6.19	37.39 ± 6.19	38.90 ± 6.27	$F_{2,15.02}$= 3.57	0.053
Min. Dist. to hen house (m)	22.83± 4.64	25.34 ± 4.64	28.16 ± 4.79	$F_{2,12.13}$= 1.44	0.274
Max. Dist. to hen house (m)	40.67 ± 9.76	53.56 ± 9.76	58.71 ± 10.01	$F_{2,15.04}$= 3.23	0.068
Activity centre (m^2)	174.14 ± 95.29	190.73 ± 95.29	224.75± 95.29	$F_{2,4}$= 0.25	0.786
CV Activity centre	116 ± 10.50	102.26 ± 10.50	108.24 ± 10.50	$F_{2,6}$= 0.48	0.642
Home range (m^2)	444.95 ± 369.13	639.93 ± 369.13	1116.23 ± 369.13	$F_{2,4}$= 2.05	0.244
CV Home range	90.87 ± 10.93	77.62 ± 18.93	96.06 ± 18.93	$F_{2,6}$= 0.25	0.785

Considering the use of space parameters indoors, total, net, maximum and minimum walked distances did not vary across age periods (Table 2). Likewise, the size of the home ranges and the coefficient of variation of the activity centres and the home ranges did not vary across age periods (Table 2). Only an increment in the size of the activity centre was detected with age period (Table 2).

Table 2. *Results of the GLMM of the effect age period (AP1, AP2 and AP3) for spatial measures (mean ± SE) in the outdoor area. Net, total, minimum and maximum walked distances were calculated in meters (m). Surfaces of the activity centres and home ranges were calculated in square meters (m^2). Coefficients of variation (CV) of the activity centres and the home ranges are presented as percentages (%). Columns with different letters (a-b) differ significantly (P < 0.05).*

	AP1	***AP2***	***AP3***	***F-value***	***p***
Net walked distance	16.21 ± 2.62	15.64 ± 2.51	14.52 ± 2.51	$F_{2,4}$=0.08	0.923
Tot. walked distance	37.64 ± 3.37	32.79 ± 3.16	26.14 ± 1.55	$F_{2,4}$=2.64	0.186
Min. walked distance	10.08 ± 1.58	10.93 ± 1.45	7.30 ± 1.44	$F_{2,4}$=1.39	0.347
Max. walked distance	23.88 ± 2.13	20.60 ± 1.94	18.43 ± 1.93	$F_{2,4}$=1.32	0.362
Activity centre	14.61 ± 3.70 ^{b}	26.76 ± 3.7 ^{a}	31.82 ± 3.7 ^{a}	$F_{2,4}$=10.70	0.024
CV Activity centre	94.32 ± 9.77	129.29 ± 9.77	113.19 ± 9.77	$F_{2,6}$=3.21	0.112
Home range	76.74 ± 11.11	86.88 ± 11.11	81.12 ± 11.11	$F_{2,4}$=0.74	0.531
CV Home range	75.92 ± 11.85	102.86 ± 11.85	84.21 ± 11.85	$F_{2,4}$=1.51	0.325

2.3.3 Space use and its relationship with morphometric measures

Spearman rank correlations between parameters defining space use and morphometric and welfare indicators are presented in Table 3. Interestingly, a strong correlation was detected between the percentage of use of outdoor area with the mean, minimum and maximum distances to the hen house and with the size of the activity centre and home ranges in the outdoor area. However, no relationship between the use of the outdoor area and the parameters characterizing use of the space indoors were detected (Table 3). As it could be expected, parameters describing use of space in the outdoor area, as well as those characterizing use of space indoors showed strong correlations among themselves, but not across.

Both, FPD and growth rate showed a negative correlation with the mean and maximum distance to the hen house, but FPD also showed a positive correlation with the total and maximum distance walked indoors. Plumage damage at the end of production was inversely correlated with the use of the outdoor area and showed a weak but positive correlation with total and minimum distance walked indoors. In addition, the results obtained showed a negative correlation between entry weight with FPD and comb peck wounds. On the contrary, a positive correlation was detected between entry weight with final weight, keel bone deformations, use of the outdoor area and minimum, maximum and mean distance to the hen house.

Table 3. Mean values and coefficients of correlation between morphometric, welfare indicators and use of space parameters. Significant correlations (P<0.05) were showed in bold. * P<0.05, ** P<0.01, ***P<0.001. Values for Foot Pad Dermatitis (FPD), keel bone deformations and comb peck wound were evaluated according to the Welfare Quality protocol (Welfare Quality ®, 2009). Scoring for plumage condition were *modified from Welfare Quality ®, (2009) and Tauson et al., (1984)*.

	Entry weight	Weight	Food pad dermatitis	Growth rate	Plumage condition	Keel bone deformation	Comb peck wounds	RFA Tarsus width	RFA Tarsus length	RFA Wing length	Final tarsus width	Out going %	Mean Dist. to the house	Min. Dist.to the house	Max. Dist. to the house	Main activity center Out.	Home range Out.	Net walked distance In.	Tot. walked distance In.	Min. walked distance In.	Max. walked distance In.	Main activity center In.	Home range In.
N	218	218	211	218	194	217	215	217	218	218	217	218	179	179	179	49	49	123	123	123	123	157	157
Mean	1370	1926	0.331	1.548	3.376	0.423	0.33	0.039	0.02	0.018	12.85	24.688	35.128	16.566	63.502	198.314	777.97	16.176	43.698	8.053	23.069	24.9484	84.616
SD	152.511	211.813	0.513	0.596	2.552	0.676	0.728	0.102	0.017	0.016	1.893	25.689	15.974	9.357	34.83	219.999	1084	11.057	31.761	7.116	12.077	27.063	64.84
Weight	**0.327*** **																						
Food pad dermatitis	**-0.393*** **	**0.176***																					
Growth rate	**-0.423*** **	**0.660*** **	**0.475*** **																				
Plumage condition	-0.103	-0.038	0.069	0.074																			
Keel bone deformation	**0.236*** **	-0.023	**-0.164***	**-0.225*** **	0.047																		
Comb peck wounds	**-0.303*** **	-0.02	**0.240*** **	**0.243*** **	-0.001	**-0.222** **																	
RFA Tarsus width	-0.026	0.004	-0.025	0.064	0.063	-0.049	0.053																
RFA Tarsus length	-0.023	**0.165***	**0.187** **	**0.165***	0.000	-0.021	0.009	0.101															
RFA Wing length	0.078	-0.122	0.06	-0.16	-0.02	0.019	-0.046	-0.043	0.06														
Final tarsus width	**0.344*** **	**0.479*** **	0.047	**0.176** **	**-0.206** **	0.033	-0.027	-0.05	0.112	-0.007													
Out going %	**0.143***	-0.096	**-0.192** **	**-0.179** **	**-0.337*** **	0.09	-0.075	-0.053	-0.075	0.01	**0.150***												
Mean Dist. to the house	**0.289*** **	-0.078	**-0.307*** **	**-0.269** **	**-0.323*** **	**0.145***	**-0.200***	-0.029	-0.07	**0.206***	0.132	**0.834*** **											
Min. Dist. to the house	**0.274** **	-0.007	**-0.264** **	**-0.240** **	**-0.277** **	0.121	**-0.176***	0.010	-0.065	0.060	0.111	**0.791*** **	**0.909*** **										
Max. Dist. to the house	**0.240** **	-0.116	**-0.304*** **	**-0.267** **	**-0.320*** **	**0.175***	**-0.173***	-0.082	0.036	0.163	0.108	**0.862*** **	**0.967*** **	**0.856*** **									
Main activity center Out.	0.083	-0.121	-0.185	-0.106	**-0.281** **	0.027	0.040	-0.080	-0.061	0.023	0.107	**0.964*** **	**0.959*** **	**0.954*** **	**0.958*** **								
Home range Out.	0.110	-0.093	**-0.192***	-0.098	**-0.290** **	0.035	0.014	-0.090	-0.029	0.067	0.129	**0.956*** **	**0.966*** **	**0.961*** **	**0.958*** **	**0.983*** **							
Net walked distance In.	0.123	0.134	0.054	-0.01	0.06	0.119	-0.095	-0.051	0.003	-0.089	0.03	-0.05	-0.022	0.001	-0.079	-0.063	-0.071						
Tot. walked distance In.	-0.103	0.069	**0.329** **	0.151	**0.254***	0.114	-0.019	0.04	0.098	-0.000	-0.106	-0.095	-0.035	0.004	-0.080	0.031	0.007	**0.504*** **					
Min. walked distance In.	-0.053	-0.066	0.137	-0.033	**0.244***	0.118	0.162	0.057	-0.001	-0.008	-0.079	-0.158	-0.182	-0.222	-0.222	-0.303	-0.335	0.072	**0.364*** **				
Max. walked distance In.	-0.024	0.107	**0.349** **	0.147	0.232	0.101	-0.038	0.122	0.137	-0.055	-0.059	-0.105	-0.047	0.035	-0.103	-0.003	-0.026	**0.623*** **	**0.925*** **	**0.296** **			
Main activity center In.	0.078	**0.217***	0.016	**0.201***	0.053	0.026	-0.000	0.066	0.085	0.059	-0.007	-0.026	-0.071	-0.021	-0.107	-0.015	-0.030	0.250	**0.332** **	0.147	0.238		
Home range In.	-0.071	0.16	0.097	0.227*	0.104	0.034	-0.019	0.013	0.102	0.049	-0.113	0.031	0.019	-0.058	0.037	0.064	0.066	**0.384** **	**0.517*** **	0.244	**0.398*** **	**0.734*** **	

2.4 Discussion

The aim of this study was to determine the factors that may influence use of space by free-range laying hens under commercial conditions, to establish the characteristics of use of space patterns and their relation with morphometric and welfare indicators. In general, the findings of the study showed that, surprisingly, time and age periods had only minor effects on the percentage of tagged birds observed in the outdoor area and over most parameters defining use of space patterns. However, one interesting finding was the evidence that the frequency of use of the outdoor area early in production had a relevant effect on its use later on. Although the results of this study only evidenced a small, but significant, improvement on plumage condition and FPD with increased use of the outdoor area, it does provide some indication on how individual use of space patterns may impact on welfare indicators.

A diurnal pattern in the use of the outdoor area was detected, with the lowest use occurring at midday (Fig. 7). This pattern appeared to be maintained throughout production as indicated by the lack of interactions among time and age period. Previous studies on the use of the outdoor area reported both, a tendency to decline during the day (Mahboub et al., 2004; Hegelund et al., 2005), and a higher use in the afternoon (Bubier and Bradshaw, 1998; Richards et al., 2011), while in this study higher levels were observed in the morning and afternoon. The variability in results across studies may relate to factors such as the climatic conditions and the season in which the studies were conducted (Hegelund et al., 2005), or to differences in the frequency of use of the outdoor area. Thus, while in this study the average use of the outdoor area of the tagged population was 32.60 ± 15.3%, a much lower use were reported by Hegelund et al., (2005) and Bubier and Bradshaw (1998), with a mean use of 9% and 12%, respectively. It is possible that when the level of use of the outdoor area is relatively low, birds may be limiting their use to the most favorable local conditions during the day, while when the use of the outdoor area is high, the morning and afternoon activity could respond to their circadian biorhythm (Channing et al., 2001; Campbell et al., 2015).

The lack of age period effects on the use of the outdoor area obtained in this study contrast with the decline observed by Hegelund et al., (2005), and with the increased found by Richards et al, (2011) and Gilani et al., (2014). Most studies that have examined the use of the outdoor area by laying hens concluded that climatic conditions have a strong effect, and that summer and autumn, or the less rainy season, promotes a higher use (Davison, 1986; Hegelund et al., 2005; Gilani et al., 2014). In this study, two of the farms began and ended the production cycle in summer while the third began and ended in winter. Therefore, it is possible that age and climatic condition effects in our study were confounded as in two of three farms the birds were young, and possibly more fearful and inexperienced during the most favorable summer season to use the outdoor area in Northern Spain. Hence, this can be a reason why no effect of the age period was detected. On the other hand, differences in the motivation to use the outdoor area might also depend on factors such as bird strain (Mahboub et al., 2004), aviary and outdoor area design (Zeltner and Hirt, 2008) or management practices (Bubier and Bradshaw, 1998; Bestman and Wagenaar, 2003; Hegelund et al., 2005; Gilani et al., 2014), but these effects are difficult to evaluate.

While accepting this confounding effect, it is important to indicate that climatic conditions of the North Coast of the Basque Country, where the farms were located, are characterized by moderate variations in temperature across seasons, with mild winters and summers (Euskalmet: www.euskalmet.euskadi.eus/) as compared to Northern European countries where other studies took place (Hegelund et al., 2005; Richards et al., 2011; Gilani et al., 2014). Given the high mean use of the outdoor area observed in this study as compared to others, it might be speculated that the mild weather conditions of the region may facilitate a high use of the outdoor area, attenuating potential differences due to seasonal variation in weather conditions or due to age effects. However, it was surprising to detect that over 49% of the tagged hens were never observed using the outdoor area along the study period, while the birds that did use the outdoor area were divided in similar proportions of light, medium and heavy users (Fig. 8). Richards et al. (2011) working with RFID tagged hens found that only 8% of the flock were never observed in the outdoor area, and an additional 12% were occasional users. Although this study was conducted over 46 weeks the results were based in direct observations collected every two wks. Therefore, it is possible that birds that only visited the outdoor area sporadically may have been missed resulting in an overestimation of the percentage of birds that were never observed in the outdoor area. No other studies conducted reported on the incidence of birds that were never observed in the outdoor area (Bubier and Bradshaw, 1998; Bestman and Wagenaar, 2003; Mahboub et al., 2004; Hegelund et al., 2005; Zeltner and Hirt, 2008; Gilani et al., 2014), therefore further research will be needed to clarify what should be normally expected.

Perhaps some of the most interesting results were obtained from the analysis considering the frequency of use of the outdoor area of individual birds. Such results evidenced that those individuals showing a high use early in production (AP1) were more likely to use the outdoor area later on (AP2 and AP3) (Fig. 9). Thus, birds categorized as heavy users during AP1 continued to show the highest use during AP2 and AP3 (42.94 ± 6.82%), that was significantly higher than those corresponding to light users (27.26 ± 6.27%), or birds that were never observed in the outdoor area during AP1 (14.74 ± 5.63%). Grigor et al., (1995b) indicated that regular exposure to an outdoor area during rearing increased birds' readiness to use the same area at 20 wks.

This study provide the first evidences of the potential impact of the early experience on the subsequent use of the outdoor area by laying hens under commercial free-range conditions.

It would be expected that as laying hens habituate to use the outdoor area they would also expand the range of exploration of the available space. Surprisingly, only a trend to increase the mean and maximum distance from the hen house with increasing age period was detected (e.g. maximum distance increased from 40.67 ± 9.76 m in AP1 to 58.71 ± 10.01 m in AP3, Table1). The size of the activity centers and home ranges did not vary significantly across age period. However, it is important to remark that the home ranges more than doubled in mean size from an area of 444.9595 ± 369.13 m^2 during AP1 to 1116.23 ± 369.13 m^2 observed during AP3. Therefore, it may be speculated that there was a tendency to expand the area used with age, although the large inter-individual variability, as indicated by the large coefficient of variation of the activity center and home range (Table 1) may have diluted the expected effects of age period.

Despite the lack of overall age period effects regarding the distance moved away from the house, a strong positive correlation was detected between the percentage of times a bird was observed in the outdoor area and the mean, minimum and maximum distance away from the hen house, as well as for the size of the activity center and home range in the outdoor area (Table 3). Therefore, these results suggest that those birds using the outdoor area more frequently were more prone to adventure themselves in the outdoor area, resulting in larger distances from the hen house and larger activity centers and home ranges (Table 3). Gilani et al. (2014) reported that laying hens ranged away from the house as they got older, increasing from 29% of the birds in the outdoor area ´away´ from the house at 16 weeks, to 42% at 36 wks, findings that would agree with the tendency to expand in the use of the outdoor area found in this study. This study, however, evidenced the large existing differences on use of space patterns among birds that use more frequently the outdoor area as compared to those that remain indoors.

The larger distances from the hen house and larger sizes of the activity centers and home ranges of the individuals using the outdoor area at higher frequency might be a consequence of habituation and increased experience in exploring the outdoor area, but could have also been influence by the availability of resources (Grigor et al., 1995c). Although grass quality was no considered in this study, it is possible that as grass close to the house would tend to deteriorate over the production period birds may have wondered away to find higher grass quality.

Similarly to the lack of a clear impact of age period on the parameters characterizing use of space in the outdoor area when considering the flock, no age period effects were detected indoors, with the exception of an increment in the size of the activity center that doubled in size from AP1 to AP3 (from 14.61 ± 3.70 to 31.82 ± 3.7 m, respectively). Although the correlations were not as strong as for the use of the outdoor area, most parameters characterizing use of space indoors were also correlated. It might be expected that the motivation for a bird to explore may be an individual trait that would be maintained regardless whether the bird is indoors or in the outdoor area. However, the results of this study showed that there was no relationship between the distances moved, the size of the activity centers and home ranges indoors and with those of the outdoor area (Table 3), suggesting that birds with good mobility indoors do not correspond, necessarily, to those ranging in the outdoor area. It is important to clarify that location data were obtained when pop-holes were open and birds could choose between the indoor and the outdoor area. Therefore, if a bird would show a preference for one of the options, the data obtained in the other location would be scarce, and would, in part, explain the lack of relationship between parameters characterizing use of space indoors and in the outdoor area.

An additional interest of this study was to determine if use of space patterns had an impact on morphometrical and welfare indicators. In this sense, the results indicate a lower incidence of plumage damage with increased used of the outdoor area (Table 3), which is in agreement with previous findings (Bestman and Wagenaar, 2003; Nicol et al., 2003; Mahboub et al. 2004). In addition, the negative correlation of plumage damage with increased distance from the house and with the size of the activity centers and home ranges suggest that those birds ranging further in the outdoor area are also the ones showing less plumage damage. On the contrary, Winckler et al., (2004) and Hegelund et al., (2006), with a mean use of the outdoor area of 18%, did not detect a benefit in plumage condition. The difference in results may depend obviously on the level of use of the outdoor area, but also on how use of outdoor area and plumage scoring is calculated. In studies considering the mean percentage of use of the outdoor area and the mean plumage scoring, the effects may be diluted as plumage condition may be assessed over birds that may not be using the outdoor area. In our study, this was directly obtained by relating considering the individual frequencies and use patterns of the outdoor area with the resulting plumage scoring of the each individual at the end of production.

The suggested explanation of the improvement on plumage condition with use of the outdoor area is that these birds may have lower probability of being feather pecked (Nicol et al., 1999, Nicol et al., 2003). It has also been suggested that plumage damage may refrain hens from exposing themselves to the outside climatic conditions (Hegelund et al., 2006). However, given the low severity of the plumage damage detected in this study this explanation seems unlikely. The most frequent (low) plumage damage in this study included the neck, head and belly areas with a 49.4, 47.60, 38.30% of birds being affected, respectively. Feather damage on the head and neck has been frequently noticed due to feather pecking and abrasion against the feed trough, and the feather loss on the belly can be seen in highly productive animals (Welfare Quality ®, 2009). Given the low rate of aggression and feather pecking observed while collecting the observations, the most likely explanation would be that individuals that remained indoors tended to have worse plumage condition majorly due to feather abrasion with the feed through or other hen house elements.

In addition to the impact on plumage condition, a positive correlation between FPD scoring and the total and maximum walked distance indoors, and negative with percentage of use of the outdoor area, mean and maximum distance from the hen house were detected. Therefore, in agreement with Niebuhr et al. (2009) that found that hens in aviary systems had worse foot conditions than hens in free-range systems, it is suggested that a higher use of the outdoor area and ranging away from the hen house reduces the risk FPD.

Finally, another interesting finding relates to the negative correlation between the frequencies of comb peck wounds and entry weight. It has been speculated that body size may be used as a signal of status which help to recognize the status of the hens in large flocks (Pagel and Dawkins, 1997). Therefore, it is possible that birds with low entry weight might have been a target of other larger hens. These birds did not seem to have any specific patterns of space use, other than for the negative correlation on the mean distance to the hen house, therefore suggesting that birds with comb wounds tend to stay closer to the hen house.

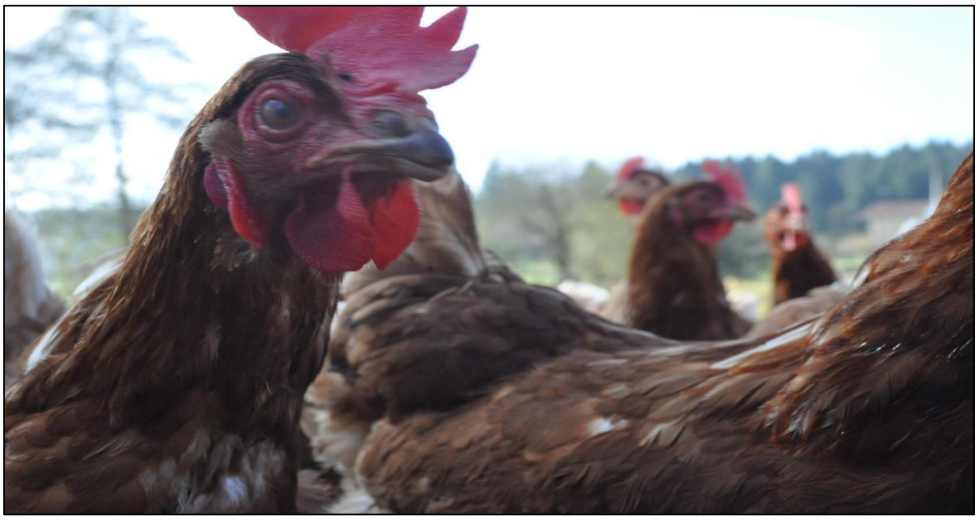
2.5 Conclusions

In conclusion, the results of this study suggest that the mean frequency of use of the outdoor area was relatively high as compared to previous studies, but was minimally affected by time of day and no affected by age period (in the general flock population) or climatic conditions. Despite the relatively high level of use of the outdoor area, almost half of the tagged population was never observed using the outdoor area, which may have been in part due to the data collection method. It is clear from the study that the frequency of use of the outdoor area early in production determined its use at later ages. Thus, individuals with high use early in production will continue to use the outdoor area at high frequencies, while those showing no use early will seldom use the outdoor area later on. The negative correlations detected between use of space parameters indoors and in the outdoor area also support the idea of subpopulations that move either indoors or in the outdoor area. The different use of space patterns appear to have an impact on welfare indicators such as plumage condition and FPD, both showing better scoring for those individuals with higher use of the outdoor area.

Chapter 3:

Aggressiveness in the domestic fowl:

Distance versus ´attitude´



Rodriguez-Aurrekoetxea. A., Estevez, I., 2014. Aggressiveness in the domestic fowl: Distance versus ´attitude´. Appl. Anim. Behav. Sci. 154, 68-74

Abstract

It has been suggested that invasion of the personal space by flock members is the main trigger of aggressive interactions in the domestic fowl (Gallus gallus domesticus). In large and dense groups of birds high frequency of attacks should be expected as the chances of invading the personal space of others is likely to occur. However, other studies suggest that after surpassing a certain group size the frequency of aggressive interactions decline. It is possible that the behaviour of the individuals themselves may be more relevant in this context. To test this hypothesis we analysed the onset of aggressive interactions in a laying strain of domestic fowl from videotaped behavioural sequences. A total of 60 interactions were analysed, for which we recorded the location (XY coordinates) of the giver (G) and receiver (R) of an aggression, the position of the two closest individuals to G (G1, G2) and to R (R1, R2), in addition to the behaviour and head orientation of all these birds with the software Chickitizer®. Distances between pairs of birds were calculated as Euclidean distances and analysed by mixed model ANOVA. Behaviours were ordered by ranges of activity and differences analysed by Kruskal-Wallis. Our results indicate that inter-individual distances at the onset of an aggressive interaction varied according to the specific pair of individuals, but contrary to the expected, distances between the G-R remained similar to the distance among the G-G1 and G-G2. R birds however, were consistently involved in more energetic demanding behaviours and with their head oriented towards G. These results suggest that aggression in the domestic fowl does not depend on the invasion of the critical distance per se, but would greatly depend on the activity level and directionality of the individuals which would be perceived as a threat by the aggressor.

Key words: Domestic fowl, Personal distance, Inter-individual distance, Aggressive interactions.

3.1. Introduction

It has traditionally been considered that aggressive interactions in the domestic fowl (*Gallus gallus domesticus*) allows priority of access to resources and maintenance of its own personal social space, and that invasion of this personal space will trigger aggressive interactions amongst group members (McBride, 1971). These interactions would occur while they are facing each other (McBride et al., 1963). However, results of a later studies by Hughes and Wood-Gush (1977), in which they found that aggressive interactions occurred at much higher frequency in spacious pens as compared to crowded cages for identical group sizes, lead the authors to suggest that aggressive interactions were more likely to occur when the birds had the opportunity to move around occasionally approaching the "personal space" of other birds, as oppose to birds being in continuous proximity.

Based in Hediger's (1955) description of spacing and the concept of individual distances, McBride (1971) defined the personal space as the area around an individual that it is attempted to maintain free from co-specifics. However, it has been documented that inter-individual distances are dynamic, and differ according to the behaviour displayed (Keeling, 1994), with the density of animals (Keeling and Duncan, 1989). If invasion of the personal space by reducing the critical distance among flock members would trigger aggressive encounters, then theoretically, under extensive aviary production conditions (in which large and densely populated groups of laying hens have a wide range freedom of movements) a high level of interactions should be expected. Contrarily, studies on the impact of density, group size and space availability in the occurrence of problematic aggressive interactions in the domestic fowl, provided strong scientific evidences that would suggest that the frequency of aggression actually declined with increased density and group size

(Carmichael et al., 1999; Estevez et al., 1997; Estevez et al., 2003; Hughes et al., 1997; Nicol et al., 1999). Originally, McBride and Foenander (1962) proposed that low aggression levels in large flocks could be maintained if birds remained within their close vicinity, allowing them to establish sub-hierarchical social structures within the large group. In reality their theory on spacing and aggression, while considered a classic paper, was not based in strong scientific evidences. This hypothesis would intrinsically imply a clear restriction in space use, for which evidence has never been documented in the domestic fowl (Estevez et al., 1997; Leone and Estevez, 2008b; Newberry and Hall, 1990). As an alternative explanation to the decline in aggression as flock size increased some authors proposed the tolerance hypothesis (Estevez et al., 1997), or the pragmatic strategy (Pagel and Dawkins, 1997). Nonetheless, to date no studies have been conducted to determine the specific context in which the aggressive interactions take place in large flocks of domestic fowl which are commonly used in commercial settings. Neither have been studied the ultimate causal factors triggering an aggressive encounter across particular individuals within the group.

It is possible that as the invasion of personal distance may act as an indicator to determine the risk of attack by another bird, its behaviour may also play a very important role. The behaviour serves as a gradual communication signal in social groups. Usually it is correlated with the disposition of the animals to perform some action, thus it gives information about their motivation (Carranza, 1994). Many scientists have tried to determine if, for example, a display of aggression by an actor can predict the subsequent behaviour or the recipient (Nelson, 1984; Piersma and Veen, 1988). In this regard, only moderate correlations between behavioural sequences of an individual were found. However, more consistent correlations were detected between the action of a first individual and the response by another

(Bradbury and Vehrencamp, 1998). If inter-individual distances vary with density, group and enclosure size or the behaviour of the birds composing the flock, it is difficult to imagine how a bird could predict the degree of threat by another individual by relying exclusively in the information conveyed by their inter-individual distances. In addition, results by Hughes and Wood-Gush (1977) and Pettit-Riley et al. (2002) indicate that interactions occur when birds are in open areas where inter-individual distances are likely larger. All these would suggest that aggressive interactions among group members in the domestic fowl are triggered by mechanisms that are more complex than the simple violation of the boundaries of the personal space.

In this study we focused on examining the influence of the critical distance between individuals as a primary factor triggering aggressive encounters in the domestic fowl (specifically a commercial layer strain) maintained in extensive type aviary systems, but exploring the role of the behaviour as a factor that may elicit the occurrence of aggressive interactions among specific individuals. We hypothesize that the behaviour of the domestic fowl may be particularly relevant to predict the direction of the aggressive encounter, beside the invasion of the personal space. We predicted that active birds would be more likely be the recipients of an interaction due to the higher immediate risk that possess to the actor as opposed to birds in more passive behavioural states.

3.2. Materials and Methods

3.2.1 Animals

For each observation day, two five minutes recordings were randomly chosen from video footage automatically collected 2 times per day (between 7.00 and 9.00 am and 11.00 and 13.00 pm). Videos were collected, three days per week, during 24 weeks, by video cameras installed at two commercial aviary egg production farms in North Carolina (USA). The video footage used for this work was part of a larger study on the behaviour of laying hens maintained under different production schemes. The birds for this study were between 40 and 66 weeks old Lohman Whites laying hens maintained under commercial conditions for egg production at a density of 5.93 and 5.6 hens/m^2 and at population sizes of 13,226 and 12,500 birds, respectively.

3.2.2 Data collection: inter-individual distances, orientation and behaviour

Video sequences were reviewed for the occurrence of aggressive interactions using ad libitum sampling. We analysed only the sequences of aggressive interactions located in a specific area, where the interaction could be correctly viewed in the computer screen, analysed and the perspective of the view allowed for correct measurement of inter-individual distances. Under these particular settings we were able to identify 30 aggressive interactions per farm (60 total).

Once an aggressive encounter was identified, we defined the individuals in the 'episode' as; the giver of the aggression (G), the individual who made the first aggressive movement towards another hen, and the receiver (R) of the aggressive interaction. G1 and G2 were identified as the two hens closest to the giver of the interaction, and R1 and R2 were the two individuals closest to R (Fig. 10). We considered that each aggressive episode began at the instant in which the giver moved towards the receiver of the interaction. While direct visual contact of the interacting birds could be considered part of aggressive behaviour, this aspect was impossible to be determined, so we considered that aggressions start when birds moved towards another.

Aggressive interactions were classified according to four types of aggression (threat, chase, fight, and aggressive pecking), according to Estevez et al. (2002). A threat was recorded when a bird with the head elevated, sometimes with the neck feathers raised, confronted directly another individual. A chase, was identified when the bird (G) run at least three steps in pursuit of another hen in an aggressive context, while aggressive pecks were recorded when a bird raised its head and directed a peck towards the receiver head area. Fights were register when two hens were facing each other and delivered more than two vigorous kicks combined or not with aggressive pecks towards the opponent.

For each aggressive episode, we recorded the type of aggressive behaviour (based on the above definitions), the directionality of the head (looking towards or away from the giver of the interaction), and the relative location in space of the G, R, the two hens closest to the giver (G1, G2) and the two hens closest to the receiver (R1, R2) right at the moment prior to the onset of the aggressive encounter.

Taking the video image of the viewed area as a model, a template of acetate with a grid was superimposed to the computer screen to aid with the specific location of the individuals. Each square on the grid had the size of a hen. Because it was not possible to make recordings from a plane parallel to the floor there was an effect of depth of field on the recordings. Consequently, not all squares of the grid had the same size, but all were adjusted to the size of a hen in perspective, which varied due to the position of the hen as a function to the distance from the video camera. For data collection, we transferred the snap shot of the moment prior to the start of the interaction to the Chickitizer software (Sanchez and Estevez, 1998). The exact locations of each individual (G, G1, G2, R, R1, R2) were recorded in a XY coordinate system, taking as reference the head position of each individual. The equivalence of the size of a bird was estimated in 10 pixels measured in the Chickitizer (25 cm). XY coordinates for each individual were recorded and Euclidean distance (Fig. 10) calculated using the Euclidean distance (distance= $\sqrt{((y2-y1)^2 + (x2-x1)^2)}$, between G-R, G-R1, G-R2, G-G1 and G-G2, as described in Estevez and Christman, (2006), Keeling and Duncan, (1989), Leone and Estevez, (2008b) and Leone et al., (2010).

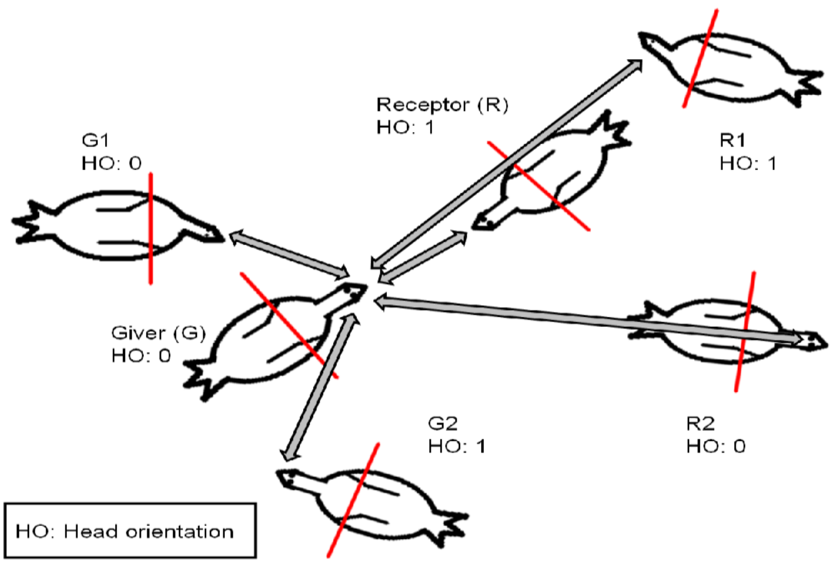


Figure 10. *Scheme of the data obtained from each aggressive encounter. Behaviour, the relative head´s orientation to the position of the giver (G), and inter-individual distances calculated as Euclidean distances from the location of the birds in XY coordinates.*

After recording the individuals location we scored the orientation of the head of each individual (G, R, G1, G2, R1, R2), noting as "0" the orientation of the head of the giver, and establishing the orientations of the head of the remaining birds by using an imaginary line that passed through the shoulders of the aggressor at angle of 180° (Fig. 10). When individuals were orienting their head in the same direction as G then, head position was recorded as "0", while when looking in the opposite direction to G then it was noted as "1".

In addition to location and head orientation, we also recorded the behaviours performed by all individuals in the 'episode' (G, R, G1, G2, R1, and R2) right before the occurrence of the aggressive interaction. Behaviours recorded were defined by Cornetto and Estevez (2001b) and Bilcík and Keeling (2000), which included; fly, forage, dust bath, rest, run, stand, self-preen, feather-peck severe or gentle, walk, wing-flapping. The viewed area had not feeders or drinkers on sight, and therefore, related behaviours were not observed.

3.2.3. Statistical analysis

Inter-individual distances obtained from the XY coordinates through calculation of Euclidean distances were analysed using a mixed model ANOVA (SAS version 9.3). Prior to testing the data, we checked for normality by Shapiro-Wilks test, and log-transformed them as needed to fit them to a normal distribution. The model included the type of pairs (G-R, G-R1, G-R2, G-G1 and G-G2), the type of aggression (threat, chase, aggressive pecking and fighting) and their interaction as fixed factors. We used "aggressive event (farm)" and "farm" as random factors. For the posteriori analysis we used a Tukey-Kramer test. We assumed that observations were independent given the large number of birds within each facility and the time span across aggressive interaction. Data for head positions were coded as a dichotomous variable 0-1 as explained above, and their frequency of occurrence analysed by Chi-Square test to compare the head's orientation of R, G1, G2, R1 and R2. We also calculated the frequency of the head orientation with regard to each type of aggressive interaction, threats and pecks. Statistical analyses for chases and fights were not performed due to the low incidence of these types of interactions.

Regarding behavioural data, we excluded the severe and gentle feather pecking behaviours because no birds involved in the episodes showed any type of feather pecking behaviours. For all other behaviours we transformed the original categorical data to an ordinal scale based on the rank of activity of the behaviours by assigning values form 1 to 8, in ascending order, as follows: (1) rest, (2) dust bathing, (3) self-preen, (4) stand, (5) forage, (6) walk, (7) run, (8) fly and wing flapping. The order used in ranking (or classifying) these behaviours, was assigned by considering the approximate energetic cost for performing each behaviour. Data were then analysed by means of a nonparametric Kruskal-Wallis test, which included individual type (G, R, G1, G2, R1 and R2) as the factor level. All statistical analyses were performed with SAS (SAS Institute and Inc., 2010).

3.2.4 Ethical note

Farms participating in this study follow the guidelines of the Free Farmed Welfare Certification Program of the American Humane Association (www.americanhumane.org). The study fulfilled the requirements of the European Directive 86/609/ECC regarding the protection of animals used for experimental and other scientific purposes.

3. 3 Results

3.3.1 Inter individual distances and head orientation

The results of the analysis for the inter-individual distances between pair of birds indicated a clear effect of the type of pairs ($F_{4,236}$ = *30.38, P < 0.0001*) but not for the kind of aggressive interactions ($F_{3,236}$ *= 0.88, P = 0.45*). Inter-individual distances between G-R and G-G1 and G-G2 were not different from each other (*P* > 0.05), but were significantly lower as compared to inter-individual distances between G-R1 and G-R2 pairs (*P* < 0.05, Fig. 11).

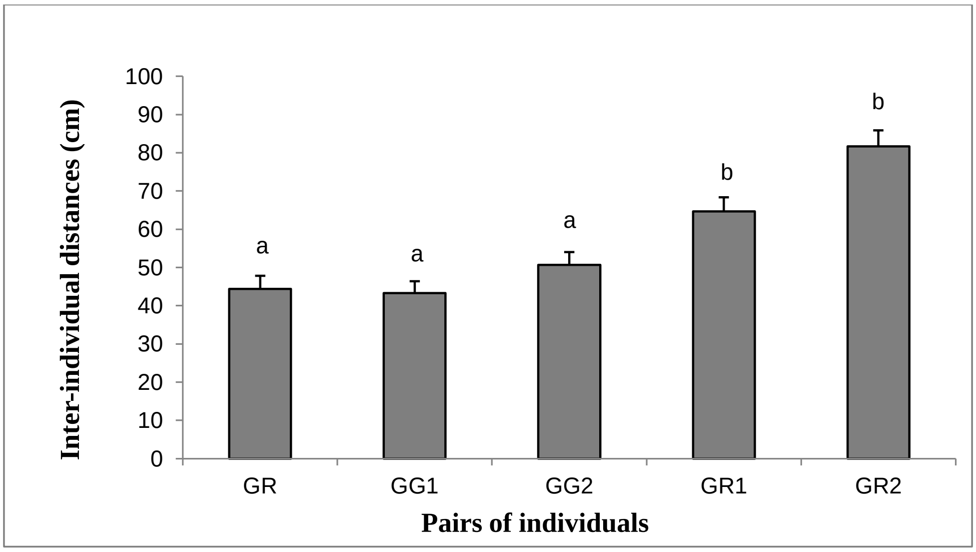


Figure 11. *Mean inter-individual distances (mean ± SE) between pairs of individuals at the onset of aggressive encounters. Means sharing any common letters are not statistically different (Tukey P > 0.05).*

It was observed that the R birds, without distinguishing between types of aggression, were the individuals significantly more often facing G birds ($X^2_4 = 13.05$, $P = 0.01$; Fig. 12). Remaining individuals (G1, G2, R1 and R2) presented similar frequencies in regard to head orientation 0-1. The analysis of the direction of the head according to the type of aggression showed that for threat, there was a significant difference in the direction of the head orientation ($X^2_4 = 10.66$, $P = 0.03$), which follow a similar pattern to the results of the overall analysis. For aggressive pecks we did not find differences ($X^2_4 = 7.32$, $P = 0.12$).

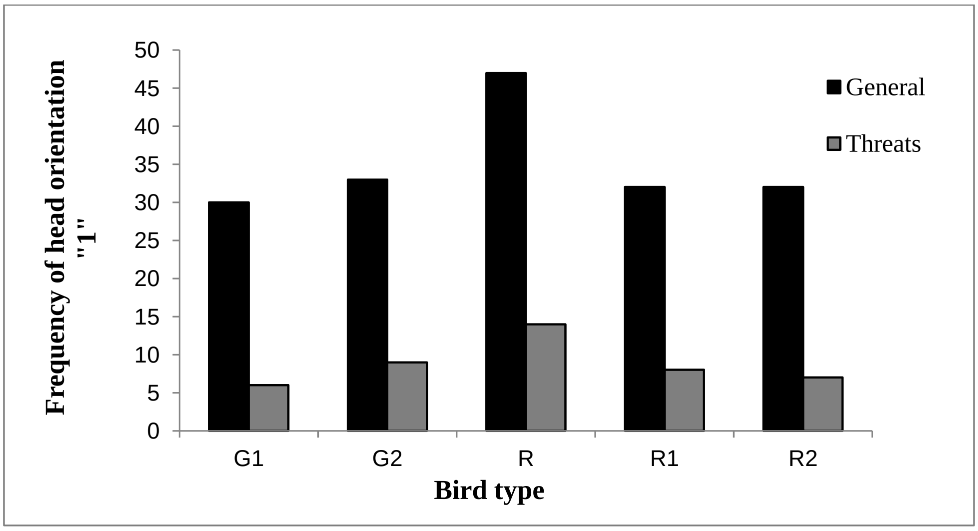


Figure 12. *Frequencies of observed head´s direction with respect to the giver (G). The direction of the head of aggressors (G) is always (0) and therefore not shown in the figure* (χ^2_4=13.05 P=0.01).

3.3.2 Behaviour prior to the aggressive interaction

In Table 4 are presented the original frequencies of behaviours observed for each individual type at the onset of the aggressive interactions, with R birds showing the highest overall activity levels such as walking and running. Regarding the mean ranks, there were significant differences in activity between individuals before the aggressive episode (H_5 = 71.82, P < 0.0001). R birds had higher mean ranks of activity, followed by G birds, while G1, G2, R1, and R2 showed lower values.

Table 4. *Contingency table of the total frequency for behaviours per type of individuals for the observed interactions (n = 60). Behaviours are sorted from low to high active behaviours. Mean ranks of activity levels for each individual type involved in the aggressive interaction (H_5 = 71.82, P < 0.0001) are presented in the last row of the table.*

	G	**G1**	**G2**	**R**	**R1**	**R2**
Rest	2	8	5	1	7	6
Dust bathing	0	1	3	0	0	0
Self-preen	3	11	9	1	5	9
Stand	22	8	15	4	11	17
Forage	15	18	23	10	27	17
Walk	14	12	5	23	10	11
Run	1	0	0	12	0	0
Fly-Wing flapping	3	2	0	9	0	0
Mean Ranks (Activity level)	186.74	158.85	140.87	276.86	167.20	152.46

3.4 Discussion

In this study, we revisited the hypothesis of the invasion of the personal space as main causal factor of aggressive interactions in a highly social species such as the domestic fowl (*Gallus gallus domesticus*). To this aim we studied the behavioural states, inter-individual distances and orientation of givers (G) and receivers (R) of the interaction, and of the two closest birds to both G and R at the onset of aggressive.

The results of this study showed that the distance between the G and R did not differ from the distance between G with G1 and G2. However, R birds were at significantly shorter distances from G as compared with R1 and R2 birds (see Fig. 11). We also found that R birds were more likely oriented facing towards G, and most importantly, that these individuals showed higher levels of active behaviours, when compared with the other birds (G1 G2 or R1 and R2) at the onset of the encounter.

The domestic fowl has a complex social structure characterized by the establishment of a hierarchical social system that takes place between 2 and 10 weeks of age (Rushen, 1982). However, it has been speculated through mathematical modelling that it would be unsuitable to form and maintain a hierarchical social system in large groups of birds (Pagel and Dawkins, 1997). In fact, studies by Estevez et al. (1997) found experimental evidences of reduced aggressive interactions in large (high density) as compared with smaller (lower density) groups of domestic fowl, suggesting the 'tolerance hypothesis'. Further evidences of reduced aggression in larger groups of domestic fowl are provided by other authors (Carmichael et al., 1999; Hughes et al., 1997; Nicol et al., 1999; Pagel and Dawkins, 1997; Rushen, 1982). Additionally, Hughes and Wood-Gush (1977) found that under conditions of controlled group size, higher level of aggressive interactions took place at higher space availabilities. The authors explained these results suggesting that the onset of an aggressive encounter would require larger relative spaces, idea that is supported by more recent studies (e.g. Pettit-Riley et al., 2002; Ventura et al., 2012) that found that most aggressive interactions in the domestic fowl took place in open areas of the enclosure. Hughes and Wood-Gush (1977) furthermore argued that under space restrictions subordinates will more likely be in close proximity to dominants, reducing the chances for aggressive encounters, and also because agonistic encounters would only take place when a bird crosses the personal space of another, and not when in close proximity.

Our results support to some extent this hypothesis, and the findings by McBride et al. (1963) indicating that domestic fowl avoid visual confrontation, as the aggression was directed towards the most active bird oriented towards G and not to G1 and G2 that, while being as close to G as R, were in a less active state (mostly foraging or standing). However, it does not appear from our results that the aggressive encounter starts as result of invasion of the personal space per se, as initially suggested by McBride (1971), but would emerge from a combination of proximity, orientation and activity or 'attitude' of the birds that would convey information about the degree of threat induced by the approaching individual.

It is well known that laying hens tend to cluster when performing passive behaviours such as egg laying, feeding, dust bathing or perching (Hughes, 1971; McLean et al., 1986), but on the contrary, distances across group members will increase when active such as while foraging or walking (Keeling and Duncan, 1991). Clustering while performing passive behaviours such as feeding, or dust bathing might be beneficial due to reduced chances of being predated (Keeling and Duncan, 1991). On the contrary, the presence of conspecifics may also lead to interference during competition for resources (Goss-Custard, 1980; Klaassen et al., 2006; Sutherland, 1983). Therefore, social animals should carefully balance inter-individual distances in order to maximize resource acquisition while minimizing predation risk, supporting the idea that inter-individual distances are dynamic.

From this perspective it could be argued that in our study R birds could have been perceived by G as a higher immediate threat of losing potential resources due to their closest proximity as compare to R1 or R2, but also to their higher activity levels and directionality in their movements that distinguish them from G1 and G2. R birds were characterized by a higher frequency of walking and running, as compared with G1 and G2, mostly characterized by higher frequencies in standing and foraging. It is possible that individuals that are walking or running, were considered a more immediate threat as they could be considered still in an active phase of searching for resources, as compared to birds that are already foraging at a 'safe' distance (R1 and R2) and/or not directly oriented towards G while in proximity (G1 and G2). Alternatively, walking or running birds directly oriented towards another bird might be perceived by the later as an immediate threat for aggression on its own.

The combination of the requirements of orientation, proximity and behaviour seem to be essential elements to instigate an aggressive encounter and may, in fact, be the mechanism that would explain why at high density/ group size lower level of interactions should be expected. Under high density or severely restricted space availability, where lower level of aggressive interactions are observed (Carmichael et al., 1999; Hughes et al., 1997; Nicol et al., 1999), the frequency of active behaviours, and in particular walking and running, is reduced due to the lack of space availability, or due to the presence of birds that would act as barriers to the movement of others (Estevez et al., 1997; Newberry and Hall, 1990). On the contrary, in larger enclosures domestic fowl walked more and maintained larger inter-individual distances (Leone and Estevez, 2008b). It is possible that when space is constrained there are indeed limited possibilities for these three requirements to occur simultaneously. The close presence of other birds in the path of movement may, for example, reduce the chances for birds to approach another individual walking or running directly oriented towards each other. A similar reasoning would explain the beneficial effects obtained by the presence of cover panels in the domestic fowl in reducing aggression (Cornetto et al., 2002).

All aggressive interactions have a cost (Krebs and Davies, 1997), but in our study it is unclear what may have been the motivation for a costly interaction when a priori there was not a clear immediate benefit as our observations were taken in areas away from points of access to water or food. However, resources are not always evident. When birds are foraging, act of scratching and pecking at the ground while moving, next to consuming small food particles, they ingest small stones which help their digestion by breaking down food in the gizzard before passing into the intestines. However the decision to protect a resource and engage in aggressive interactions depends on its distribution and the density or population size (Grant, 1993; Estevez et al., 2002). Although the density, defined as floor area per individual in the farm does not change, the space available for each individual at specific times varies depending on the movements of other individuals. This dynamic in flock movement can easily generate open areas where the density of individuals at a particular location may be reduced, sometimes substantially. It has been found that is in these locations where the risk of encounters would be the highest (Pettit-Riley et al., 2002; Ventura et al., 2012). We could not estimate the relative bird density based on our personal observations but we agreed that aggressive encounters occurred in open areas when a low number of birds were in the proximity. Nevertheless, aggressions were not directed towards individuals who showed passive behaviours, which are the ones that require smaller inter-individual distances (Table 1). However, why birds engage in a costly interaction, considering that feed was provided ad libitum and data were collected away from feeding areas is difficult to explain. It is possible that even though in a commercial facility there is no feed available on the floor, as we mentioned before, there could be small food particles and stones that hens ingest.

These particles, perhaps, could motivate the protection of an area when the number of competitors is low. Individual decisions on engaging in attacking another bird may depend on the perceived risk of loss of resources, present or expected (Bradbury and Vehrencamp, 1998), and has been argued that the behaviour is a good indicator of an individual's motivation to compete for resources (Carranza, 1994). Thus, our results could indicate that G may perceive a different degree of motivation to take a competitive action depending on the behaviour of approaching bird. Alternatively, or in combination, they may be simply reacting to the 'perceived' degree of threat to an unknown directly incoming active bird, as group sizes in this study were above 13,000, and likely birds do not know each other.

3.5 Conclusions

The results of our study evidenced that aggressive encounters emerge as a combination of proximity, orientation and activity (or 'attitude') of the birds, and not by simple incursion of another's space. The requirement of these three elements to instigate an aggressive encounter provides a mechanism to explain the recurrent findings of reduced frequency of aggressive interactions when space availability is reduced since most of those encounters in domestic fowl take place in open areas.

Chapter 4:

Environmental complexity and use of space in slow-growing free-range chickens

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Chapter 4: *Environmental complexity and use of space in chickens*

Abstract

Production environments for meat poultry are generally bi-dimensional open areas where birds tend to cluster along the walls. We investigated the impact of increasing environmental complexity (EC) on slow-growing free-range chickens raised under commercial conditions. The study was conducted in four farms, each with three independent houses and outdoor ranges that were outfitted with panels, perches or remained under standard management (control). Forty birds per house were individually tagged and their locations (in XY coordinates) recorded from weeks of age 6 to 12. Total and net distance travelled, use of the central areas indoors, and core areas were calculated from the indoor and outdoor locations. Core areas define the areas of activity according to assigned probability levels, (50, 75 and 100% in this study). Production and welfare indicators were collected at slaughter; incidence of footpad dermatitis, fluctuating asymmetry and growth rate. Results showed an EC treatment effects ($P < 0.05$) with more birds using the central indoor area in the panel and perch treatment than controls ($P < 0.05$). The interaction between treatment and temperature affected total distance travelled indoors ($P < 0.05$), while net distance was affected by weeks of age and treatment interaction ($P < 0.05$). No treatment or age period effects ($P > 0.05$) were found for core areas indoors. The use of the outdoor area was affected by the birds weeks of age and the temperature ($P < 0.05$), although the use of the outdoor area remained low, with an average of 63.08 ± 5.37% of the tagged birds never observed outside. The size of the core areas outside at 50 and 100% increased with age period ($P < 0.05$). No treatment effect was found for production and welfare indicators. Although the benefits of the EC treatments may have been restricted by the small number of devices introduced, these results suggest that EC facilitates a more homogeneous use of the space inside the houses and that the use of the outdoor area increased with the experience provided by age.

Key words: Environmental enrichment, Panels, Perches, Use of space, Free-range chickens.

4.1. Introduction

In the wild, animals move about their environment in order to optimize feeding rate, increase their reproductive success, and seek shelter from potential predators (Krebs and Davies, 1997). Indeed, the factors that motivate an animal to move are complex. For example when food sources are located away from cover, animals establish trade-offs between foraging efficiency and security from predators (Andrusiak and Harestad, 1989). However, in commercial poultry settings feed supplies are freely available at predictable locations, predation risk is minimal and the simplicity of the environment does not challenge the birds to explore, potentially reducing the birds' motivation to use such environments fully (Newberry, 1999). Even under free range conditions poultry have a strong tendency to remain in close proximity of the house (Keeling et al., 1988; Zeltner and Hirt, 2003) which may create management problems with the range. For commercial chickens raised indoors it has been documented that they have a tendency to stay near the pen walls (Newberry and Hall, 1990; Pamment et al., 1983). An uneven bird distribution increases the birds´ relative density at specific locations, which may contribute to litter quality deterioration. Dermatitis, either on the foot or breast, is directly related to environmental degradation and particularly to poor litter quality (Cravener et al., 1992; De Jong et al., 2014; Martins et al., 2013; McIlroy et al., 1987).

The limited scientific information available regarding the use of outdoor ranges in poultry suggests that only a fraction of the birds uses the outdoor areas. For example, Dawkins et al. (2003) estimated that a maximum of 15% of broiler chickens used outdoor areas in free range systems, whereas in layers the average ranges between 7 and 38% (Hegelund et al., 2005). Even when using the outdoor areas most birds tend to remain close to the rearing house (Zeltner and Hirt, 2003; Hegelund et al., 2005), increasing the risk of parasitic infestation due to the overuse of these areas (Permin et al., 1999).

In natural environments birds use vegetation or natural barriers for protection from the weather, to hide from conspecifics (Newberry and Shackleton, 1997) and predators, or to reduce inter-animal conflict by decreasing visual contact (Estep and Baker, 1991). Artificial structures, such as panels and perches, have been used successfully to increase the environmental complexity (EC) in poultry houses with the aim of improving the overall welfare of birds (Newberry, 1995; Bailie et al., 2013; Cornetto and Estevez, 2001b; Cornetto et al., 2002; Estevez et al., 2002; Leone et al., 2007; Ventura et al., 2010). Perches also provide birds the opportunity to use the vertical space, and it has been shown that perches located in central areas improve the use of this generally underused space (LeVan et al., 2000). Further, use of perches may be associated with increased exercise and improved leg condition in layers (Haye and Simons, 1978) and broilers (Bizeray et al., 2002a; Ventura et al., 2010). Therefore, the provision of panels and perches in slow-growing extensive chicken farms may not only favour a more homogeneous use of space, both inside and in the outdoor area, but may also improve bird health and welfare.

The aim of this study was to determine the potential benefits associated with increased EC in the form of panels and perches on use of the central indoor and outdoor areas at commercial free range slow-growth meat chicken farms, and relate these to welfare indicators and final production quality. We expected that panels and perches would improve the use of the outdoor areas and favour a more homogeneous use of the areas inside the house. Moreover, we expected that this enrichment would have a positive effect on the welfare of chickens as measured by production and welfare indicators.

4.2. Material and Methods

4.2.1. Farms and animals

This study was conducted from March to September, 2012 in four commercial free range slow-growing chicken farms located in the Basque Country (Northern Spain). All farms produced under the Eusko-Label Certified Program, which has tight specifications for housing, management practices and bird type. The Eusko-Label certification only permits natural ventilation; therefore temperature after brooding is not controlled.

Each farm under study consisted of three independent 110 m^2 houses, each with its own fenced outdoor area (approx. size around 2700 m^2). A total of 3900 one day old chicks of Atlantic strain (Sasso T44) were housed at each farm. Due to standard management practices of the Eusko-Label certified program all birds were brooded together in one house for the first month, after which birds were divided evenly across the three houses (1300 birds/house). Bird density was 12 chickens/m^2, indoors and 2 birds/m^2 in the outdoor area, which is the maximum permitted density for flocks older than 3 weeks of age. Feed and water were provided ad libitum inside the houses only.

4.2.2. Experimental design

We used a completely randomized block design with three treatments: panels, perches or control (standard management). Each was assigned randomly to one of the houses in each farm. Eighteen lightweight panels or wooden perches were set up in the panel and perch treatments, respectively. Half of the enrichment was placed inside the houses at 2 m intervals within the central area and perpendicular to the pop-holes. The remaining nine panels or perches were placed in the outdoor area parallel to the pop-holes (Fig. 13). Panels were constructed of 2.5 cm diameter PVC pipe, 0.5 m tall and 0.5 m wide, overlaid with opaque plastic green mesh (Cornetto and Estevez, 2001b; Cornetto et al., 2002). Perches were 0.5 m long, 25 cm high and 4 cm wide, following the design of Ventura et al. (2010). Perches and panels were available to approximately 7% of the birds in each house, assuming 10 cm of space per bird with 1300 birds per house. The low availability of devices in this study was due to the fact that we were working under commercial conditions and did not want to interfere with regular management practices.

In order to acclimate birds to the panels and perches, one of each was placed in the brooding house for the first month. Plastic ID tags were also placed at different locations to get the animals used to them as well. Once the birds were distributed in the three houses 40 birds per house were tagged with individual IDs. Birds were neck tagged on each side with laminated labels following the procedure described by Cornetto and Estevez, (2001b) and weighed prior to placement in their respective house.

4.2.3. Data collection

Outdoor areas for free range chickens under Eusko-Label Certification are usually accessible to the birds starting on the 5th or the 6th weeks of age, depending on weather conditions. In this study observations started on week 6 and were conducted once per week until the end of rearing, at 12 weeks of age.

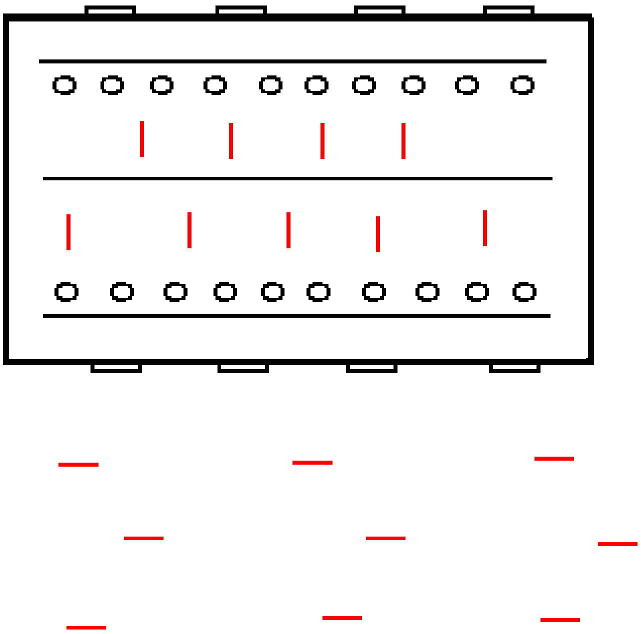

Figure 13. *Diagram showing the location of the perches or panels inside the house and in the outdoor area.*

Observations took place between 10:00 (approximated time when outdoor areas were accessible) and 17:00 h, with six observations per day. Data were collected by walking along predefined paths covering the entire indoor and outdoor areas of each house within the farm and locating as many tagged birds as possible. When a tagged bird was sighted, its location in XY coordinates and ID were collected with the Chickitizer software (Sanchez and Estevez, 1998) installed on a portable computer, with the help of a scaled reference blueprint of the indoor and outdoor areas that was attached to the screen of the computer. The independent variables recorded included farm, treatment, day, hour, and temperature. The temperature was measured using an electronic thermometer at the beginning of each scan (inside and outside). The time interval between the first and last observation per day (inside or outside) was approximately six hours. The starting point and house order was randomly chosen each day of observation.

At the end of the production cycle tagged birds were weighed at the slaughterhouse. These birds were also examined for the presence or absence of foot pad dermatitis (incidence was too low to use a full scoring scale and, if present, it was mild), bumble foot, hock burns, breast dermatitis, breast blisters, keel bone deformations and comb peck wounds using the Welfare Quality ® scale (Welfare Quality ®, 2009). Birds were also measured for fluctuating asymmetry of tarsal length and width as well as wing length. Measures were taken with a digital calliper following the procedure described by (Campo and Prieto, 2010; Bizeray et al., 2002a). Relative fluctuating asymmetry (RFA) was defined as the absolute difference between the right and left legs, or wings, divided by the mean of the left and right measures (Møller et al., 1995).

4.2.4. Use of space calculations

4.2.4.1 Indoors

For the use of space analysis indoors we delineated two areas, the central area, where the enrichment in the form of panels or perches was placed, and the exterior area near the walls. We included a one meter buffer zone around the edge of the panels and perches in the central area. For statistical analysis we calculated the ratio of tagged birds in the central area compared with the total number of tagged birds observed.

From the locations in XY coordinates of the tagged birds we calculated the total, net, maximum and minimum distances travelled as described previously by Leone and Estevez (2008b). In brief, total distance was defined as the sum of Euclidean distances$(d(x, y) = \sqrt{(\ y2 - y1\ ^{2} + \ x2 - x1\ ^{2})})$ between sequential locations for each tagged bird on a given day. Net distance was calculated as the Euclidean distance between the first and last location for each bird on each day. The maximum distance was defined as the furthest distance, and the minimum distance was the smallest distance, between consecutive locations travelled by a tagged bird within a day. Because the number of sightings for each tagged bird varied for each day we only used data from birds which were observed a minimum of three times for a given day.

In addition, from the XY coordinates we determined long term use of space by calculating the core area of tagged birds at 50, 75 and 100%. Core areas are common parameters used to calculate areas of activity, or home ranges, and depicts the area which represent the likely probability (50, 75 or 100%) of observing a bird within the given core area (Estevez et al., 1997; Estevez and Christman, 2006; Leone et al., 2007; Mallapur et al., 2009). These percentages were chosen to represent varying degrees of space use. To estimate core areas we used nonparametric Kernel density estimation, which determines the probability of observing a subject at each point in space without making any assumptions about the distribution of the observation points (Worton, 1987, 1989). We calculated core areas using the 'adehabitat' package for R 2.14 (2008). Individual bird core areas were calculated using all observed locations during two distinct age periods, from 6 to 8 weeks of age (period 1) and from 10 to 12 weeks of age (period 2). The coefficient of variation (CV) at each core areas percentage was also calculated to determine the variability in space use among birds. Although other traditional methods are available for calculating space use, core areas consider both the location visited as well as the frequency of the visit to such location or nearby locations. The advantages of calculating core areas in captive animals as compare to other methods are discussed in detail in Estevez and Christman (2006).

2.4.2 Outdoor area

The use of the outdoor areas was much lower than expected, and therefore it was difficult to find the same tagged birds in the outdoor space throughout the day. This resulted in an insufficient number of tagged bird locations to perform the core area analysis as performed for the indoor data. Because of this limitation it was only feasible to calculate the mean percentage of use of the outdoor area, percentage of birds from the flock that were never observed outside, and net distance travelled. Net distance travelled in the outdoor area was calculated as the Euclidean distance between the first and last observed location for a bird on a given day. To estimate general use of the outdoor areas core areas at 50, 75 and 100% were calculated using the locations of all tagged birds combined.

4.2.5. Statistical analysis

The percentage of birds using the central area, percentage of birds that were never observed in the outdoor area, and the incidence of foot pad dermatitis (presence/absence) were analysed with a generalized linear mixed model (GLIM-

MIX) using SAS V9.2 (SAS Institute, Cary, NC, USA). To score foot pad dermatitis we followed the Welfare Quality ® scale (Welfare Quality ®, 2009) and calculated the mean score of the two pads; in individuals with scores equal to or below one food pad dermatitis was categorized as absent and for those with scores greater than one it was present. The remaining welfare indicators could not be statistically analysed due to the low frequency of occurrence.

Total, net, maximum and minimum distance travelled, core areas, the number of birds in the outdoor area, fluctuating asymmetry, and weight were each analysed using a linear mixed model (PROC MIXED) with a Kenward-Roger adjustment for the degrees of freedom (Littell et al., 2006). Because fluctuating asymmetry was not normally distributed, a log-transformation was used. For all parameters the means for the indoor and outdoor areas for each house were used in the statistical analyses.

In each linear mixed model we included treatment and weeks of age as fixed factors, and farm as random factor. To account for repeated observations at each farm we included a repeated measure term for weeks of age. For total, net, maximum and minimum distance, and the number of birds in the outdoor area, we also included temperature as a covariate. We included all two factor interactions in each model.

4.2.6 Ethical note

Farms participating in this study follow the guidelines of the Eusko-Label Certification Program of the Kalitatea foundation of the Basque Government. The study fulfilled the requirements of the European Directive 86/609/ECC regarding the protection of animals used for experimental and other scientific purposes.

4.3. Results

4.3.1 Use of the indoor areas

The results obtained indicated that the use of the central area was affected by EC treatment ($F_{2,6.53} = 5.65, P = 0.0376$), with a higher use in houses with panels and perches as compared to controls (64.10 ± 18.9; 67.22 ± 19.03; 24.31 ± 19.24% mean ± SE for panels, perches and control, respectively; panels vs. control $P = 0.026$, perches vs. control $P = 0.020$). No effects of weeks of age or the interaction with treatment were significant ($F_{6,63.14} = 1.43, P = 0.218; F_{12,51.42} = 0.68, P = 0.759$, respectively).

Regarding net distance travelled, we found no effect of treatment ($F_{2,36.3} = 1.90, P = 0.160$), weeks of age ($F_{6,40.8} = 1.58, P = 0.175$), or their interaction ($F_{12,39.2} = 1.43, P = 0.194$). The average net distance travelled between the first and last observation was 4.942 ± 1.076 m (mean ± SE), which increased with temperature ($F_{1,42.4} = 4.12, P = 0.048$). No significant effects were found across weeks of age for total distance ($F_{6,34.8} = 1.38, P = 0.25$), nor was there an interaction of treatment by weeks of age ($F_{12,31.8} = 0.72, P = 0.722$). However we did find an effect of treatment ($F_{2,35.8} = 4.06, P = 0.025$), and an interaction between treatment and temperature ($F_{2,37.5} = 5.40, P = 0.0087$) on total distance travelled (Fig. 14). These results indicate a negative effect of the temperature in the distance travel in the control treatment, while had no effect for the perch and panel treatments. No effects of EC treatment, weeks of age or their interaction were detected for minimum distance ($P > 0.05$; 2.587 ± 0.752 m, mean ± SE), or maximum distance ($P > 0.05$; 6.174 ± 1.253 m; mean ± SE).

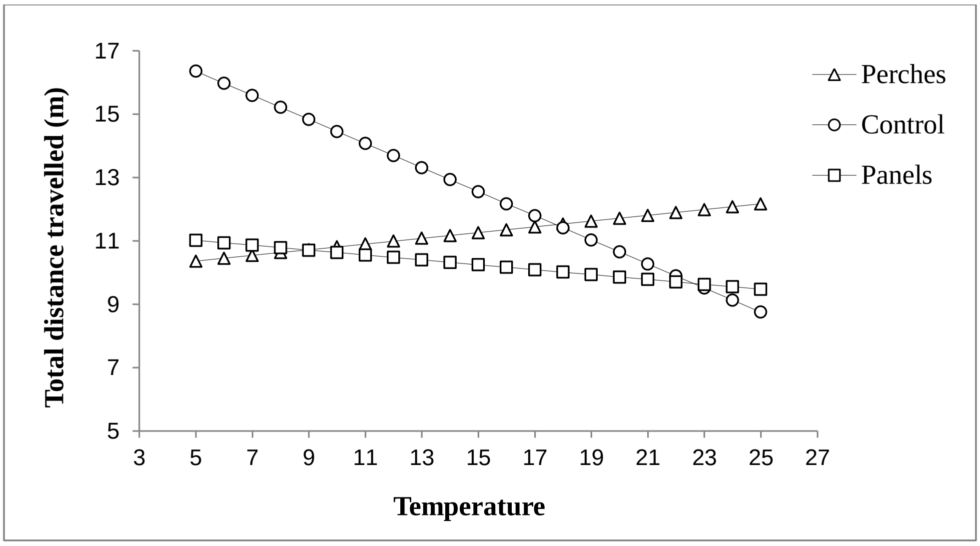

Figure 14. *The effect of temperature on the total distance travelled inside the houses according to the perch, panel and control treatments (treatments significantly different, P < 0.01).*

No significant effects were detected for treatment ($P > 0.05$), weeks of age ($P > 0.05$), or their interaction ($P > 0.05$, for 50, 75 and 100% core areas, respectively). Likewise, the coefficient of variation for each period and core area level did not differ ($P > 0.05$). Mean core areas inside were 4.365 ± 0.370, 10.744 ± 0.739, 26.025 ± 1.278 m^2 (mean ± SE) for 50, 75 and 100%, respectively with a coefficient of variation in the size of core areas was 28.860 ± 6.502, 23.703 ± 5.173, 14.056 ± 2.649% (mean ± SE).

4.3.2 Use of the outdoor areas

Presence of EC treatment did not have an effect on the number of birds using the outdoor areas ($F_{2,33.2}$ *= 0.45, P = 0.64*) or on the proportion of birds that were never observed using it ($F_{2,9}$ *= 0.71, P = 0.51*). An average of 1.842 ± 0.198 (mean ± SE) tagged birds were located outside per observation. This would be equivalent to 4.6% of the flock, assuming that tagged and non-tagged birds used the outdoor area in equal proportion. The mean percentage of tagged birds that were never observed in the outdoor area was 63.081 ± 5.378% (mean ± SE). A significant interaction between weeks of age and temperature was found ($F_{6,45.7}$ *= 2.87, P = 0.018*) for the number of birds using the outdoor area (Fig 15). These results indicate that the use of the outdoor area generally increased with weeks of age, but in older birds the use of the outdoor area was noticeable affected by the temperature.

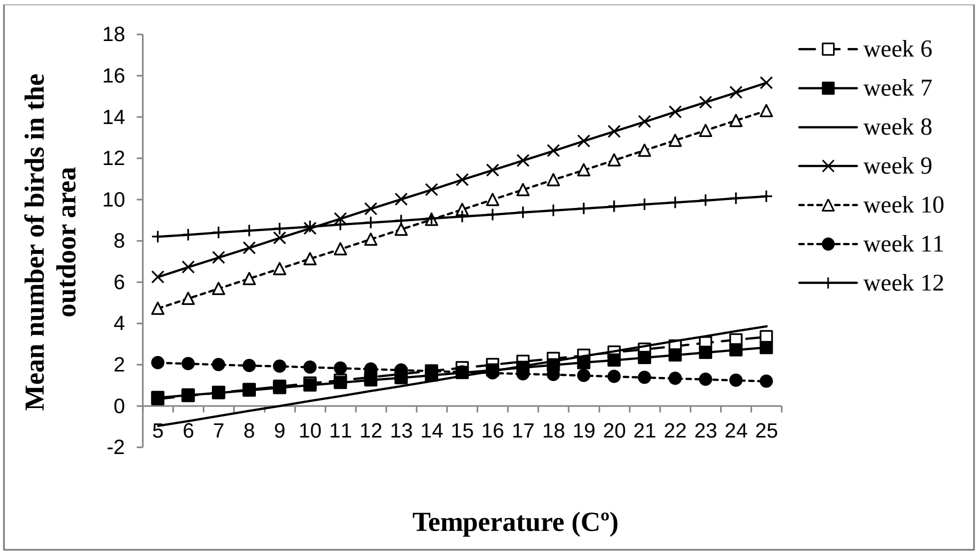

Figure 15. *The effect of temperature on the mean number of birds using the outdoor area across weeks of age (P < 0.05).*

EC treatment had no effect on the net distance travelled outside ($F_{2,5.52} = 0.33$, $P = 0.729$), but we did find a trend of greater net distances with increasing weeks of age ($F_{6,12.96} = 2.69$, $P = 0.063$), and a significant interaction between treatment and weeks of age ($F_{12,13.31} = 3.01$, $P = 0.032$, Fig. 16). These results show that net distance travelled in the outdoor area was increasing along weeks of age for birds in the perch treatment, while in panel and control treatments there results were variable.

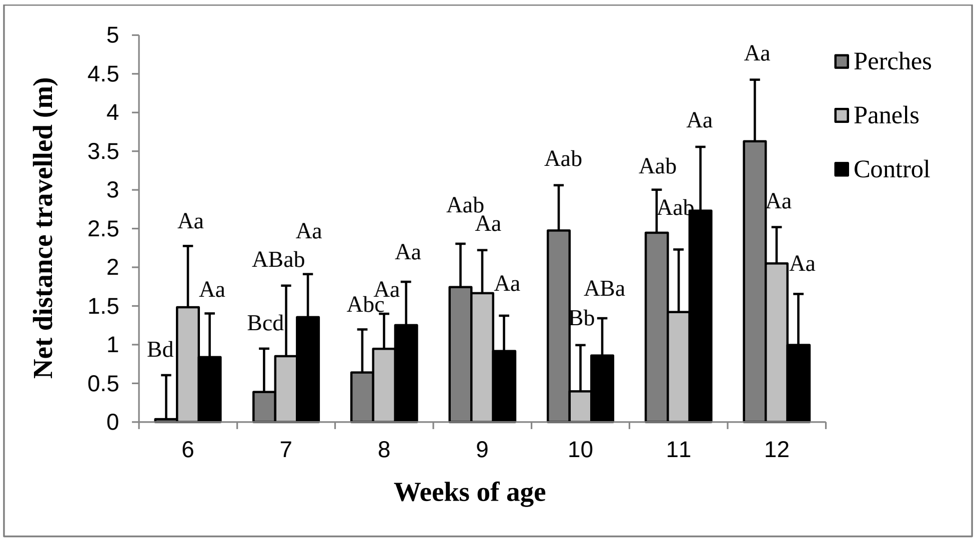

Figure 16. *The interaction between treatment and weeks of age on net distance travelled in the outdoor area (means ± SE). Small letters represent differences within treatments across weeks of age and capital letters represent differences across treatments within weeks. Means sharing any common letters are not statistically different (P > 0.05).*

Regarding the general use of outdoor space defined by the combined core areas, we found no effect of treatment ($P > 0.05$, at 50, 75 and 100% core areas, respectively).

However age period had a significant effect on the 50 and 100% core areas, which increased with age period ($F_{1,12}$ *= 13.94, P = 0.002;* $F_{1,12}$ *= 9.90, P = 0.008*, respectively, Fig. 17). Surprisingly there was no effect of age period detected for the 75% core area (*P > 0.05*). The interaction between treatment and age period was no significant (*P > 0.05*, for 50, 75 and 100% core areas, respectively). Further analysis indicated an effect of age period on the variation (CV) in the size of the core areas, at 50% and 75% ($F_{1,2}$ *= 34.62 P = 0.027,* $F_{1,2}$ *= 109.86 P = 0.009*, Fig. 18), which decrease in the second age period, but not for 100% (*P > 0.05*).

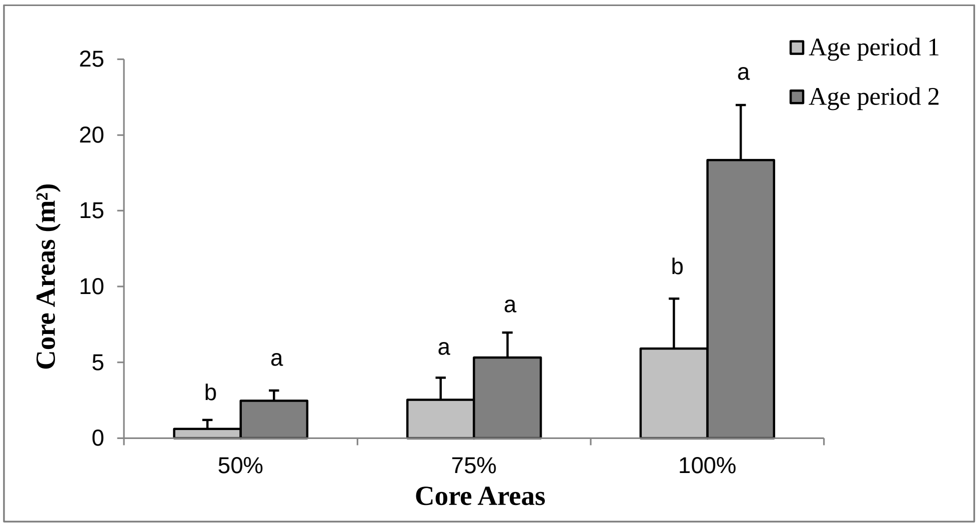

Figure 17. *Effect of age period on the 50, 75 and 100% outdoor core areas. Period 1 corresponds to weeks of age 6 to 8 while Period 2 corresponds to weeks of age 10 to 12. Means sharing any common letters are not statistically different (P > 0.05).*

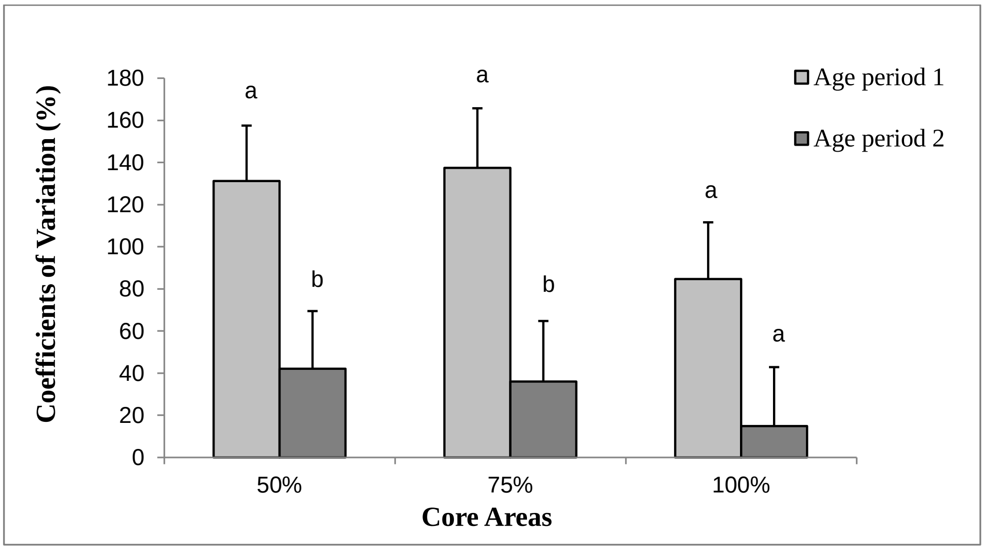

Figure 18. *Coefficient of variation for the 50, 75, and 100% core areas according to age periods. Period 1 corresponds to weeks of age 6 to 8 while Period 2 corresponds to weeks of age 10 to 12. Means sharing any common letters are not statistically different (P > 0.05).*

4.3.3 Welfare indicators

No differences were found across treatments for the relative fluctuating asymmetry values in wing length ($F_{2,6} = 0.74, P = 0.5167$), tarsus width ($F_{2,9} = 0.56, P = 0.589$), or tarsus length ($F_{2,6} = 1.70, P = 0.259$). Similarly, we did not find any differences between treatments for foot pad dermatitis ($F_{2,9} = 0.16, P = 0.854$) or daily growth ($F_{2.6} = 0.04, P = 0.962$). As indicated previously the incidence of bumble foot, hock burns, breast dermatitis, breast blisters, keel bone deformations and comb peck wounds were detected too infrequently to allow statistical analysis.

4.4. Discussion

This experiment was designed to study the potential benefits of environmental interventions in the form of vertical panels and perches on use of space and welfare in free-range slow-growing chickens. Our hypothesis was that the presence of vertical panels and perches would result in a more uniform distribution of birds and a higher use of the outdoor areas.

As hypothesized we found that increasing EC by provision of vertical panels or perches improved the use the central house areas, which increased from a 24% use in the control, to a 67% use in the perch treatment. These results agree with previous studies in broilers which found an increased used of central areas when panels were added (Cornetto and Estevez, 2001a). As suggested by the initial studies on the use of panels for poultry management (Cornetto et al., 2002), these devices create protected areas where birds can safely rest, avoid disturbances, or get away from aggressive conspecifics.

Theoretically the main function of the perches was to provide birds with perching opportunities. However, in our study the perches were likely high enough to provide birds with a protective effect from possible interactions with other birds. We would not have expected this finding if perches were only used as a roost. Previous studies have shown that simply placing frames in the central area, which provide no visual cover or barrier, can improve use of the central area (Cornetto and Estevez, 2001a; Newberry and Shackleton, 1997). Having an object in the central space may help to produce an aggregation effect, especially when performing comfort behaviours (Keeling, 1994). It is possible that when some birds found an appropriate location close to the perches other birds were attracted to those areas because of the cohesive effect of the flock.

The effect of the EC treatments in this study remained constant across ages, whereas (Cornetto and Estevez, 2001a) found a lower use of the cover panels in the first 2 weeks of age of the rearing period for broiler chickens. In their study sampling ages ranged from 1 to 6 weeks of age, while we observed birds from week 6 to 12 weeks of age. Therefore, the differences in age, the differences in growth rate and activity, and the lower rearing density used in the Eusko-Label Certified Program (12 birds/m^2) may explain the differences in results between the studies.

Results showed that in the case of perches there was a positive relationship between temperature and total distance travelled, while the opposite effect was observed in the control houses, and no effect was observed in the panel treatment. It has been suggested that birds use perches for thermoregulatory purposes (Estevez et al., 2002) so it is possible that when the temperature increased birds searched for a perch to use in order to facilitate thermoregulation by getting off the floor, thus increasing total distance travelled. On the other hand, in control treatments perhaps birds were more reluctant to expend additional energy and move at high temperatures, resulting in less total distance travelled.

In terms of use of space inside the house, as measured by the core areas, we did not find an effect of the EC treatments, which is the same result found by Leone et al. (2007). Nor did we find an effect of age period on core areas. The amount of space used, as measured by the 100% core area, was 26.025 ± 1.278 m^2, which represents about one quarter of the total amount of space available in the house. The lack of age period effects may be due to the fact that the observations started on weeks of age 6, when the birds were already habituated to the housing conditions. Most likely there was no reduction in the use of space over the weeks as slow-growing chickens maintain good activity levels throughout rearing and leg condition is excellent compared with data obtained from studies of broilers (Ekstrand et al., 1997; SCAHAW, 2000). Previous studies suggest that some birds use more restricted areas while others use all of the available space (Hughes et al., 1974; Appleby et al., 1989). Given that the 100% core areas only represented about a quarter of the total house space available it would suggest that there was a certain limitation in space use. However, plots of the 100% core areas across the entire study period, and the relatively low coefficient of variation, suggest that most birds used the majority of the house across the entire period of study (pers. observation).

We expected that provision of EC in the outdoor area would increase the frequency of use of the outdoor areas, which we did find. On average only 4.6% of birds used the outdoor area from weeks of age 6 to 12. This is similar to the frequency of use of outdoor areas by free range broilers (Dawkins et al., 2003; Weeks et al., 1994), with a peak use of 15% reported in Dawkins et al. (2003). The greatest use was observed during the summer, while less than 5% were observed in the outdoor area during winter and spring months. Although these results are not totally comparable due to weather differences between the regions and strain differences, our results suggest that temperature had an effect on use of the outdoor areas, and that this effect varied with weeks of age (Fig. 15). At the beginning of the study temperature had no effect, probably because the birds were not used to the new area and they needed time for habituation. The effect of the outside temperature seemed to be particularly relevant in the middle weeks of age (weeks 8, 9 and 10), as birds were using the outdoor areas and temperatures were warmer. Nevertheless, in the last weeks of age of rearing the effect of temperature again faded, although the reason for this is unclear.

Dawkins et al. (2003) indicated that domestic fowl prefer covered areas with trees and bushes and Kells et al. (2001) demonstrated that broilers were more active when provided with straw bales. Novel objects have previously been shown to stimulate movement in broiler chickens (Newberry, 1999). Theoretically then panels and perches could work as artificial substitutes for vegetation. We therefore expected that their presence would provide protective areas and increase use of the outdoor areas. The lack of effect of the EC treatments on the number of birds outside may have been due to the fact that they were perhaps placed too close to the pop-holes, decreasing the incentive for birds to venture farther into the outdoor area. We also noticed that during high winds the panels moved, potentially frightening the birds. Based on these results we believe that a larger number of EC devices are necessary in the outdoor area to provoke greater interest in the outdoor areas.

A significant treatment by weeks of age interaction on net distance travel outside was observed as distances grew with age in the perch treatment alone. The lack of positive effects of the panel treatment on net distance outside may have been due to the unstable structure of the panels under windy conditions, as mentioned earlier. For most weeks the net distances covered by birds in the perch treatment was greater (although not significantly so) than the controls. These results seem to suggest that greater perch availability in the outdoor areas may have some benefit on the distance travelled by the birds, and perhaps also increase use of the outdoor areas.

In general, the number of birds using the outdoor areas increased with weeks of age, as did the amount of space used, as measured by the general core areas. This could be due to habituation, meaning that the birds explored more of the space as they got accustomed to the area. Although the insufficient number of tagged birds in the outdoor area did not allow a more detailed analysis of the core areas outside, the general analysis based on locations from all tagged individuals indicated that the area use by all tagged birds was very small no more than 20 m^2 used from an average space availability of 2700 m^2. Clearly these results, together with the finding that over 60% of the birds were never seen in the outdoor area suggest that management practices should be developed and implemented in order to improve the use of the outdoor areas.

It has been suggested that increasing activity via environmental enrichment improves leg condition in broilers (Hester, 1994; Balog et al., 1997). Although previous studies with perches suggested that perch use in broilers was too low to have any relevant impact on leg condition (LeVan et al., 2000; Pettit-Riley and Estevez, 2001; Bizeray et al., 2002b), the use of perches has been shown to be effective at improving the degree of foot pad dermatitis (Ventura et al., 2010). Therefore, we expected to find an effect of the EC treatment on welfare indicators, particularly in regard to the incidence of foot pad dermatitis, but no effects were found. These results were most likely related to the slow-growth bird strain, the low rearing densities, and management practices under the Eusko-Label Certified program.

4.5 Conclusions

The results of this study showed that both perches and panels lead to a higher use of the central areas of the house, resulting in an improved use of the indoor space available. However, the EC treatment had very limited effects in regard to use of space of the outdoor area, with only mild effects detected in net distance travelled in the presence of perches. The use of the outdoor area was much lower than expected and many of the birds were never observed using the outdoor area. However, the small number of devices used in this study may, in part, explain the results obtained. Perhaps higher perch availability outdoors might have resulted in greater benefits. Furthermore, we consider that the structural design and stability of the environmental enrichment is an important factor in obtaining desired benefits. It remains clear that improved management practices are necessary to increase use of the outdoor areas for free range chickens.

Chapter 5:

Effects of panels and perches on the behaviour of commercial slow-growing free-range meat chickens

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Abstract

Environmental enrichment has the potential to benefit the welfare of farm animals. In poultry, panels and perches are two of the most commonly used forms of enrichment but few studies have determined their effects under commercial conditions. The aim of this work was to assess the impact of these forms of enrichment on the behaviour of slow-growing free range meat chickens. The study was conducted in four commercial farms each with 3900 birds, housed in three independent houses with access to an outdoor area. One house in each farm was outfitted with indoor and outdoor panels, the second with perches, and the third house was used as a control and had no enrichment. In each house 40 birds were tagged for individual recognition. Focal observations were performed from 6 to 12 weeks of age, with thirty 5 min focal samples collected in each house in the indoor and outdoor areas alternatively. In addition, the location (in XY coordinates) of tagged birds inside and out, and their behaviour, was also collected. We did not find a main effect of treatment on the behaviour inside or outside the house ($P > 0.05$). However, the interaction between treatment and week of age for standing ($P < 0.05$) indicated a general increase with week of age only for the perch treatment inside the houses, and for only the perch and control treatments in the outdoor area ($P < 0.05$). Resting decreased until week 9, while locomotive behaviours increased until week of age 10 in the outdoor area ($P < 0.05$), with both trends reversing afterwards. A higher percentage of birds performed locomotive behaviours more often in the central area of the house in the panel treatment as compared to the control ($P < 0.05$), with perch treatment showing intermediate values. Overall, environmental complexity had a limited effect on the behaviour of slow-growing meat chickens, although the perch presence translated into a higher percentage of standing. It is likely that the reduced effects of the environmental enrichment treatments would have been greater if more devices were introduced.

Key words: Environmental enrichment, Panels, Perches, Behavior, Free-range meat chickens.

5.1 Introduction

The physical environment of commercial chicken farms tends to provide limited protection areas with the exception of walls, bell drinkers and feeders that birds may use as 'cover' for protection. The lack of protective areas frequently results in birds clustering along the walls, leaving unused space in the centre of the houses (Newberry and Hall, 1990; Cornetto and Estevez, 2001a; Buijs et al., 2010; Rodriguez-Aurrekoetxea et al., 2014). When birds have access to an outdoor area, even though it may offer a wide variety of behavioural opportunities, a few dispersed trees may be the only structure that can be used for protection. A deficiency of areas where the birds can feel safe often results in a high reluctance of the birds to move away from the chicken house (Grigor and Hughes, 1993; Dawkins et al., 2003) where they can shelter in case of any perceived danger. This uneven use of indoor and outdoor space leads to a difference in the effective density of birds throughout the space which may lead to some problems such as increased disturbances in the wall area (Cornetto et al., 2002), overuse and erosion of the outdoor areas closer to the house (Breitsameter et al., 2014), or an excess of nutrients in such areas (Aarnink et al., 2006).

The provision of cover offers to animals' visual isolation from predators or conspecifics, a suitable habitat for resting, and protection from weather conditions (Elton, 1939). Because of these benefits, different forms of cover have been broadly used in captive animals with positive effects on their behaviour (Estep and Baker, 1991; Whittington and Chamove, 1995; Holierhoek and Power, 1995). In broiler chickens and layers, provision of panels indoors has been shown to be effective at modifying birds´ behaviour, increasing their motivation to explore new areas and stimuli, improving the use of central underused indoor areas, and reducing the incidence of disturbances (Newberry, 1995; Newberry and Shackleton, 1997; Cornetto and Estevez, 2001 (a, b); Cornetto et al., 2002). A study on the effects of panels in commercial broiler breeder houses found increased male home ranges, improved female reproductive performance and a consequent economic benefit for farmers (Leone and Estevez, 2008a).

Roosting is a natural behaviour of both jungle (Collias and Collias, 1967) and the domestic fowl (Blokhuis, 1984). Perches offer birds the possibility to use the third spatial dimension, and can increase movement and exercise while jumping on and off of them as birds move around (Bizeray et al., 2002b). Several studies have investigated the use of perches by broiler chickens with variable results (LeVan et al., 2000; Bizeray et al., 2002b; Ventura et al., 2012). The highest perch use was found in the study by Ventura et al. (2012) with up to 25% of perches used during the day at 4 and 5 weeks of age. Nonetheless, perch provision can have additional benefits as it has been shown to decrease the frequency of disturbances and aggressive interactions and to improve the use of central areas (Ventura et al., 2012), similar to the findings by Rodriguez-Aurrekoetxea et al. (2014). Therefore, using panels and perches to make the outdoor areas more complex and interesting for slow-growing meat chickens may increase their usage by the birds.

It has been shown that in free-range broiler chickens, the use of the outdoor area depended on environmental factors such as temperature, sunlight, or tree cover (Dawkins et al., 2003). Nevertheless, even under optimal weather conditions the use of the outdoor areas was shown to be low with a maximum of 14.3% of use (Dawkins et al., 2003). It is possible that increasing the complexity of the environment, favouring vertical space use, and providing cover in the outdoor areas may encourage greater use of outdoor space and a wider behavioural repertoire, especially in more active slow-growing meat chickens. Additionally, the presence of the same enrichment devices inside and outside could improve the use of the outdoor area as a consequence of imprinting or familiarity (Grigor et al., 1995c). Therefore, providing the same enrichment inside and in the outdoor area could be a good strategy to increase use, dispersion, and activity in the outdoor area.

The aim of this study was to determine the potential benefits of panels and perches both indoors and in the outdoor areas on the behavioural activity of slow-growing free range meat chickens under commercial conditions. We predicted that perches and panels would modify the time that birds spent performing different behaviours and the location in which they were performed. Also, we expected an increase in the variety of behaviours that birds performed in the outdoor areas.

5.2 Material and Methods

5.2.1 Farms and animals

Four commercial free-range slow-growing meat chicken farms were selected for this study. All farms had similar features and management because they produced under the Eusko-Label Certification Program. The birds in all four farms were slow-growth Sasso T44 females, with a minimum rearing period of 82 days which is required by the certification program. The T44 bird is characterized by a lower growth rate as compared to a slow-growing broiler chicken that may also be used for free-range production in other regions.

Each farm in the study consisted of three 110 m^2 houses, each with its own fenced outdoor area measuring approximately 2700 m^2. Birds had access to the outdoor area from 10:00 a.m. until dusk (19:00-21:00). At each farm a total of 3900 female chicks were housed together for brooding from arrival, on day one, until 5 weeks of age. Then they were divided in equal group sizes of 1300 across the three houses at a density of 12 birds per m^2 (27 kg/m^2 approximately). Once birds were moved to their respective rearing houses with the corresponding treatments, 40 random birds per house (120 birds per farm) were tagged in the neck for individual identification. Tags were made of laminated cream paper disks 4 cm in diameter with a unique two digit black number printed on both sides (modified from Cornetto and Estevez, 2001b). This study was carried out from March to September of 2012.

5.2.2 Experimental design

This experiment was designed as a completely randomized block design consisting of three treatments; panels, perches, and control which were assigned randomly to each house. For the panel treatment we placed nine lightweight panels inside the houses and nine panels in the outdoor area. The interior panels were placed in two rows perpendicular to the pop-holes along the house, at a distance of 2 m from each other. The outdoor panels were placed in three rows, starting 2.5 m away from the pop-holes, in parallel to the house at 2 m distance from one another. The setup was exactly the same for the perch treatments, except that we used perches in place of panels. The panels were built in a square (0.5 × 0.5 m) from PVC pipe (diameter 2.5 cm) with two 30 cm legs, coated on the inside with green plastic mesh (modified from Cornetto et al., 2002). The design of the perches was modified based on results from previous studies in broiler chickens. Perches in our study were higher than in Bizeray et al. (2002b) and Ventura et al. (2012), as we expected slow-growing meat chickens to be more agile than broiler chickens. Wooden perches were 50 cm long, 25 cm high and 4 cm wide, attached to a base (two 20 cm × 5 cm × .5 cm) that was hidden in the litter. Considering an average of 10 cm of space per bird, perches and panels were available for a maximum of 7% of the birds to perch simultaneously, 3.5% inside and 3.5% in the outdoor area of the birds' house (see also Rodriguez-Aurrekoetxea et al., 2014 for further details).

For the first month, to avoid impairment of spatial cognitive skills (Gunnarsson et al., 2000) and to habituate birds to the enrichment devices and identification tags, we randomly placed one perch, a panel and several ID tags in the brooding houses upon arrival of the birds.

5.2.3 Data collection

Starting at 6 weeks of age, observations were performed once per week, between 10:00 and 17:00, until the end of rearing at 12 weeks of age by the same observer. Observations were collected with the software Observer XT v. 10.0 (Noldus Information Technology, Netherlands). Each day of observation we collected 5 min focal samplings of 15 randomly chosen tagged birds, inside the house and in the outdoor areas, for a total of 30 focal observations per day and treatment (90 per farm).

The data collection was carried out in alternating sets of five focal observations inside each house with another five observations in the corresponding outdoor area. This procedure was repeated three times during the day until the 30 focal observations per treatment were completed (15 inside and 15 in the outdoor area). A 5 min period for habituation to the presence of the observer was allowed before data collection. The ethogram used consisted of a combination of states and events as described by Estevez (1994) and Cornetto and Estevez (2001b) (Table 5). The independent variables recorded for each focal observation were farm, treatment, day, time and temperature, although the temperature was not used in the behavioural analysis. In some cases, as the frequency of birds in the outdoor area was low, we could not take focal observations from all five tagged birds. Therefore, observations were conducted on the maximum number of birds available for the corresponding observation set. When a bird was outside and went inside or vice versa, the observation was discarded and when possible, a new bird was randomly selected.

After the collection of each set of 5 focal birds, the observer walked slowly along predefined transects through the interior or the outdoor area of the house recording the location of all tagged birds in XY coordinates and their instantaneous behaviour with the software Chickitizer (modified from Sanchez and Estevez, 1998). For further details on the methodology see Rodriguez-Aurrekoetxea et al. (2014), where the results regarding space use were reported. In this manuscript XY data were used to determine specific behavioural differences between central and wall areas.

3.2.4 Statistical analysis

From the focal observations, the percentage of time in each behavioural state and the frequency of events were calculated per bird. The mean percentages of time, or frequency for each behaviour, were calculated per location (indoors or in the outdoor area) for each house. Due to the smaller number of observations obtained in the outdoor area as compared to indoors, we did not consider it appropriate to compare the time budgets across locations. Therefore the 'location' factor was not included in the model, and time budgets were analysed separately for each location.

Table 5. *Ethogram used during observations (Modified from Cornetto and Estevez, (2001b) and Estevez, (1994)).*

Behavioural States	
Foraging	Bird pecks and scratches the substrate while standing or slowly walking forward with head below rump level.
Resting	The bird lies down with the sternum resting on the substrate. A resting bout ends when the birds rights itself.
Standing	The bird maintains an upright position on extended legs for at least a second.
Walking	Starts when bird takes one or more steps forward in succession
Running	Starts when bird takes two or more steps forward in rapid succession
Self-preening	Bird arranges or oils her feathers with the beak
Flying	Starts when bird extends and flaps her wings and moves a distance through the air.
Wing-flapping	Bird shakes her wings at least twice. Can move forward or remain stationary.
Dust bathing	Bird lies down and pulls loose substrate close to its body to toss it and distribute above its body with a series of legs and wing movements.
Perching	The bird is positioned on the perches or panels, either in a standing or resting position
Eating	The bird place its head inside a feeder
Drinking	The bird drinks in the drinker or outside in a puddle.
Behavioural Events	
Threating	The bird raises the head and neck quickly, staring at the opponent directly and appearing to be about to peck aggressively.
Face-off	Two birds remain with the head facing the same height over two seconds.
Fighting	Two birds facing, heads and necks raised to the same height, kicking and pecking at least twice.
Chasing	The bird runs at least three steps in pursuit of another bird.
Aggressive peck	A fast and strong pecking directed towards the head of another bird.
Jumping	One bird or both jumps towards one another. No pecks are observed.
Sparring	Two birds simulate a fight with no physical contact.

Data were checked for normality and homogeneity of variance and analysed using a mixed model in SAS V9.3 (SAS Inst. Inc., Cary, NC) with treatment (nested within farm), week of age, and their interaction as fixed factors, farm as a random variable and week of age as the repeated measure. A Kenward-Roger adjustment for the degrees of freedom was used for all the analysis (Littell et al., 2006).

Due to the low occurrence of some behaviours we pooled all walking and running behaviours into the category 'locomotive behaviours', and all self-preening, dust bathing, and wing flapping behaviours in the 'comfort behaviours' category. Aggressive behaviours included all chase, fight, jump, aggressive peck, stand-off and threat events. For the analysis of locomotive and comfort behaviours occurring in the outdoor area, data were log transformed because they were not normally distributed. In other instances, despite the regrouping of the behaviours, the frequency of aggressive events was too low to permit any statistical analysis, even when considering their sum of aggressive episodes during the whole study period. As drinkers and feeders were not available in the outdoor area, these two behaviours were only considered indoors, and perching behaviour was only considered in the perch treatment.

We also analysed the behavioural differences according to the birds' position in the house while indoors. This was done by dividing each house into two areas, the wall area (46 m^2) and the interior (64 m^2), which contained either perches, panels or was empty (control). A one meter buffer zone around the edge of the perches and panels was included to calculate the interior area. We used a generalized linear mixed model assuming a binomial distribution to compare the percentage of birds performing resting, standing, and walking, in the central area. The model included treatment (nested within farm), week of age, and their interaction as fixed factors, farm as random factor and week of age as the repeated measure. We only used resting, standing and locomotive behaviours because these were the only behaviours which provided sufficient data for analysis.

5.2.5 Ethical note

Farms participating in this study followed the guidelines of the Eusko-Label Quality program of the Basque Government. The study fulfilled the requirements of the European Directive 86/609/ECC regarding the protection of animals used for experimental and other scientific purposes.

5.3 Results

We found no effect of the treatments on the percentage of time spent performing the behaviours of interest indoors (Table 6). Age only affected the proportion of time spent in comfort behaviours which increased until the 8th week of age and then decreased (Table 7). There was an interaction between treatment and week of age for standing (Table 6). As shown in Figure 19 differences in time standing between treatments were only observed during the 8th and 10th week of age. In the 8th week the proportion of time standing was higher in the control than in the panel treatment while for the 10th week was higher in the perches treatment than in the control treatment. No differences across treatments or for the interaction between treatments and week of age were observed for any other behaviour (Table 6).

Table 6. *Results of the ANOVA for the effects of treatment, week of age, and their interaction for behaviours inside the house.*

	Treatment		**Week of age**		**Treatment * Week of age**	
	F-value	**p**	**F-value**	**p**	**F-value**	**p**
Standing	$F_{2,9}$ =1.16	0.356	$F_{6,54}$ =1.46	0.210	$F_{12,54}$ =2	**0.041**
Resting	$F_{2,6}$ =2.93	0.130	$F_{6,54}$ =0.46	0.836	$F_{12,54}$ =0.7	0.744
Foraging	$F_{2,9}$ =1.55	0.265	$F_{6,54}$ =1.09	0.383	$F_{12,54}$ =1.45	0.174
Drinking	$F_{2,8.07}$ =0.29	0.759	$F_{6,39.1}$ =0.92	0.494	$F_{12,38.6}$ =1.29	0.265
Eating	$F_{2,9}$ =1.09	0.377	$F_{6,54}$ =1.33	0.258	$F_{12,54}$ =0.34	0.977
Comfort Behav.	$F_{2,6}$ =0.05	0.952	$F_{6,54}$ =2.48	0.034	$F_{12,54}$ =1.20	0.307
Locomotive Behav.	$F_{2,9}$ =0.18	0.838	$F_{6,54}$ =1.23	0.307	$F_{12,54}$ =0.81	0.640

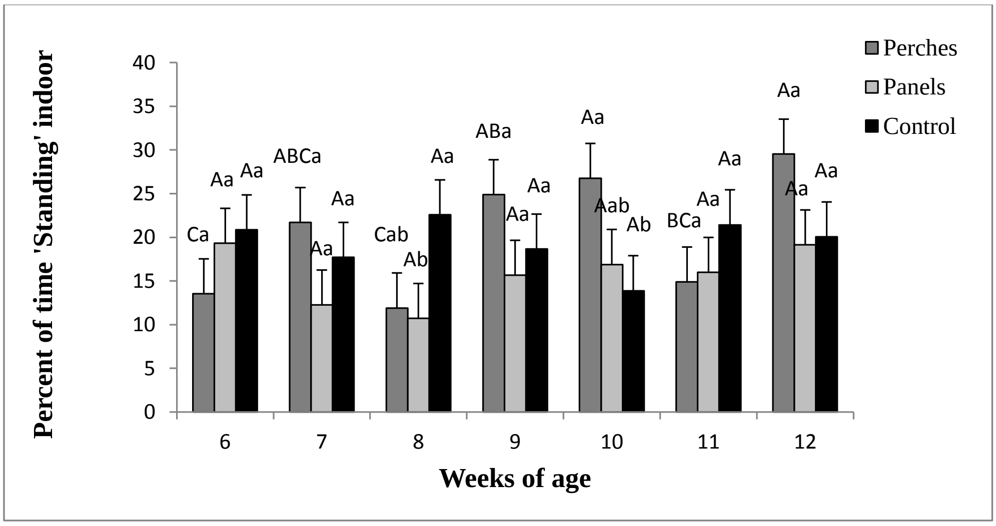

Figure 19. *Effect of the interaction between treatment and week of age on the percentage of time standing inside the house (mean ± SE). Capital letters refer to differences across weeks of age within treatment. Lower case letters refer to treatment differences within week of age. Within each comparison different letters (a, b or A, B) are statistically different (P<0.05).*

Table 7. *Effect of age on the percentage of time (mean ±SE) spent performing different behaviours inside the house. The Perching and Aggression columns show the frequency observed during the 5 minutes focal observation. Means within columns with different letters (a-b) differ significantly (P<0.05).*

Age (wk)	Resting	Standing	Foraging	Eating	Drinking	Perching	Comfort behaviours	Locomotive behaviours	Aggression
6	45.52 ± 4.00	19.09 ± 2.20	4.81 ± 1.23	11.16 ± 2.27	3.63 ± 1.10	2.39 ± 1.51	7.23 ± 1.30 [a]	6.13 ± 0.90	0.01± 0.01
7	49.60 ± 3.81	17.08 ± 2.10	4.58 ± 1.40	6.81 ± 1.92	4.15 ± 1.08	2.78 ± 1.28	7.16 ± 1.52 [ab]	7.81 ± 1.63	0.12± 0.05
8	48.75 ± 3.83	16.01 ± 2.30	6.42 ± 1.94	5.32 ± 1.41	2.78 ± 0.88	3.44 ± 1.66	11.19 ± 2.18 [a]	6.06 ± 1.18	0.13 ± 0.06
9	54.15 ± 3.80	20.04 ± 2.48	3.12 ± 0.77	4.17 ± 1.26	2.78 ± 1.01	1.63 ± 1.39	8.84 ± 1.57 [a]	5.22 ± 1.29	0.10 ± 0.04
10	49.12 ± 3.87	20.97 ± 2.24	4.64 ± 0.97	5.34 ± 1.81	3.94 ± 1.17	0.29 ± 0.29	10.51 ± 1.72 [a]	5.16 ± 0.95	0.18 ± 0.12
11	54.93 ± 3.39	18.56 ± 2.18	4.65 ± 1.25	6.58 ± 1.79	1.31 ± 0.57	1.59 ± 1.34	7.90 ± 1.49 [a]	4.43 ± 0.61	0.02 ± 0.01
12	47.91 ± 4.27	26.70 ± 2.97	3.60 ± 1.00	7.24 ± 2.28	5.77 ± 1.58	0 ± 0	4.75 ± 1.32 [b]	3.99 ± 0.60	0.07 ± 0.5

To better determine the effect of the panels and perches, separate analyses were carried out on the behaviour performed in the central indoor area where the panels and perches were placed as compared with the wall areas. A treatment effect was found for the percentage of birds performing locomotive behaviours in the central area ($F_{2,20.33} = 6.45$, $P = 0.046$) but not across week of age ($F_{6,45.16} = 0.58$, $P = 0.744$), or for their interaction ($F_{12,31.35} = 0.93$, $P = 0.534$). The percentage of birds performing locomotive behaviours in the central area was higher in the panel treatment compared with control ($P = 0.013$, 60.491 ± 3.98, 51.642 ± 3.47, 45.540 ± 3.81 (mean ± SE) for panel, perch and control, respectively). No other significant effects across treatments, weeks of age or their interaction were detected ($P > 0.05$).Similar to the results obtained inside the houses we found no main effect of treatment on the behaviour of the birds in the outdoor area only an interaction between treatment and week of age for the percentage of standing (Table 8). Standing occurred more often during the 7th week of age in the panel treatment as compared to the perch treatment and the control. Contrarily, during the 11th week of age, birds in the control treatment stood more than in the other treatments (Fig. 20). The time birds spent standing in the outdoor area increased across age in the control treatment, but remained constant in the perch treatment and did not show any clear trend in the panel treatment (Fig. 20).

Table 8. *Results of the ANOVA for the effects of treatment, week of age, and their interaction for behaviours observed in the outdoor area.*

	Treatment		**Week of age**		**Treatment * Week of age**	
	F-value	**p**	**F-value**	**p**	**F-value**	**p**
Standing	$F_{2,32}$ =0.21	0.814	$F_{6,32}$ =2.11	0.080	$F_{12,32}$ =2.59	0.015
Resting	$F_{2,11.9}$ =1.65	0.234	$F_{6,24.4}$ =5.71	<0.001	$F_{12,22.3}$ =1.33	0.271
Foraging	$F_{2,14}$ =0.46	0.638	$F_{6,25.7}$ =1.04	0.425	$F_{12,23.6}$ =0.69	0.742
Comfort Behav.	$F_{2,11.4}$ =1.28	0.316	$F_{6,21.6}$ =2.46	0.057	$F_{12,19.7}$ =1.78	0.123
Locomotive Behav.	$F_{2,4.58}$ =3.51	0.120	$F_{6,30.4}$ =2.42	0.049	$F_{12,28.8}$ =0.92	0.543

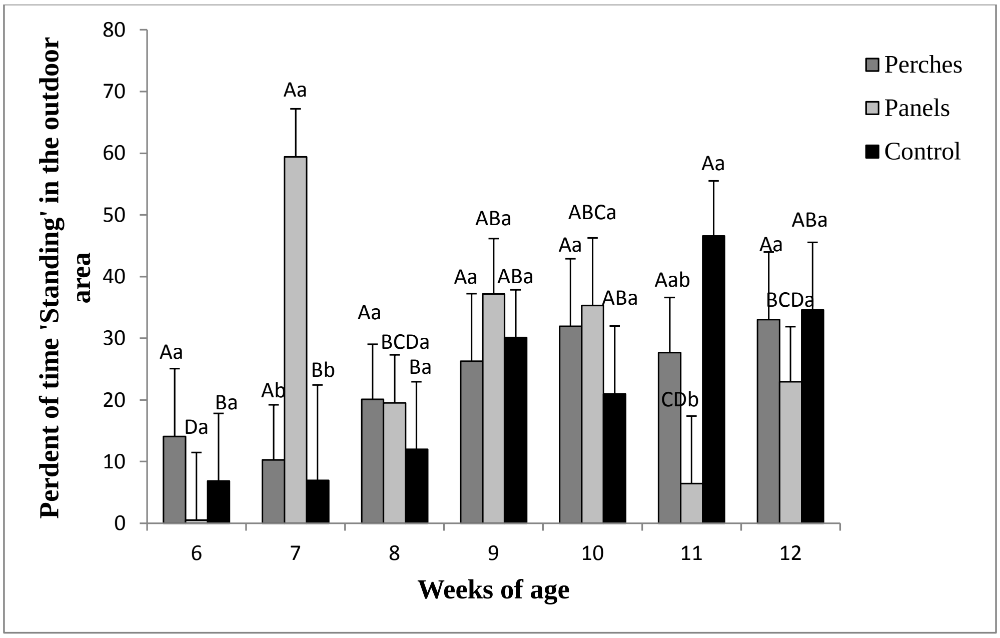

Figure 20. *Effect of the interaction between treatment and week of age on the percentage of time standing in the outdoor area (mean ± SE). Capital letters refer to differences across week of age within treatment. Lower case letters refer to treatment differences within week of age. Within each comparison different letters (a, b or A, B) are statistically different (P<0.05).*

Besides these effects there were differences across week of age for resting and locomotive behaviours, and comfort behaviours in the outdoor area was nearly significant (Table 8). Resting tended to decrease with age until week 9, while locomotive behaviours tended to increase until week 10, with both trends reversing afterwards (Table 9). Aggressive interactions occurred only sporadically and did not permit statistical analyses. However, means observed per week of age for aggressive interactions and for perching inside and in the outdoor areas are reported in Tables 7 and 9, respectively.

Table 9. *Effect of age on the percentage of time (mean ±SE) spent performing different behaviours in the outdoor area. The Perching and Aggression columns show the frequency observed during the 5 minutes focal observations. Means within columns with different letters (a-b) differ significantly (P<0.05).*

Age (wk)	Resting	Standing	Foraging	Eating	Drinking	Perching	Comfort behaviour	Locomotive behaviour	Aggression
6	75.26 ± 6.77[a]	8.84± 3.02	5.95 ± 5.40	0 ± 0	0 ± 0	0 ± 0	4.90 ± 0.57	5.02 ± 0.45[b]	0 ± 0
7	38.02 ± 9.81[b]	24.99 ± 5.67	17.56 ± 4.90	4.41 ± 4.41	0.59 ± 0.59	0 ± 0	1.83 ± 0.57	10.43 ± 0.46[ab]	0.30 ± 0.15
8	44.90 ± 7.98[b]	17.45 ± 3.25	17.17 ± 5.91	0.19 ± 0.19	0.24 ± 0.24	0 ± 0	8.13 ± 0.48	9.40 ± 0.38[b]	0.44 ± 0.29
9	28.06 ± 6[b]	28.63 ± 3.76	14.07 ± 3.81	0.26 ± 0.26	1.22 ± 0.85	0 ± 0	6.50 ± 0.48	18.16 ± 0.40[a]	0.24 ± 0.13
10	8.04 ± 4.74[c]	37.45 ± 6.56	23.32 ± 7.12	0.69 ± 0.69	0 ± 0	0.42 ± 0.33	2.18 ± 0.58	27.87 ± 0.46[ab]	1.31 ± 0.90
11	21.34 ± 8.19[bc]	32.25 ± 6.52	18.25 ± 6.42	0.06 ± 0.06	4.08 ± 1.94	0 ± 0	2.29 ± 0.49	17.82 ± 0.40[a]	0.16 ± 0.12
12	30.22 ± 8.63[bc]	29.39 ± 4.83	6.25 ± 2.39	0 ± 0	1.22 ± 0.89	0 ± 0	13.65 ± 0.52	9.81 ± 0.42[ab]	0 ± 0

5.4. Discussion

In this study conducted under commercial conditions we found only mild effects of the enrichment treatments on behaviour of slow-growing free-range meat chickens. An interaction of treatment with week of age was found for standing, both indoors and in the outdoor area, together with a higher percentage of birds in locomotion detected in the central areas for the panel treatment as compared to wall areas.

These results contrast with previous results for broilers in which provision of panels and perches resulted in clear increased resting and decreased disturbances, aggressive interactions, standing, and foraging behaviours (Cornetto and Estevez, 2001b; Cornetto et al., 2002; Donaldson and O'Connell, 2012; Ventura et al., 2012). In slow-growing meat chickens enrichment interventions in the form of panels under experimental conditions resulted in a reduced frequency of aggressive interactions (Estevez et al., 1998), and increased preening and standing in layers (Newberry and Shackleton, 1997). It should be noted that although in this study the rearing density used indoors (12 birds/m^2) was comparable to the range of densities used in Cornetto and Estevez (2001a, b), Cornetto et al. (2002) and Ventura et al. (2012), group sizes were very different. At constant density, larger group sizes imply greater total area available to move around, assuming no social restriction or territoriality in the use of the indoor and outdoor space, as was suggested from the results of the use of space analysis (Rodriguez-Aurrekoetxea et al., 2014), and can be seen by the lack of aggressive interactions found in this study. Therefore, it is possible that the greater space availability may have either limited or diluted the effect of the enrichments. Additionally, although the flocks were exposed to one panel and perch during the brooding period for habituation, full exposure to each treatment was only possible once the brooding flock was split and moved to their respective rearing houses at five weeks of age. It is possible that the much later exposure to the full perch and panel treatments may have reduced their impact as compared with broiler studies where the full treatment was introduced from day one.

In addition to the differences related to density, group size and total space available across studies, the existing disparities in growth rate between fast growing broilers and the slow-growing meat chicken strains can shape their behaviour (Bokkers and Koene, 2003). As indicated by our results, slow growing chickens were far more active both indoors and in the outdoor area. This was evidenced by the lower proportion of time resting and higher level of standing or time spent foraging and in locomotion (Tables 7 and 9) as compared to broilers (Cornetto and Estevez, 2001b). However, because of their higher activity, lack of leg problems and their ability to jump on and off the perches, it could be expected that the enrichments would have had a greater effect in these birds as compared to less active broilers (Bokkers and Koene, 2003), but this was not the case in our study. This trend of high activity remained throughout most of the observation period, except for the 6th week of age, when resting was considerably higher. This result could reflect the novelty of access to the outdoor area, as this was the first week when birds had access to the outdoor area.

Given the higher activity level of slow-growing birds, a clear effect of the treatments on the decrease of aggressive interactions was expected. On the contrary, the frequency observed (Tables 7 and 9) was so low that it was not feasible to perform statistical analyses. These results differ from the effects of panels, perches and straw bales on the decrease of the frequency of aggressive interactions and disturbances previously reported in broilers (Cornetto et al., 2002; Pettit-Riley et al., 2002) and broiler breeders (King, 2001). It is possible that the low level of aggression might have been due to the social tolerance that is expected in large groups (Estevez et al., 1997). According to this theory we would expect the frequency of attacks to be lower in a large group as this strategy is more cost effective (Pagel and Dawkins, 1997). In addition the large areas where the birds could disperse and freely roam may reduce the chances for interactions, and therefore, dilute the effects of the enrichments.

In our study, we detected an interaction of treatment with week of age for standing inside the house (Fig. 19) and in the outdoor area (Fig. 20). Inside the house, the effect of the treatment was evident at 8 weeks of age, with less standing in the panel and perch treatments as compare to the control. On the contrary, at 10 weeks, less standing was observed in the control treatment as compared to the perch treatment (Fig. 19). We also found that birds provided with panels performed more locomotive behaviours in the central area indoors (over 10% more) as compared with the control; the perch treatment remained at intermediate values. These results are in line with the higher bird movement found by Kells et al. (2001) when straw bales were provided in broiler houses, although in contrast Bailie et al. (2013) failed to find an effect on time budgets.

The lack of consistency in some of the results obtained in this study is difficult to explain. The use of space analysis indicated a higher use of the indoor central area with enrichment, especially with cover panels (Rodriguez-Aurrekoetxea et al., 2014). It is possible that until the 8th week of age, both panels and perches may have offered protection to the birds, as the perches were high enough to offer some form of cover while the birds were relatively small. Later in the growing phase, when perches may have lost their protective effects due to the larger size of the birds, and even though the use of the perches was low during the day, occasional jumping birds may have disturbed others around the perches causing them to stand up as observed during week 10, and to a certain extent in week 12 (Fig. 19).

Standing in the outdoor area was the only behaviour that was significantly affected by the interaction of treatment and week of age. It followed an even more variable pattern, with more standing during the 7th week of age for the cover treatment, which decreased by week 11. While perches may not have provided suitable cover during the entire growing period, we did expect panels to be effective due to the positive results shown in the scientific literature. However, although the panels worked well indoors, it is possible that in this study they were not sufficiently attractive to the birds in the outdoor area due to their light weight (to facilitate management and reduce cost) making them unstable in windy conditions and possibly frightening the birds. Furthermore, the use of the outdoor area was much lower than initially expected (Rodriguez-Aurrekoetxea et al., 2014) and it is possible that available fences and some natural cover in the outdoor area were sufficient to provide cover to the reduced number of birds using the outdoor area.

Additionally, we found that the frequency of locomotive behaviours in the central area was greater in panel treatment compared to controls, but did not differ from the perch treatment. The increased locomotion have been a consequence of the birds going back and forth between the resting areas provided by the enriched environment and the drinkers and feeders located in adjacent areas of the house.

On the other hand, the percentage of time of perch use by tagged birds inside was 1.7% similar to the 2% found by in LeVan et al. (2000) but for perches provided in the outdoor area it was only 0.06% of the time. Total perch availability only allowed 7% of the birds to use them at any one time. Perch use may have been greater if more perching space was available, as found by Nielsen, (2004) or Ventura et al (2012). However in no instances during the observation period were perches fully occupied. From the 9^{th} week of age onwards, as birds were reaching heavier body weights, perch use decreased

(Table 8). This effect of age is somewhat similar to findings in broilers (LeVan et al., 2000, Pettit-Riley and Estevez, 2001, Ventura et al., 2012) in which perch use declined from 4 weeks of age onwards.

It is likely that outdoor areas represented a novel environment for the birds. As the birds grew older they may have become more used to these areas and therefore less hesitant to explore this space. While a relationship between the availability of vegetative cover and use of the outdoor areas has been documented (Dawkins et al., 2003), the accessibility of perches or panels in our study did not affect the birds' time budgets when using the outdoor area. Neither did we detect an effect of treatment on the percentage of birds using the outdoor areas (Rodriguez-Aurrekoetxea et al., 2014). Nevertheless our previous findings showed that as birds became older they travelled longer distances when the outdoor area included perches. However, the limited effects would probably improve if more perches were available and if the structure of the outdoor panels was improved for outdoor conditions.

5.5 Conclusions

The results of this initial study suggest that the provision of panels and perches in slow-growing free-range meat chickens has limited impact on the behaviour, although their effects were somewhat stronger indoors. There was a clear effect of age on the behaviour of birds, but the changes occurred predominantly in the outdoor area as birds got used to the new space which led to increased locomotion and decreased resting in the outdoor area. However it is necessary to keep in mind the limitations of this study in terms of the availability of panels and perches. It is possible that higher availability of devices and a more stable panel structure would have led to different, more promising results.

Chapter 6:

General discussion

6.1 The use of the outdoor area

The goal of this study was to understand how free-range laying hens and slow-growing meat chickens use the available space, and how the use of space patterns and environmental complexity interventions may influence their behaviour and welfare.

The results for the laying hen study indicated that peak activity in the outdoor area occurred during the morning and afternoon periods, which may respond to normal internal biorhythms, characterized by a reduction in activity during the central hours (Channing et al., 2001). The use of the outdoor area in this study was relatively high, (32.60 ± 15.3%) as compared to previous studies (e.g. Hegelund et al., 2005; Gilani et al., 2014). Such level of use of the outdoor area remained stable across age periods and climatic conditions in which the study was conducted. The maximum used of the outdoor area observed in the laying hen study was 40.92%. On the contrary, for slow-growing meat chickens, the use of the outdoor area was substantially lower, with an average of 4.6 ± 0.49% and a maximum of 12% observed in the 9th week of age, a maximum value similar to the results obtained by Dawkins et al., (2003) for meat chickens. The use of the outdoor area was also more dependent on the climatic conditions, being particularly evident towards the end of the rearing period.

The use of the outdoor area was lower than expected, especially for slow-growing meat chickens, since access to an outdoor area is consider a primordial factor to improve poultry welfare. Keeling et al., (1988) suggested that a low use of the outdoor area might be due to: 1) the gregarious nature of the birds that may result in a strong tendency to remain indoors with most of the flock, and for those using the outdoor area to be drawn back inside, 2) birds may be fearful of going to a new outdoor environment, especially if no many birds are using such area, and 3) due to a low need, or motivation, to investigate the outdoor area given that most of the basic resources are available indoors. Nevertheless, despite of the strong effects of the social group, the availability of an attractive pasture to forage might be a factor that stimulates the use and dispersion of the birds in the outdoor area.

In laying hens no effect of age period was detected when looking to the results in general, although there was a trend to increase. However, a more detailed analysis did show that those individuals that used the outdoor area at a high frequency during AP1 continued to show a high use frequency during AP2 and AP3. It is possible that birds that used the outdoor area from the onset of production had more opportunities to experience and habituate to the outdoor area, becoming more familiar with their surroundings and showed a tendency to increase both the distance to the hen house, as well as the size of their core areas. In laying hens, it was observed that the grass initially available close to the hen house was consumed during the first age period. Therefore, it is speculated that the need to travel further away to find better pastures might be also one of the factors responsible for this tendency.

The large inter-individual differences in the use of the outdoor area detected for laying hens could be due to differences in personality traits. For example risk-taking behaviour in great tits (*Parus major*) is repeatable and it is positively correlated with their exploratory behaviour (van Oers et al., 2004). Therefore, although birds are motivated to explore new environments (Newberry, 1999) and the external stimulus per se would be the same for the entire flock, the perception of the risk and the motivation to explore the new area may be different according to their differences in the personalities. These inter-individual differences in the inner motivation to explore the new accessible outdoor area would be positively reinforced by habituation and experience.

In slow-growing meat chickens, an increment on the number of birds in the outdoor area was detected with age, effect that was not observed in laying hens. This difference may relate to the fact that when laying hens were first exposed to the outdoor area they were already adults, whereas chickens were still young birds, probably more fearful of exploring a new environment, and certainly, more vulnerable to unknown dangers (Hocking et al., 2001). In addition, at 6 to 12 weeks of age slow-growing meat chickens might also be more vulnerable to weather conditions and less willing to explore the outdoor area if plumage was not fully developed. It may be argued that the use of the outdoor area by slow-growing meat chickens in this study was more affected by temperature in older birds that in younger ones. However, use the outdoor area by young birds was low, possibly due to the variable and cold spring weather conditions that were encountered during the study. In addition, as previous experience is known to affect the use of the outdoor area (Grigor et al., 1995b), it would seem logical to expect a lower use given the limited time they had to experience the outdoor area, as compared to laying hens. An increment in the size of the core areas with age was observed for slow-growing meat chickens which may have been due to a similar habituation process.

6.2 The effects of environmental complexity in the outdoor area

Regarding the effect of increasing environmental complexity for slow-growing meat chickens, only minor effects were observed. The availability of artificial devices in the outdoor area had no effect on the frequency of use or on the use of space parameters considered, with the exception of net distance travelled in the outdoor area that increased with age in the perch treatment. Although the frequency of perching was very low, perches were high enough to serve as some form of cover and could have motivated the birds to move around the outdoor area, causing the net distance to increased. On the other hand, the lack of the expected positive effects of the panel treatment may have been related to the instability of the panels during high winds which may have frightened the birds. A second possible reason for the lack of stronger effects of the environmental complexity treatments in general would have been the reduced number of devices included in each house. This was the first study conducted in commercial facilities and we tried to avoid including too many devices that could be unpractical or disruptive for the farmers´ daily routines. It is likely that the results could have been better if additional devices were included, if distance to the first device would have been greater, or both, in order to motivate birds exploring further areas to the chicken house.

Likewise, increasing environmental complexity in the outdoor area had only minor effects on the behavioural response of slow-growing meat chickens. Time standing increased with age in the control treatment, but remained constant in the perch treatment and did not show any clear trend in the panel treatment. Probably, the panel treatment was somewhat beneficial in no, or low wind conditions, but disruptive under high winds, as explained previously. If the perches served as a form of (more stable) cover, birds would have been less likely to be in an alert standing position as in the control, explaining the differences across these treatments. Previous studies in broilers found that provision of panels and perches increased resting and decreased standing (Cornetto and Estevez, 2001b; Ventura et al., 2012). It is suggested that domestic fowl might seek out the protection of the vertical artificial devices to reduce vulnerability to potential predators (Newberry and Shackleton, 1997). Indeed domestic fowl have a strong tendency to stay near these locations (Preston and Murphy, 1989; Newberry and Hall, 1990). However, it is possible that the characteristics of the vertical structures that are effective indoors are not practical for the outdoor area because the potential danger if they are not stable enough.

6.3 Use of space patterns and its impact on welfare indicators

The behaviour and activity of the animals has a direct effect on their physical condition (Prayitno et al., 1997), and their activity depends, at least in part, on the characteristics of the physical environment (Newberry and Hall, 1990; Dawkins et al., 2003; Leone et al., 2010; Bailie et al., 2012; Ventura et al., 2012; Gilani et al., 2014). Therefore, it was speculated that the characteristics of the environment in terms of environmental complexity and the birds' movement patterns may have had an impact over the morphological and welfare indicators in slow-growing meat chickens. However, no relationship was found between their use of space patterns in outdoor area, or the presence of devices with any of the morphological or welfare indicators considered in this study. The lack of effects was not surprising given the low frequency of use of the outdoor area observed.

On the other hand, some positive relationship between movement patterns and welfare indicators were detected for laying hens. The frequency of use of the outdoor area was negatively correlated to plumage damage, indicating that birds showing a higher use of the outdoor area exhibited less plumage damage. The reasons for this may relate to the lower bird density that birds encounter in the outdoor area, therefore reducing the risk of feather pecking (Huber–Eicher and Audige, 1999; Green et al., 2000), or because hens using the outdoor area at higher frequencies might have been less exposed to erosion damage caused by feeders, drinkers or other devices indoors. Additionally, positive correlations between FPD and total and maximum walked distance indoors, and negative with the percentage going out, mean and maximum distance from the hen house were detected. Aarnik et al., (2006) found that the presence of ammonia in the outdoor area was higher close to the house probably due to the greater concentration of dejections in comparison with further away areas. Therefore, birds that adventured themselves further away from the house may have been less exposed to ammonia. Regarding the negative correlation between the use of the outdoor area and the presence of FPD, it can be speculated that birds remaining indoors were more exposed to a litter of lower quality compared to those who stay longer in the outdoor area.

6.4 Use of space and behaviour indoors

Contrary to the minor effects of increasing environmental complexity in the outdoor area, use of space patterns in slow-growing meat chickens were altered by the presence of devices indoors. The results obtained indicated a clear increment in the use of the central area in the perch and panel treatments (64 and 67%, respectively) as compared to the control (24%) in which a much higher use of the wall areas was detected. These results are similar to the consistent effects of the presence of cover found by Newberry and Hall, (1990) for laying hens, and Cornetto and Estevez, (2001a) for broilers, and can be explained by the protecting effects from conspecifics and predators (Elton, 1939) provided by panels and perches. On the contrary, when no devices were located in the central areas birds tend to stay close to the walls (Pamment et al., 1983) as found in this study.

The main purpose of adding perches as a complexity treatment was to provide birds with perching opportunities. However, the perches in this study seemed to have had a protective effect similar to the effects of adding panels, even though they were lower than the panels provided. It is possible that including any type of devices in the central areas may increase its use by producing an aggregation effect, especially when performing comfort behaviours (Keeling, 1994), and therefore increasing the permanence of the birds in the central areas. For example, it has been shown that by simply placing frames in central pen areas improved the use of such space in broilers (Cornetto and Estevez, 2001a). Possibly, as it happens in the wall area, when birds find an appropriate location close to any vertical obstacle, such as perches, additional birds could be attracted to those areas because of a cohesive effect of the flock.

In addition, the results showed that in the perch treatment there was a positive relationship between temperature and total distance travelled, while the opposite effect was observed in the control, and no effect was observed in the panel treatment. It has been suggested that birds use perches for thermoregulatory purposes (Estevez et al., 2002). Thus, it is possible that when the temperature increased the birds searched for a perch to use in order to facilitate thermoregulation by getting off the floor which may have resulted in an increased in the total distance travelled. On the other hand, in control treatments, perhaps the birds were more reluctant to move at high temperatures, resulting in less total distance travelled.

Regarding the area of the house used by slow-growing meat chickens calculated as core areas (50, 75 and 100%), no differences across treatments or age period were detected. Likewise, Leone et al., (2007) found no effect of increasing environmental complexity on the size of the core areas in 16 m^2 pens for broilers. In this study the total space available indoors was 110 m^2 while the mean used area per age period was 26.025 ± 1.278 m^2 (mean ± SE). The lack of age period effects was a surprising result which may obey to the fact that birds were already habituated to the housing conditions when the data collection started, and may be indicating the mean area used to satisfy all their needs for feed, water and resting purposes. However, such result should not be interpreted as if the birds were limited to a 26 m^2 area. Most likely the birds were moving through different locations within the house, but mean areas remained similar across age periods. It is also possible that due to the central position of the complexity devices and the small size of the house the effect of the treatments and age over the used surface was not noticeable. For laying hens, an increase in the size of the 50% core areas was detected with age, but in this case the data collection started at the arrival of the hens to the farm. Besides, it is possible that the bigger size of the hen houses, compared to chicken houses, facilitated the random exploration of new areas.

6.5 Factors triggering aggression in the domestic fowl

Spatial factors, and specifically the invasion of the personal space by flock members, have been considered the main factor in determining the occurrence of aggressive interactions in the domestic fowl (McBride, 1971). Given the focus of this work in the use of space by the domestic fowl in alternative production systems, it was relevant to determine to which extent spatial parameters played a role in the development of aggressive interactions in laying hens, and if so, what would be the critical distance that triggers such interaction. Differences in the behaviour of the interacting birds moments prior to the interaction were also considered. The results of this study indicated that the close distance between the giver and receiver was not the only relevant factor in triggering the aggressive interaction. The recipient of the interaction was at significantly shorter distances from the giver as compared to other birds located in the frontal area, but not as compared to the other two birds considered in the close proximity of the giver. The recipients of the interaction were also more likely oriented facing the giver, and showed higher levels of active behaviours as compared to other birds in close proximity to the giver. These results suggest that aggression in the domestic fowl does not depend on the invasion of the critical distance per se, but would greatly depend on the activity level and directionality of the individuals which would be perceived as a threat by the aggressor.

Previous studies demonstrated that the majority of aggressive interactions takes place in central areas (Cornetto et al., 2002; Pettit-Riley et al., 2002), and that the presence of panels, perches and straw bales decrease the frequency of aggressive interactions in broilers (Cornetto et al., 2002; Pettit-Riley et al., 2002) and broiler breeders (King, 2001). Considering that birds are more likely to perform active behaviours in the centre of the house, and that this could trigger aggressive encounters, one might expect that the frequency of attacks decreased in the areas with high environmental complexity. The presence of the panels, and to some extent the perches, could create spaces of visual isolation, where birds would hide momentarily from other birds. However, aggressive interactions were rarely observed during the study and therefore, it was no possible to perform any statistical analyses.

6.6 Implications for the poultry industry

The findings obtained in the studies concerning free-range laying hens and slow-growing meat chickens are relevant for industry, especially because they were conducted in commercial farms and thus the results are directly applicable to real commercial conditions. Although no major problems were detected regarding plumage quality or the incidence of FPD of the free-range laying hens under the study conditions, still a higher use of the outdoor area was shown to benefit birds welfare in these two aspects. It is known that a poor plumage quality translates into increased food consumption for thermoregulation, and on the other hand, the presence of FPD may compromise the birds´ health, besides being considered a welfare problem. Therefore, encouraging the birds to use the outdoor area could help to assure a good plumage quality and feet conditions, perhaps having some impact in reducing feed costs, or cost associated to a reduction in health status. The frequency of use of the outdoor area was much higher for laying hens as compared to slow-growing meat chickens. This is may be one of the main reasons explaining the lack of benefits of using the outdoor area in slow-growing chickens. However, it is possible that if use of the outdoor area would be higher, similar benefits as to those found for laying hens could be potentially detected.

Since maintenance of an outdoor area carries a great economic and environmental cost, it would be desirable to maximize its use, therefore optimizing the benefits it can bring in terms of health, performance, improved marketability and profitability. One of the main practical findings of the study in laying hens was the detection that birds´ early experience on the use of the outdoor area determined to which extent laying hens will continue to use the outdoor area in subsequent production phases. Therefore, encouraging the use of the outdoor area at the beginning of the production period should help to improve the use of the outdoor area in general. In this respect, actions to encourage an early access to the outdoor area could include; provide additional, but limited, feeding opportunities during the first few days of access, or by making the outdoor area initially more attractive by scattering some straw bales when laying hens are starting to experience the outdoor area which may facilitate exploration while offering some protective cover. A higher use of the outdoor area may be more relevant in the near future as a mean to reduce risk of feather pecking and cannibalism if a ban on beak trimming was to be adopted in MS countries.

However, to improve the frequency of use of the outdoor area might be not as easy as it could be expected by means of increasing environment complexity. While the results obtained by applying environmental complexity treatments indoors was satisfactory for slow-growing meat chickens, the results of the interventions in the outdoor area were not very encouraging. The complexity treatments indoors, both perches and panels, had the desired effect of improving the use of the central areas. On the contrary, the benefits encounter in the outdoor area were only marginal. Thus, from a practical stand point when the implementation of complexity treatments are to be conducted in the outdoor area the stability of the treatments should be critically assessed, especially in areas exposed to windy conditions.

Based on the results of this study, it may be interesting to play with the idea of providing birds with stable devices that would serve both as perch and as protective devices simultaneously, for example, by providing birds with lower sturdy panels, but with a grip surface in which birds could perch on. Such devices may be used both by slow-growing chickens and laying hens, although height should be adjusted accordingly in each case. A similar device may help in reducing aggressive interactions in problematic flocks as it was evidenced in this work that aggression arises due to a combination of distance, orientation and behavioural components. Therefore, it is possible that spatial partitions, strategically located in the open areas, may reduce the risk of birds directly confronting each other.

A second relevant aspect evidenced in this work was the fact that some birds, both in laying hens and slow-growing meat chickens, do not use, or use only sporadically the outdoor areas. Use of space patterns were clearly different among them, but the reasons why they remain indoors are unclear. Further investigation to determine the cause of such behaviour would be highly relevant for designing better flock management procedures.

Finally, it should be indicated that sustainability is a critical aspect that must be consider in all animal production systems to assure their existence in the long term. Animal welfare is regarded as a fundamental pillar of sustainability and thus, management strategies that can benefit the welfare of poultry while simultaneously improve and health and performance should be consider as a helpful tool.

Chapter 7:

Conclusions

7. Conclusions

This work provided some relevant insights into characteristics of the use of space and behaviour of laying hens and slow-growing meat chickens housed under free-range conditions. The main conclusions of the study are:

A) In **free-range laying hens:**

- The mean frequency of use of the outdoor area was relatively high as compared to previous studies, but was minimally affected by time of day and no affected by age period, or climatic conditions.
- Almost half of the tagged hen population was never observed using the outdoor area, which could in part be explained by the data collection method used in the study.
- In addition, when using the outdoor area, laying hens remained close to the hen house, as indicated by the mean and maximum distance, and used only a small portion of the available space during the entire production period.
- The results of the study demonstrated that frequency of use of the outdoor area during AP1 determined it usage at later age periods. Thus, birds that showed a high use during AP1 continue to use it at higher frequencies later in production. On the contrary, those that were not observed in the outdoor area during AP1 continued to show a low frequency of use.
- The size of the activity centre of the hens inside the house increased with the age period.

- The negative correlation detected between the use of space parameters indoors and in the outdoor area also supports the idea of subpopulations that move either indoors or in the outdoor area.
- Differences in use of space patterns appear to have some impact on welfare indicators such as plumage condition and FPD, both showing better scoring for those individuals that use the outdoor area at higher frequencies.
- Regarding analyses of the factors that may trigger aggressive encounters the results of the study indicate that the encounters emerge as a combination of proximity, orientation and activity (or ´attitude´) of the birds, and not by simple invasion on the birds´ personal space.

B) In **slow-growing free-range meat chickens:**

- Increased environmental complexity by means of providing perches and panels lead to a higher use of the central areas of the house, resulting in an improved use of the indoor space.
- Temperature in the control treatment had a negative effect on the total distance travelled indoors.
- Age period had no effect over the minimum distance, maximum distance , net distance, total distance or size of the core areas indoors.
- The use of the outdoor area was much lower than expected, and more than the half of the tagged birds were never observed using it during the study.

- Use of the outdoor area in slow-growing meat chickens increased with age period as indicated by the number of birds using the outdoor area and the size of the core area at 50 and 100%.
- Temperature affected to the use of the outdoor area, especially to older birds, although this was probably due to the fact that when young use of the outdoor area was low.
- Increased environmental complexity had only minor effects in regard to their potential to modify use of space patterns in the outdoor area, only evidenced by the increase net distance travelled in the presence of perches.
- A clear effect of age ~~period~~ on the behaviour of birds was detected, with main changes occurring in the outdoor area with increased locomotion and decreased resting.
- The provision of panels and perches had a limited impact on their behaviour, although their effects were somewhat stronger indoors, with ~~increased standing with age in the perch treatment. In addition,~~ a higher percentage of birds performed locomotive behaviours in the central area of the house in the panel treatment as compared to the control. In the outdoor area standing increased with age in the perch and control treatment.
- Environmental complexity treatments did not appeared to have an effect the any of the production and welfare indicators considered in this study.

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