Using Spatial Distribution and Behaviour to Determine Optimal Space Allowances for Poultry and Rabbits

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Abstract

Previous research on the effect of stocking density on welfare has focused on adverse effects on health and behaviour. Absence of such effects does not mean that space allowance is optimal from the animals' point of view. This thesis aimed to assess optimal space allowances by studying spatial distribution and behaviour. The importance of lower densities was studied using a combination of preference and motivation testing.

Broilers were increasingly attracted to the pen walls as stocking density increased. This attraction seems to stem from an attempt to minimize disturbances by conspecifics, which increased with stocking density (paper I). Such environmental influences on spacing need to be corrected for when studying the social component of spatial distribution: attraction/avoidance between animals. When such corrections were made, broilers were found to avoid each other if stocked at densities above 2.4 birds/m^2 (paper II). Broiler chickens showed a considerable motivation for densities below 15 birds/m^2. To get to lower densities, they crossed barriers that deterred 20-25% of broilers from obtaining feed after 6 hours of feed deprivation (paper III).

When environmental influences were accounted for, fattening rabbits avoided their conspecifics at all densities studied, suggesting that the optimal stocking density lies below 5 animals/m^2 in this species. Furthermore, they seemed less attracted to each other when a wooden enrichment structure was present (paper IV). Fattening rabbits spent more time lying sternally at higher densities, possibly because other behaviours were increasingly impeded. In enriched cages less time was spent on cage manipulation, social contact and drinking. This time was instead spent gnawing and exploring the structure, suggesting that in barren cages such behaviour was redirected towards conspecifics and cage materials (paper V).

The results show the importance of correcting for environmental influences when assessing the social component of spatial distribution. Additionally, the use of multiple distribution indices is recommended.

Keywords: spatial distribution, behaviour, space allowance, stocking density, enrichment, broiler, rabbit

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Contents

List of Publications

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

I Buijs, S., Keeling, L.J., Vangestel, C., Baert, J., Vangeyte, J. & Tuyttens, F.A.M. (2010). Resting or hiding? Why broiler chickens stay near walls and how density affects this. *Applied Animal Behaviour Science* 124, 97-103.

II Buijs, S., Keeling, L.J., Vangestel, C., Baert, J. & Tuyttens, F.A.M. (2011). Neighbourhood analysis as an indicator of spatial requirements of broiler chickens. *Applied Animal Behaviour Science* 129, 111-120.

III Buijs, S., Keeling, L.J. & Tuyttens, F.A.M. (2011). Using motivation to feed as a way to assess the importance of space for broiler chickens. *Animal Behaviour* 81, 145-151.

IV Buijs, S., Keeling, L.J., Vangestel, C., Baert, J., Vangeyte, J. & Tuyttens, F.A.M. Assessing attraction or avoidance between rabbits: a comparison of methodologies to analyse spatial distribution. Manuscript.

V Buijs, S., Keeling, L.J., & Tuyttens, F.A.M. Behaviour and use of space in fattening rabbits as influenced by cage size and enrichment. Submitted.

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Abbreviations

21L:3D	21 hours of light followed by 3 hours of darkness
$area_{adj}$	size of the adjustable side of the density preference test pen
CVDPA	coefficient of variation in Dirichlet polygon areas
CVIID	coefficient of variation in inter-individual distances
DPA	Dirichlet polygon area
e.g.	exempli gratia, *for example*
ENR	enriched
EU	European Union
exp	exponent
F	female
Fig.	figure
i.e.	id est, *that is*
IID	inter-individual distance
l × w × h	length × width × height
LSMEANS	least-squares means
lx	lux
M	male
NH_3	ammonia
NND	nearest neighbour distance
ns	not significant
PVC	polyvinyl chloride
R-COR	resource-corrected random distribution
RAND	random distribution
r_s	Spearman correlation coefficient
SD	standard deviation
SEM	standard error of the mean
UNE	unenriched

Introduction

This thesis describes experiments that were aimed at determining the optimal space allowance from the animals' point of view, by studying their spatial distribution and behaviour. This topic is of importance because presently used welfare indicators often lead to contradicting conclusions on which density should be considered optimal. Furthermore, the absence of health problems or behavioural adaptations measured by such welfare indicators does not necessarily indicate that an animals' space preferences are met.

The introduction starts with a description of the differences between the housing systems of our two subject species (broiler chickens and fattening rabbits), and highlights the limitations that these systems put on natural spatial distribution and behaviour. The unavoidable confounding that occurs in stocking density experiments is explained, followed by an overview of the effects of stocking density on welfare. Then, spatial distribution is introduced as an indicator of optimal space allowance from the animals' point of view, and the need to account for environmental influences on spacing is described. Also, the possible occurrence of socio-environmental influences is introduced. Then, some attention is devoted to the relation between behaviour and spatial distribution. The introduction ends with a description of methodology to assess the importance of achieving an optimal space allowance.

Introduction of the species and the husbandry systems

The two species studied in this thesis are broiler chickens (*Gallus gallus domesticus*) and fattening rabbits (*Oryctolagus cuniculus*). Fattening rabbits are sometimes also referred to as *broiler rabbits*, a term which will not be used in this thesis to prevent confusion with the term *broiler*, which will be used to refer to broiler chickens. Both broiler chickens and fattening rabbits are used for meat production, but the way they are housed in modern husbandry differs greatly.

Global broiler meat production exceeds 70 million tonnes per year (USDA, 2010). These broilers are usually kept in groups of several thousand individuals in climate controlled housed with solid, litter covered floors (SCAHAW, 2000). Broilers grow to their slaughter weight (around 2 kg, although the targeted end-weight differs between countries) in about 42 days. The length of this rearing period has decreased greatly since 1970, when it took over 10 weeks to reach this weight (SCAHAW, 2000). When broiler chickens and red jungle fowl (their most probable ancestor) were reared under the same circumstances, broilers were 9 times as heavy as jungle fowl at 42 days of age (Zulkifli, 2008). Modern broiler chickens spend about 65% of their time sitting or lying inactive (Hall, 2001; McLean et al., 2002). Although they are still motivated to be active, their high bodyweight (Bokkers and Koene, 2004; Rutten et al., 2002) and painful joints (McGeown et al., 1999) impede activity.

Rabbit production is a much smaller sector, with an estimated global production of 1 million tonnes per year. The main producers of rabbit meat are China, Italy, Spain and France (FAO, 2001). Fattening rabbits are most commonly kept in groups of 2 to 6 animals in wire cages (Verga et al., 2007). They are placed in these cages after weaning (around 4 weeks of age) and are usually slaughtered around 10 weeks of age, at a weight of 2.5 kg. Rabbits are not fully grown at this time, commercially bred adult breeding does reach body weights of 4-5 kg (Trocino and Xiccato, 2006). The adult body weight of wild rabbits is much lower (1-2 kg, Cabezas et al., 2007; Gage et al., 2006; Williams and Moore, 1989). However, this difference in bodyweight is nowhere near the 9-fold increase reported for broiler chickens. Like broiler chickens, fattening rabbits spend most (60-65%) of their time sitting or lying without performing any discernible activity

(Martrenchar et al., 2001; Morisse and Maurice, 1997), although it needs to be remarked that resting is also a very common behaviour in wild rabbits (Vastrade, 1984).

Space use under (semi-) natural conditions

Being able to live in a way that is natural for a species is seen as an important aspect of welfare (Fraser, 2009). For this reason, behaviour of farm animal species is often compared to that of their wild counterparts, or to domestic animals kept in semi-natural conditions (e.g., Jensen and Wood-Gush, 1984; Schmid and Wechsler, 1997). This section describes such studies for both species, as well as factors that limit their extrapolation to commercial situations.

Because the modern broiler chicken is the result of generations of intense selection, it does not have a clear wild counterpart. However, indications of 'natural spacing' have been obtained by studying zoo populations of red jungle fowl, the domestic chicken's most probable ancestor. These formed groups of 6 to 30 adults, which ranged over an area of 3 000 to 17 000 m^2 around their roosting tree (Collias and Collias, 1996; Collias et al., 1966). Little is known about the spacing behaviour of broiler chickens under semi-natural conditions. Studies carried out in commercial free range systems show an attraction to trees and the area around the broiler house (Dawkins et al., 2003; Jones et al., 2007). However, ranging behaviour is negatively correlated with growth rate, and thus the ranging behaviour of conventional broiler strains is limited (Dal Bosco et al., 2010; Eriksson et al., 2010; Nielsen et al., 2003). When lighter, but also domesticated, laying hens were studied in a large semi-natural enclosure, flocks of 15 birds occupied an area of 412 m^2 on average (Keeling and Duncan, 1991). This may give some indication on how much space broilers would use if not hindered by their weight, although such an extrapolation should be made cautiously as strain differences affect flock area (Keeling and Duncan, 1991).

In contrast to wild chickens, wild rabbits are common. Their behaviour differs little from that of domestic rabbits (Hoy, 2006). The mean home range of wild rabbits was estimated at 2200 m^2 for males and 1600 m^2 for females, whilst inter-pair distances of adult fattening rabbits in semi-natural conditions were 21-24 m on average (Vastrade, 1987).

Animal density under naturalistic conditions differs greatly from that under commercial conditions for both subject species. However, the specific aspect of density that differs is not the same for the two species. Commercial broiler flocks may have access to an area as large as the 3 000 m^2 home range described for some groups of jungle fowl (Collias and Collias, 1996; Collias et al., 1966). However, this area is populated by thousands of birds, instead of the 6 to 30 individuals reported under naturalistic conditions. In contrast, fattening rabbits are kept in groups of 2 to 6 individuals, which roughly corresponds with their natural group size, but have access to an area that is far smaller than their natural home range (Verga et al., 2007).

Studies under (semi-) natural conditions can give us a general idea of how much space is used when animals are not limited by enclosure size. However, this does not necessarily mean that this is the amount of space domestic species would need (or use) in captivity. For instance, wild rabbits use less space when food availability is high (Lombardi et al., 2007), indicating that space use in the wild may reflect the area needed in order to get sufficient nourishment. Since fattening rabbits are usually fed ad libitum diets and do not need to search for their food, they may need far less space in captivity. In addition, space between animals is also influenced by aggression levels (Lill, 1968). Domestic rearing has been suggested to increase the threshold for aggressive behaviour (Price, 1999). A decrease in aggression may thus lead to a decreased space need in domestic animals as compared to wild ones. For instance, Desforges and Wood-Gush (1975) showed that domestic ducks were less aggressive than wild mallards and rested at shorter inter-bird distances. However, domestication is not always associated with a decrease in aggression, as layer chicks were more often involved in agonistic interactions than jungle fowl chicks when observed in an open-field test (Vaisanen and Jensen, 2004). Because of these influences, tests that analyse the amount of space needed in confinement should preferentially be carried out in the relevant husbandry setting, and should use the relevant domestic breeds.

Quality of space under commercial husbandry conditions

In addition to differences between the quantity of space available in natural and commercial settings, the quality of this space also differs. Commercial housing improves the quality of space by protecting animals from, e.g., adverse climatic conditions and predation. However, captive animals are often housed in relatively barren environments, due to economic and hygienic considerations. The quality of space can be improved by providing environmental enrichment, i.e., modifications in the environment that seek to enhance physical and psychological well-being by providing stimuli meeting the animals' species-specific needs (Baumans, 2005). As the effect of enrichment on chickens was not studied in this thesis, enrichment strategies for this species will not be discussed in detail (although, see the section on environmental influences for the effects of vertical panels). Instead, in the remainder of this section enrichment strategies for fattening rabbits are described.

Although often misclassified as a rodent (from the Latin *rodere*, to gnaw), rabbits belong to the order of the Lagomorphs (together with hares and pikas). However, just like rodents, rabbits have a strong motivation to gnaw (Huls et al., 1991). The commonly used cages for fattening rabbits consist of floors, walls and roofs constructed of metal wire, which seem little suited to satisfy the rabbits' motivation for gnawing. Several materials can be used as gnawing enrichment. Although rabbits seem to prefer roughage over wooden sticks (Lidfors, 1997), research on enrichment for fattening rabbits has mainly focused on such sticks. These seem to have a favourable influence on welfare, as less aggression and abnormal behaviour is reported in cages with sticks (Jordan et al., 2006; Princz et al., 2007; Verga et al., 2004). Other reported effects include increased social contact, allogrooming, general activity and locomotion (Jordan et al., 2004; Princz et al., 2007; Princz et al., 2008; Zucca et al., 2008) and less grooming and lying (Jordan et al., 2006; Luzi et al., 2005; Verga et al., 2004). However, these results are not consistent over studies. Furthermore, such changes in behaviour can be difficult to interpret in terms of increased or decreased welfare. For instance, increased activity can indicate that animals are less apathetic due to the presence of the enrichment, or that the resting behaviour of one rabbit is disturbed by another's response to the enrichment.

The use of platforms as enrichment is more common for breeding does, but these can be used for fattening rabbits as well. Platforms lead to increased environmental complexity, as well as to an increase in the total floor space available. They may also lead to segmentation of the cage into different functional areas, as Postollec et al. (2008) found that fatteners used the area underneath a wire platform to rest without being disturbed by more active animals. In such cases, segmentation can be seen as a positive contribution to welfare, as it increases the amount of control the animal has over its environment. However, segmentation of the cage may also hinder locomotor behaviour, thus impacting negatively on welfare.

Factors that covary with stocking density

Stocking density (i.e., space allowance per animal) is inherently confounded with either the number of animals in a group, or with the total amount of space available to this group. In addition, altering total space availability affects either the perimeter length or the shape of the cage/pen. Group size, total space allowance, perimeter length and shape may all affect behaviour and spatial distribution of animals.

Group size

Group size is reported to influence anti-predator behaviour in both wild birds (Dias, 2006; Fernandez et al., 2003; Harkin et al., 2000; Newey, 2007) and domestic fowl (Newberry et al., 2001). Vigilance is generally decreased in larger groups, and several explanations have been suggested for this. First, the chance that one of the group members will detect a predator, and alert other group members, may be increased in larger groups. Second, larger groups lead to a decreased chance of being predated at the individual level, as there are more prey animals for the predator to target (Beauchamp, 2003). Third, the behaviour of larger groups may confuse the predator, decreasing its attack success (Ioannou et al., 2009). Although farm animals seldom encounter any predators in commercial indoor settings, both broilers and rabbits still display anti-predator behaviour (Baumans, 2005; Newberry et al., 2001; Verga et al., 2006). Wild chickens and rabbits rely on cover for predator avoidance (Collias and Collias, 1967; Cowan and Bell, 1986; Lombardi et al., 2003). In commercial husbandry the walls are usually the main (or even only) sources of cover, and thus spatial distribution may be affected by group size because smaller groups show a stronger tendency to stay near walls.

Total space allowance

If the amount of space per animals is kept constant whilst group size and total cage size are increased simultaneously, this leads to an increased amount of shared free space - space not taken up by the physical presence of the animals (McGlone and Newby, 1994). As such, an increase in types of behaviour that require more space can be expected. In addition, increasing total cage size may allow for types of behaviour that require a longer distance to perform. For instance, it is physically impossible for rabbits to hop in small individual cages, as one full hop requires 70 cm (EFSA, 2005). When rabbits are group housed at an equal space per animal, these 70 cm lines may become available to the rabbit when its cage mates are huddling on the other side of the cage (Figure 1).

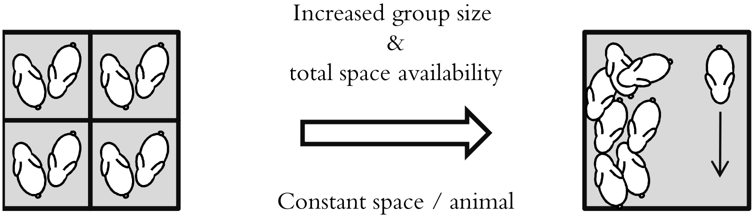

Figure 1. **The effect of simultaneously increased group size and total space availability.** Hopping behaviour is physically impossible in the small cages. When most rabbits stay together on one side of the larger cage, free space becomes available for an animal to hop.

Shape and perimeter

When total space availability is decreased, the perimeter of the pen will be decreased as well (unless pen shape is altered, Figure 2). Perimeter length is of importance because some animals are reported to prefer to stay near walls, and the provision of extra "wall length" by placing partitions in a pen can decrease the disturbances by conspecifics in broiler chickens, and increase the time spent resting (Cornetto et al., 2002; Cornetto and Estevez, 2001b; Newberry and Shackleton, 1997).

Confounding area size and perimeter length can be avoided by changing the shape of the pen. But pen shape itself may influence how efficiently animals can use the area available. For instance, Stricklin et al. (1979) showed that crowded beef steers tended to orientate towards the edge of the enclosure, and that as a result, enclosures with a greater perimeter-to-area ratio could be used more efficiently. This ratio is greater in rectangular than in square enclosures. Another effect of rectangular pens is that the maximum distance between the far cage walls is increased, which may facilitate locomotor behaviour (EFSA, 2005), for example the hopping behaviour referred to in the previous section.

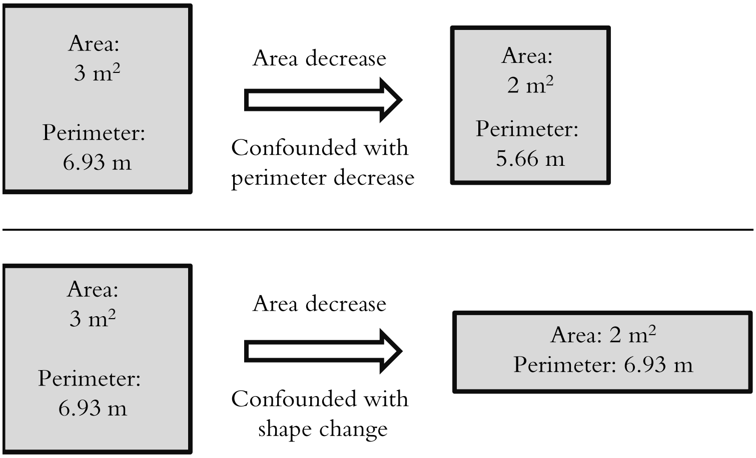

Figure 2. **When area is decreased, either perimeter length is decreased or pen shape changes.** In both examples, perimeter to area ratio is increased, but this change is more pronounced when area decrease is achieved by changing pen shape.

Identifying the causative factor

Varying all the factors associated with stocking density systematically leads to extremely large experiments. This is especially so when a wide range of densities needs to be studied because it is unknown around which density changes in behaviour are to be expected. Therefore, stocking density studies usually draw final conclusions on the causative factor by comparing the effects observed in several studies in which a different factor (either space per animal, group size or total space availability) was kept constant, whilst the other two were varied simultaneously (Averos et al., 2010; Buijs et al., 2009; Faerevik et al., 2008). In most stocking density experiments, animals are subject to a certain treatment throughout the experiment in order to evaluate long term effects. In contrast, preference testing may be used to gain insight into the causative factor in a more efficient way, as multiple choices can be offered to animals in short succession using such techniques (Frommen et al., 2009; Held et al., 1995; Lindberg and Nicol, 1996). However, such tests will of course only show short term preferences.

Stocking density effects on welfare

Stocking density is a much discussed topic in animal science. Increasing stocking density generally leads to a decrease in welfare in many farm animal species (Estevez, 2007; Petherick and Phillips, 2009; Szendro and

Luzi, 2006), although the lowest density from which density induced welfare problems start to arise will of course differ between species. A recent survey (Vanhonacker et al., 2008) showed that citizens perceive stocking density as a top priority for animal welfare, and are concerned about the stocking densities currently used in commercial livestock production. However, the economic effect of reducing stocking density is large. Although high densities can diminish individual growth in both chickens and rabbits (Dawkins et al., 2004; Estevez, 2007; Szendro and Luzi, 2006), the economic benefit per square meter increases with stocking density (Cravener et al., 1992; Feddes et al., 2002; Verspecht, 2009), providing an economic incentive for high densities. As a result, many species are stocked at high densities under commercial conditions. The species studied in this thesis are no exception to this. For broiler chickens, the EU has recently set a maximum stocking density of 42 kg/m^2, approximately 19 birds/m^2 (EU Council Directive 2007/43/EC). No such regulations have been implemented for fattening rabbits, although some EU member states have national legislation or guidelines concerning cage size (Luzi et al., 2003).

Research on the effects of stocking density on broiler chicken welfare has been conducted over a wide range of densities (5 - 72 kg slaughter weight/m^2). Several indicators of welfare are influenced negatively when stocking density is increased. Walking ability was found to decrease with increasing density, both when studied under experimental circumstances (Buijs et al., 2009a; Thomas et al., 2004) and in a field study (Dawkins et al., 2004). The weight of the Bursa of Fabricius is also reported to decrease with increasing stocking density, indicating stress and immunosuppression (Heckert et al., 2002; Ravindran et al., 2006). The incidence of footpad dermatitis - an ulcerative skin disorder caused primarily by contact between the skin and irritating substances in the faeces (Bradshaw et al., 2002) - also increases with stocking density (Arnould and Faure, 2004; Cravener et al., 1992). However, the effect of stocking density may be obscured by other factors that differ between farms, as no effect of stocking density on footpad dermatitis was shown in field trials (Dawkins et al., 2004; Ekstrand et al., 1997). Increases in fearfulness have also been reported for higher stocking densities (Andrews et al., 1997; Buijs et al., 2009a).

Studies on the behaviour of broiler chickens also suggest a negative influence of high densities on welfare. Resting and preening are increasingly disturbed at high densities (Cornetto et al., 2002; Hall, 2001; Lewis and

Hurnik, 1990). Both behaviours can be expected to impact on bird welfare. Rest is important for all animals, but specifically for young ones like broiler chickens (Malleau et al., 2007). Preening shows a 'rebound effect', i.e., it is increased after a period of restriction, suggesting that chickens have an internal drive to perform such behaviour (Nicol, 1987). Decreases in locomotion and foraging suggest that broilers' freedom of movement is increasingly limited at higher stocking densities (Blokhuis and Van der Haar, 1990; Sanotra et al., 2002). In addition to these effects, increased stocking density may cause heat stress, as McLean et al. (2002) reports increased deep panting (a behaviour performed to facilitate heat loss) at a higher stocking density. This effect may be ameliorated by adjusting ventilation capacity, but at high density air gets trapped in pockets between animals, thus decreasing ventilation efficiency (Reiter and Bessei, 2000). When studied in field trials on commercial farms, the only confirmed effects of increased density on behaviour were a decrease in walking bout length, and an increase in disturbances by other birds (Dawkins et al., 2004). As mentioned previously for footpad dermatitis, this indicates that in practice other factors may overshadow stocking density effects.

Apart from effects on growth and behaviour, little is known about the effects of stocking density on fattening rabbit welfare (Szendro and Luzi, 2006). There are indications however, that increased stocking density impacts negatively on rabbit welfare by increasing stress levels, as increased corticosterone levels have been reported (Onbasilar and Onbasilar, 2007). Also, subordinate wild rabbits studied in semi-natural surroundings showed constantly elevated heart-rates, which declined after the dominant rabbits were removed (Eisermann, 1992). Co-habitation with a dominant conspecific could thus be speculated to increase stress levels in subordinate fattening rabbits as well. However, such effects may be prevented by the fact that the dominance hierarchy is usually not established before fattening rabbits' commercial slaughter age (Lehmann, 1991).

Stocking density also influences the behaviour of fattening rabbits. In contrast to what was previously described for broiler chickens, increased grooming and resting were found at higher stocking densities, and cage manipulation also increased with density (Morisse and Maurice, 1997). It is possible that rabbits genuinely feel a greater need to perform such behaviour when stocked more densely. However, these types of behaviour replaced locomotion and social interaction (Morisse and Maurice, 1997) which are both impeded by high stocking densities. This suggests that grooming and resting may serve as 'time filler' activities (Keeling, 1995), and should not be interpreted as a sign of increased welfare in this case. Cage manipulation can be interpreted as an abnormal behaviour (Chu et al., 2004), which is a sign of unfavourable housing conditions (Mason et al., 2007). Another indication that high stocking density influences rabbit welfare negatively is an increase in aggression (Szendro and Luzi, 2006; Verga et al., 2007), although this can be greatly reduced by slaughtering before 80 days of age (Rommers and Meijerhof, 1998).

The number of studies in which fattening rabbit behaviour was observed at different densities is limited. More often, studies have focused on housing fattening rabbits at an equal stocking density, whilst group size and total cage size increased simultaneously. As explained previously, this can be expected to have similar effects as lowering stocking density (McGlone and Newby, 1994). Such studies indicate an increase in either the frequency or the vigour of locomotion when more space is available (Martrenchar et al., 2001; Postollec et al., 2006; Princz et al., 2008; Zucca et al., 2008).

As discussed above, high stocking densities may impact on several physical and behavioural indicators of welfare. However, the density from which these indicators are influenced can differ between indicators. For instance, when broilers were studied at different densities (Buijs et al., 2009a), leg strength showed a steep decrease between 6 and 23 kg/m^2, whilst hock dermatitis rose between 35 and 56 kg/m^2, and footpad dermatitis and fearfulness were only significantly higher at 56 kg/m^2. This makes it harder to define a specific threshold density from which animal welfare can be said to be impaired. Furthermore, the absence of physical problems does not ensure that enough space is provided to satisfy the animals' needs (Fraser, 2009). Instead of studying the density at which physical or behavioural problems start to occur, the optimal space allowance for animals housed in groups (from the animals' point of view) may be assessed by studying their spatial distribution, as detailed in the next section.

Divergence from random spacing as an indicator of optimal space allowance

Animals generally approach stimuli which are attractive, and avoid or withdraw from those that are aversive (Brown, 1948; Schneirla, 1959). As shown by preference and motivation studies (Huls et al., 1991; Lindberg and Nicol, 1996; Seaman et al., 2008), both chickens and rabbits are social animals that show an aversion to social isolation. However, an aversion to social isolation does not necessarily mean that animals want to be close to each other. A social companion can be an aversive stimulus when too close, as well as an attractive stimulus when too far away. When the conspecific is too close, animals will react by increasing their distance to this conspecific and when conspecifics are too far away, animals will move towards a conspecific (Gueron et al., 1996). Thus, by studying distances between animals, we can identify whether they are avoiding each other, or attracted to each other.

For captive animals, spatial distribution is restricted by the confines of their housing. This not only affects the observed spatial distribution, but also the distribution that would be expected to occur by chance. In larger enclosures randomly moving animals will on average be further apart than in smaller enclosures, simply because greater distances between animals are possible. Therefore, assessing avoidance/attraction cannot be done by looking at the absolute distances between animals. Instead, the observed distribution should be compared to the distribution that would be expected if animals were indifferent to the location of their conspecifics. When domestic animals are kept in a confined space which forces them closer together than preferred, they will attempt to move away from each other. This will result in greater distances between individuals and a more homogeneous distribution over the available space than would be expected by chance (Brown and Orians, 1970; Keeling, 1995). Conversely, when animals are observed to be distributed as expected by chance, or even closer together than expected, it can be assumed that animals are not forced into closer proximity than preferred by a lack of space. This means that enough space is available to satisfy the animals' proximity preferences in the particular setting in which they were studied. By increasing their distance to conspecifics animals may be able to avoid, or at least decrease, the deleterious effects of crowding which are measured by more traditional welfare indicators.

Several broiler chickens studies have measured how the observed distribution diverged from expected values. Such expected values are usually based on a random distribution of animals over the study area. For instance, (Febrer et al., 2006) compared the observed distribution of broilers stocked at densities between 30 and 46 kg/m^2 (14 to 21 birds/m^2) to simulations. Simulated "birds" were placed on a randomly selected coordinate within the simulation area one by one, and accepted or rejected this coordinate based on the distance to the other simulated “birds”. Rejection distance and probability were altered to fit the observed variation in inter-individual distance (an index of clustering explained in more detail in the materials and methods section). For all densities, the observed and the simulated distribution were most similar when animals were modelled to be attracted to each other. In line with this, Leone et al. (2007) found that broilers were closer to their nearest conspecific than expected if assuming a random distribution, when studied at densities of 0.3, 0.6 and 1.2 birds/m^2. Maximum inter-individual distances (i.e., the distance between the two flock members furthest apart) were also smaller than expected. In contrast, the minimum inter-individual distance (i.e., the distance between the two closest flock members) did not diverge from expected values in the groups stocked at 1.2 birds/m^2. In this study (Leone et al., 2007), density and group size were confounded, and as such it cannot be determined reliably which factor caused the deviation from random spacing. However, in other studies (Leone et al., 2010; Leone and Estevez, 2008) varying density and enclosure size at a stable group size had a more pronounced effect on the deviation from random spacing than varying group size and enclosure size at a stable density. This indicates that where the deviation from random spacing is concerned, stocking density may have a greater impact than group size. In contrast to the smaller than expected distances described for lower stocking densities, birds stocked at 6.7 birds/m^2 were usually further apart than expected assuming a random distribution (Leone et al., 2010; Leone and Estevez, 2008). Thus there is a large discrepancy between studies, with some reporting social aversion already at stocking densities less than 7 birds/m^2 and another reporting social attraction at densities as high as 21 birds/m^2. Although these differences may be genuine effects of the different genetic lines and housing conditions, they may also be a consequence of the methodology that was used, since none of the studies took environmental influences on spacing into account.

Environmental influences on the divergence from random spacing

Methods that compare the observed distribution with expected values based on a random distribution assume that animal presence is equally likely in all parts of the study area (Clark and Evans, 1954). This is not always a realistic assumption, since animals are more likely to be found in parts of the study area that contain important resources (Collins et al., 2011; Folmer et al., 2010). When animals are attracted to a certain area because of its environmental characteristics, and thus cluster in this area, comparisons with a random distribution will lead to the erroneous conclusion that they are attracted to each other. This section describes several environmental influences that lead to a non-random use of space, and explains how these influences can be accounted for.

In broiler chickens, the area near the wall is often reported to be preferred over the centre of the pen. This effect was found both in small flocks (80 birds) and in large ones (3000 birds) stocked at densities above 7 birds/m^2 (Cornetto and Estevez, 2001b; Newberry and Hall, 1990). However, this tendency seems to be density dependent. Arnould and Faure (2004) also found more birds in the wall area when studying flocks stocked at 15 birds/m^2, but in flocks stocked at 2 birds/m^2 more birds were found in the central part of the pen. Possible reasons for this wall preference are discussed in detail in paper I. The influence of feeders and drinkers on spatial distribution also seems to be influenced by density. Arnould and Faure (2004) found that broiler chickens housed at 2 birds/m^2 mainly stayed near the feeders and drinkers, whereas broilers stocked at 15 birds/m^2 mainly stayed in the area that did not contain such equipment. Additionally, management may influence spacing: young broilers are attracted to heaters when ambient temperature is low (Aviagen Ltd., 2002), areas with poor litter quality may be avoided as broiler chickens avoid sitting down in wet places (Weeks et al., 2002) and broilers preferentially use areas with a specific type of light (Kristensen et al., 2007). Less is known about features that attract domestic rabbits, apart from an attraction to gnawing sticks (Princz et al., 2007) and pen corners (Huls et al., 1991). Wild rabbits are attracted to areas that provide shelter from predators (Cowan and Bell, 1986; Lombardi et al., 2003). As walls are usually the only structures in a fattening rabbit cage that offer some shelter from predators (by impeding the predators approach from one side, and by offering limited visual cover) they may be speculated to attract rabbits.

For some environmental features it may be possible to distribute these homogeneously over the study area, to minimize differences in area use due to these features, thus also minimizing their influence on the divergence from random spacing. However, this may lead to other problems, e.g., when extra panels are placed in the centre of the pen to draw animals away from the wall area, this may limit free movement throughout the pen. Making feed and water available at every location in the pen may have similar effects. In addition, factors like temperature and airflow are difficult to get completely even, and presently unknown factors may influence spacing as well. Instead of attempting to create a fully homogenous environment, it may be preferable to analyse avoidance/attraction in a way that accounts for all environmental influences, whether known or not. This can be done by comparing the observed distribution to a resource-corrected random distribution. Such a resource-corrected random distribution reflects unequal space use due to environmental circumstances, but is not influenced by social factors, i.e., distances between animals (Burgess, 1980), see "Creation of expected values of spatial distribution" in the materials and methods section for a more detailed explanation). By comparing an observed distribution to such a resource-corrected distribution we can thus see if animals are attracted to conspecifics (i.e., closer to each other than if they were indifferent of each other's location) or avoid conspecifics (i.e., further away from each other than if they were indifferent to each other's location, Keeling and Duncan, 1989). By studying avoidance/attraction at increasing stocking density, the density at which a switch from attraction to avoidance occurs can be pinpointed. This density can be seen as optimal from the animals' point of view. Although densities below this point are not necessarily less preferable from the animals' point of view, the density at which the switch occurs is called optimal because it allows animals to keep their preferred spatial distribution, without supplying animals with what could possibly be an abundance of space, as this last is of course undesirable from an economic point of view.

Socio-environmental influences on the divergence from resource-corrected random spacing

In the previous paragraphs two components of spacing were described: a social component (attraction or avoidance between animals) and an environmental component (attraction or avoidance of certain areas). In addition to these two components, a socio-environmental component may be present. That is to say, environmental features may affect the extent to which animals avoid each other (or are attracted to each other). For instance they may be more tolerant of each other's proximity when they are outside each other's field of vision, because they are on opposite sides of a panel or other type of cover structure. This suggestion is supported by the fact that the provision of cover structures decreases disturbances by conspecifics and aggression in many species (Aschwanden et al., 2009; Coe et al., 2009; Cornetto and Estevez, 2001a).

The existence of socio-environmental influences can be studied by comparing the divergence from the resource-corrected random distribution in the presence or absence of, for instance, cover structures. If such structures would only influence the environmental component of spacing, the divergence from the resource-corrected random distribution would not differ for situations with and without cover structures, since the resource-corrected random distribution reflects the environmental component. Thus, if comparisons with the resource-corrected random distribution show decreased avoidance (or increased attraction) in the presence of a cover structure, this means that the socio-environmental influence of the structure caused the animals to be more tolerant of each other's proximity. It needs to be remarked that such a socio-environmental influence will only be detected if it increases simultaneous use of an area without altering total use of this area. When increased tolerance of each other's proximity instead leads an increase in total use of the area near the cover structure, this will be reflected in the resource-corrected distribution, and will thus not be apparent in the divergence from this distribution.

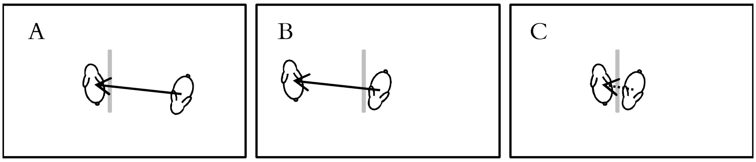

Figure 3. **Detection of a socio-environmental influence.** The figure shows three examples in which rabbits show an attraction to a panel providing visual cover (depicted by the grey line). When animals avoid coming closer to each other than a certain distance, this will force them to take turns using the panel (as shown in Figure 3A and 3B, with the solid arrow depicting the avoidance distance). However, if animals are more tolerant of close proximity when separated visually by the panel, they can use their preferred space near the structure simultaneously (Figure 3C, dashed arrow depicts the decreased avoidance distance). If this causes the rabbits to spent twice as much time near the structure, the socio-environmental influence is indistinguishable from the environmental influence of the panel (i.e., the increased use of the area near the panel will be reflected in the resource-corrected distribution). In contrast, if the panel allows simultaneous use without increasing the total time spent in the area near the panel, the resource-corrected random distribution is not influenced. In this case, the observed distribution is altered by the panel, but the resource-corrected random distribution is not. Thus the presence of the structure will alter the divergence from the resource-corrected random distribution.

Behavioural influences on spacing

Apart from social, environmental and possible socio-environmental influences, behaviour may also influence spacing. Animals may move away from their conspecifics in order to perform behaviours that require more space, and may group together to perform other behaviours. For instance Keeling and Duncan (1991) found that hens were further apart when foraging than when preening, and Collins (2008) found that walking broilers kept longer distances from walking, feeding, standing and preening neighbours than from sitting neighbours. Rabbits are reported to cluster when resting (Matics et al., 2004; Postollec et al., 2006) and when feeding (Cowan and Bell, 1986). Alternatively, these observations may be explained by an influence of spacing on behaviour. Nicol (1989) found effects on behaviour when spacing was manipulated systematically: more preening and body shaking were observed in hens caged close to a conspecific than in hens caged further from a conspecific. Determination of cause and effect is of importance for methods assessing avoidance/attraction between animals. This is because if behaviour is the causative factor, the optimal space allowance may differ according to the behaviours performed. This could for instance lead to different optimal stocking densities for different parts of the day. The expected distributions should preferentially account for such influences, taking into account both the frequency and the synchrony of behaviour expected in that environment and at the time of interest. Conversely, if behaviour is the result of spatial distribution, a correction of the expected distribution is not necessary. However, as of yet it has not been possible to discern cause and effect, and in fact both mechanisms may occur, depending on the situation and the type of behaviour. Because the causative factor is unclear, no correction for behaviour was included in the models used to study avoidance/attraction between animals in this thesis. However, with a view to the future use of expected distributions that are also corrected for behaviour, we did measure spatial distribution during different types of behaviour.

The importance of achieving optimal space allowances

Although analysis of spatial distribution at different stocking densities can help us identify the optimal space allowance from the animals' point of view, this does not give information about how important it is for the animal to achieve such a space allowance. To gain insight into the importance of a certain preference, motivation testing can be used. In motivation tests, animals are taught to perform a certain action (e.g., pushing a button, or pushing open a weighted door) to get access to a reward (Dawkins, 1983). The amount of work (number of pushes, maximum weight pushed) that is performed in order to get the reward is seen as an indication of the importance of the reward to the animal. There are many ways to evaluate this importance, most of which are based on measures from economics. In this thesis, the maximum price paid (i.e., the amount of work performed for a single reward) was used. This measure has been suggested as the most suited to studies of animal welfare because of its dependence on the animals' internal motivational state and its independence of external cues (Seaman et al., 2008; Warburton and Mason, 2003).

Little is known about broiler chickens' motivation for decreased stocking density. One reason for this may be that broiler chickens' rapid growth, poor walking ability and short lifespan make it hard to perform motivation tests, as such tests often require an extensive learning phase as well as behavioural activity. Motivation tests have been carried out in which layers could influence their cage size by pecking a button, but results seemed inconsistent as the layers were found to work for smaller cages as well as for larger ones (Faure, 1985; Faure, 1994). Such inconsistencies may have occurred because the animals were first trained to peck buttons in order to get a food reward, and no check was made later on to see if the birds had actually understood that the keys now influenced pen size. This suggestion is supported by the fact that birds directed 25% of their pecks to an ineffective button in the first experiment. When rabbits were exposed to the same training method (i.e., first teaching the animals to press the key to get food, and subsequently allowing the use of the same key to increase cage size) they also worked both to increase cage size and to decrease it (Bessei et al., 2006). In contrast, individually tested adult pet rabbits are reported to show a greater motivation for access to a large space than for access to a small space (Dixon and Cooper, 2010).

Aims of the thesis

The general aim of this thesis was to study the effects of stocking density on the welfare of broiler chickens and fattening rabbits, by looking at their behaviour and spatial distribution.

More specifically, the aims of the broiler studies were:

- To identify a threshold density above which the behaviour of broiler chickens changed more rapidly, by studying the behavioural time budget of broilers stocked at different densities created by altering the number of birds per pen

- To investigate the underlying reason for broiler chickens' attraction to the wall area, by studying their distribution over the pen when housed at different densities

- To assess broiler chickens' preferred space allowance, by determining the highest stocking density at which they did not avoid the proximity of their conspecifics

- To study the motivation of broiler chickens for a lower stocking density, by titrating their motivation for a lower stocking density against their motivation to feed

Whereas the aims of the rabbit studies were:

- To assess the influence of stocking density and environmental enrichment on the behaviour of fattening rabbits, by studying the behavioural time budget of evenly sized groups in barren and enriched cages of various sizes

- To assess fattening rabbits' preferred space allowance, by determining the highest stocking density at which they did not avoid the proximity of their conspecifics

- To see if the provision of a cover structure would influence avoidance/attraction between animals

In addition to studying the effects of stocking density on welfare, this thesis had two more theoretical aims:

- To compare the outcome of three different indices of spatial distribution when applied to the same datasets

- To study the difference in outcome of comparisons with expected distributions that accounted for environmental influences, and comparisons with expected distributions that did not account for such environmental influences

Materials and methods

Animals and housing

All studies were carried out at the test facility of the Institute for Agricultural and Fisheries Research (ILVO) in Merelbeke, Belgium.

The broiler chickens (Ross 308) hatched at a commercial hatchery (Belgabroed, Merksplas, Belgium) and were moved to the facility as day-old chicks. They were housed in floor pens covered with wood shavings. Temperature was 31°C when the animals arrived, and was decreased by one degree each day until a temperature of 21°C was reached. Where individual broilers needed to be marked, this was done using non-toxic spray cans. Other details on broiler housing and management varied between the experiments, and are thus described in the paper specific methods.

The fattening rabbits (hybrids of several commercial breeds) were bred at the test facility. Until weaning each litter was housed with the doe, in a wire cage with a nest box lined with wood shavings and hay. Pups were cross fostered when litters were too large or when does could not supply enough milk. Since rabbits would regularly experience human contact during the experiment, which apart from the studies described in this thesis included TI-testing and regular lesion scoring (Buijs et al., 2009b), the rabbit pups were 'handled' systematically once before and once shortly after the eyes opened. This is known to decreases fear of humans (Bilko and Altbacker, 2000). The handling consisted of taking a pup from the nest, stroking it gently and then putting it into a box with wood shavings for about 5 minutes, after which pups were returned to the nest. At 28 days of age, animals were weaned, tattooed for individual recognition and allotted to an experimental cage. Each cage contained 4 males and 4 females. Cages never contained siblings, as kinship is known to influence spatial distribution (Farnsworth and Beecham, 1997). Dead animals were replaced throughout the first two weeks after weaning. After this time, no replacements were carried out to avoid disruption of behaviour caused by the introduction of new group members. Mortality was generally low (1.8 %) and there were never less than 7 individuals in a cage. Temperature was kept at 20°C throughout the experimental period.

All animals had ad libitum access to feed and water throughout the experimental periods (with the exception of the broiler chickens used in the feeding motivation test described in paper III). Any obviously sick animals, and broiler chickens with a gait score of 4 or 5 (Kestin et al., 1992) were culled using a captive bolt device (Cash Poultry Killer and Rabbit Killer Kieferle, for broilers and rabbits respectively). At their normal slaughter age (6 weeks for broilers, 10 weeks for rabbits), animals were either sold to a commercial slaughter plant, or culled by lethal injection, and used for morphological studies that will be described in future papers.

Behavioural observations (including location scoring)

All behavioural observations were carried out using digital video recordings. No people were present in the experimental rooms when these recordings were made, as this could have altered animal behaviour, e.g., by causing fear which may cause broilers to clump together (Marin et al., 2001). For paper I and II continuous focal sampling of 5 minute videos was carried out. Frequency, average bout length and total time spent were determined for each type of behaviour, using The Observer 8.0. In addition, a scan sampling of the first behaviour of 8 marked focal animals was carried out to study the association between behaviour and spatial distribution. However, the broilers were inactive most of the time. This made it harder to assess the association between spacing and some of the more active types of behaviour reliably. Therefore, more observations were carried out per treatment in the rabbit study (paper IV and V), although this meant that only scan sampling could be carried out, due to time restraints. For paper V, scan samples of all rabbits in the pen were carried out in the middle of each 10 minute video clip, and results were noted directly in Excel. For both species two separate ethograms were used simultaneously (Table 1 and 2): one that scored postures and one that scored activities. Postures are referred to as "major behaviours" in paper II. In addition to true postures, the posture category included different types of locomotor behaviour. This was done because locomotion could be performed whilst simultaneously performing an activity (for instance walking whilst tail wagging, or running whilst avoiding another animal), but could not be performed simultaneously with a posture. Activities are referred to as "minor behaviours" in paper II.

Table 1. The ethogram used in the broiler studies (paper I and II)

Posture	Description
Standing	Not moving, body not touching the floor
Sitting	Body and both hocks touching the floor underneath or directly on either side of the bird
Lying	Lying on its side, with both feet on the same side of the bird
Walking	Locomotion, the first foot is put down on the floor before the second one is lifted
Running	Rapid locomotion, the second foot is lifted before the first is set down
Activity	Description
Adjusting	Changing the sitting or lying posture without fully standing up. Usually animals swayed from side to side and/or crawled a few centimetres
Drinking	Pecking at the drinker, followed by tilting of the head
Preening	Moving the beak over the feathers
Ground pecking	Pecking at the litter
Eating	Pecking at the feed in the feeder, or between two such pecks
Agonistic behaviour	Fights including pecking at another chicken
Ground scratching	Stepping backwards whilst raking the feet across the floor
Dust bathing	Scratching and bill-raking the litter, followed by vertical wing shaking, head rubbing, bill raking and/or scratching with one leg whilst lying, and then shaking the dust from the plumage
Leg stretching	Elongation of the leg not associated with walking
Head flicking	Rapid head movements in the horizontal plane
Comfort behaviour	Includes wing flapping, body shaking, feather ruffling and tail wagging, but not preening
Displacing	Pushing another bird away from the feeder/drinker
Being displaced	Being pushed away from the feeder/drinker by another bird
Other	All activities that did not fall into the activity categories mentioned in this ethogram. Usually animals showed no other obvious behaviour than scored in the posture category

Table 2. *Ethogram used in the rabbit study (paper V)*

Posture	Description
Lateral lying	The side of the rabbit is in contact with the floor
Sternal lying	The abdomen of the rabbit is in contact with the floor
Sitting	Hocks in contact with the floor, forepaws stretched and feet touching the floor
Standing	Four feet on the floor, abdomen lifted from the floor
Rearing	Front legs not touching the floor, hind legs as in sitting or stretched out
Locomotion	Displacement of the whole body, consisting of one or more hops
Running	At least three consecutive quick hops
Jumping	No contact with floor
Frolicking	Locomotor play consisting of running, jumping and head flicking
Adjusting	Changing the lying or sitting posture without hopping
Crossing	Hopping or climbing over another rabbit
Activity	Description
Aggression	Threatening, biting, attacking, fighting, pushing, chasing or scratching another rabbit
Avoidance	Withdrawing, fleeing, crouching (freezing with nose below that of approaching rabbit) from or for another rabbit
Stretching	Stretching the limbs or the body
Drinking	Mouth in contact with drinking nipple, or between two such contacts
Eating	Head in feeder or performing caecotrophy
Enrichment manipulation	Sniffing, licking or gnawing the enrichment
Grooming	Licking/nibbling/scratching/stroking the own head or body
Cage manipulation	Digging/scratching/licking/gnawing/sniffing walls, floor or cage equipment
Sexual	Mounting another rabbit
Social contact	Sniffing, grooming, gnawing or rubbing another rabbit, or pushing the head underneath the chest of another rabbit. These behaviours can be interpreted either positively or negatively by the target rabbit.
Miscellaneous	Any activity not described in this list, but usually no other behaviour than scored in the posture category

Where spatial distribution was studied (Papers I, II, IV and V), cameras were positioned at an approximately 90 degree angle from the floor to minimize image distortion. Furthermore, images from each camera were calibrated separately using a calibration plate and the Halcon 7.1 software package to reduce any image distortion due to internal and external camera parameters (e.g., wide-angle lens distortion and tilt of the camera). Single frames were isolated from the videos for the analysis of spatial distribution. In the broiler study, 6 photographs were made per pen per week, in weeks 4-6. In the rabbit studies 27 photographs were made per pen per week, when rabbits were 6 and 9 weeks old. Subsequently, animal coordinates were scored by clicking on the centre of each animal in a custom built extension of the Halcon 7.1 software package, and the spatial distribution indices (NND, CVIID and CVDPA) were calculated from this data in R (www.r-project.org), using the add-on packages spatstat (Baddeley and Turner, 2005) and deldir (Turner, 2009).

Indices of spatial distribution

Many spatial distribution indices are available (for an overview see for instance Krebs (1998) or Liu (2001)). These indices can be divided into two categories: quadrat-based methods, which compare the number of animals in different areas, and distance-based methods, which measure the distances between animals (Goodall and West, 1979). For this thesis, one quadrat-based index and three distance-based indices were chosen.

Quadrat scoring was used to study the environmental influences on spacing, by comparing the number of animals in areas with and without a supposedly attractive factor. To study avoidance/attraction, the distribution of animals in relation to each other was assessed using distance-based methods, as these methods have a greater resolution than those based on quadrats (Campbell and Clarke, 1971; Collins et al., 2011). More specifically, nearest neighbour distance (NND) was used to measure absolute distances, whilst the coefficient of variation in inter-individual distances (CVIID) was used to study relative distances. Variation in Dirichlet polygon areas (CVDPA) was also used to study avoidance/attraction, although this index is also directly influenced by environmental features (i.e. proximity to the edges of the study area). These three distance-based indices highlight different aspects of spatial distribution, by assessing different aspects of spacing.

Nearest neighbour distance is an indicator commonly used in ethology. It has been used previously to study the distribution of broiler chickens (Collins, 2008; Leone et al., 2007; Leone et al., 2010; Leone and Estevez, 2008). Although NND can in principle include the distance to any specified number of nearest neighbours (Burgess, 1980; Clark and Evans, 1954), this number is usually restricted to the first nearest neighbour (e.g., Evans and Harris, 2008; Polidori et al., 2008; Radford and Ridley, 2008; Sibbald et al., 2000). Where NND is used in this thesis, it refers to the first nearest neighbour only, unless stated otherwise. It could be argued that analysing NND is an oversimplification of the complexity of spatial distribution, since animals other than the nearest one are likely to influence an individual as well. Furthermore, distributions that include both short and long NNDs, may lead to the same average NND as a distribution in which all individuals are at an intermediate distance from their neighbours (Campbell and Clarke, 1971, Figure 4). However, an advantage of using NND is that this index is more easily influenced by the individual, as it requires interaction with one other animal only. CVIID and CVDPA, in contrast, result from more complex interactions with multiple individuals.

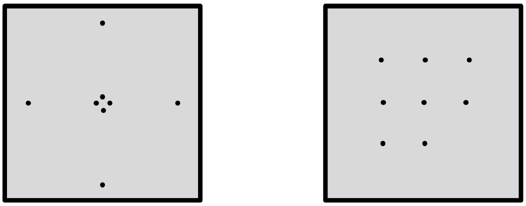

Figure 4. **Two examples of a distribution of 8 individuals.** Average nearest neighbour distance (NND) is equal for the two situations, but the coefficient of variation of inter-individual distance (CVIID) varies.

Inter-individual distance is sometimes used synonymously with NND (e.g., Mooring et al., 2004). However, whenever inter-individual distances are mentioned in this thesis, these include the distance between all possible pairs of animals in a group (Febrer et al., 2006). The coefficient of variation (i.e., the standard deviation divided by the mean) of all inter-individual distances in one observation was used in this thesis, because increased CVIID had recently been suggested as an indicator of social avoidance/attraction (Febrer et al., 2006). In contrast to NND, which is insensitive to the variation in the distance between pairs, CVIID specifically focuses on such variation. As such, it measures subgroup formation, as the variation in inter-individual distance will increase when animals form discrete clusters (i.e., the distance between animals in the same cluster is decreased, and the distance between animals in different clusters is increased). However, CVIID should not stand alone as an index of spacing. This is because it only measures relative distances, and animals may be much further apart in absolute terms whilst still having the same CVIID, as long as the proportions of the distances are equal. Even more problematically, CVIID will also increase when animals avoid specific individuals in a group, but are indifferent to the location of other individuals. In contrast to the possible underestimation of the complexity of spacing noted for NND in the previous paragraph, CVIID may represent an overestimation of the complexity of spacing. Recent studies suggest that animals may adjust their spacing to a fixed amount of their closest neighbours, rather than to all animals in the group (Ballerini et al., 2008). Complex patterns may emerge even when animals adjust their spacing to their closest neighbours, which could influence CVIID. However, animals are likely to adjust their position to conspecifics within the same cluster, as these are the most proximate. As a result, inter-cluster distance is unlikely to be a source of consideration for the animals. In contrast, inter-cluster distance has a major effect on CVIID.

Dirichlet polygons have been used in studies of territory size (Doncaster and Woodroffe, 1993; Valcu and Kempenaers, 2008). They are also known as Voronoi or Thiessen polygons, and encompass the area around a point that is closer to that point than to any other point in the study area (Halls et al., 2001). CVDPA is the standard deviation / mean of the Dirichlet polygon area of all individuals in a group, and thus shows the homogeneity of space division (Byers, 1992). Spatial complexity as reflected by Dirichlet polygon analysis is somewhere between that of NND and CVIID, as two individuals separated by a third do not influence each other's polygon size (Figure 5). In light of the finding that animals may adjust their spacing to a few close individuals only (Ballerini et al., 2008), CVDPA may represent an interesting scale for the study of spatial distribution.

The size of a Dirichlet polygon is not only limited by that of other Dirichlet polygons, but also by the edges of the study area. In this thesis, the edges of the study area were formed by the walls of the pen. As such, this limitation was a realistic representation of the space available to the animals, and it was not necessary to exclude polygons on the edge from analysis - as is sometimes done when studying animals in non-captive conditions (Byers, 1992; Valcu and Kempenaers, 2010). This meant that animals near the wall, and especially those in corners, were likely to have smaller Dirichlet polygon areas than those in the centre of the studied area. For this reason, CVDPA is the only index of the three mentioned in this paper that is directly influenced by the animals' location within the study area (Figure 5).

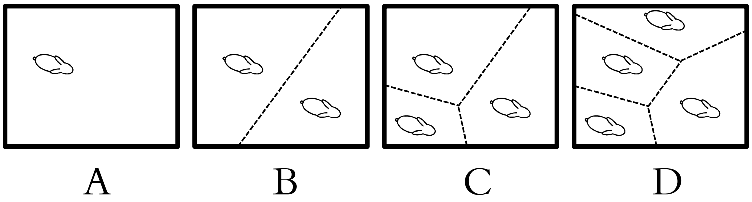

Figure 5. **Division of space by Dirichlet polygons.** 5A: Only one animal is present, and its Dirichlet polygon encompasses the whole pen. 5B: Two animals are present, and space is divided by the perpendicular bisector line between the animals. 5C: Three animals are present, polygons are built up by connecting the perpendicular bisector lines. Notice that although distances between all animals are approximately equal, the animal in the lower left corner has a smaller Dirichlet polygon because the pen walls limit its space. 5D: The last animal that was added does not influence the polygon area of the animal in the lower left corner, because they are separated by the polygon of an intermediate animal.

NND is the only index used in this thesis that measures proximity between individuals directly. But when animals increase the distance from conspecifics in a finite area, this leads to a more homogeneous division of animals over the available space (Febrer et al., 2006). When animals are distributed more evenly, CVIID and CVDPA are decreased. This means that greater than expected NND, and smaller than expected CVIID and CVDPA are all signs that animals are avoiding each other.

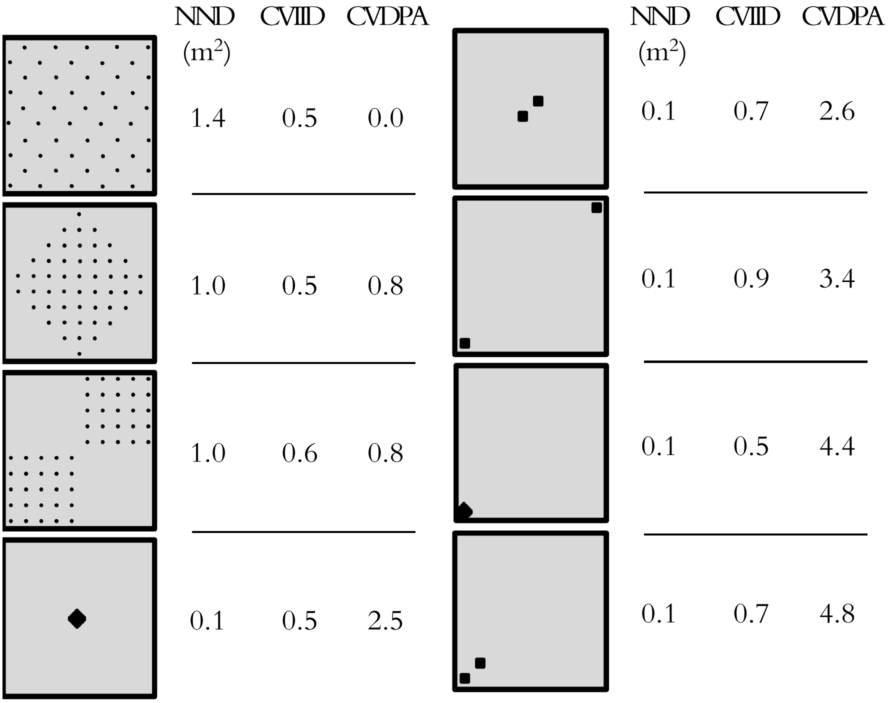

Figure 6. **Examples of the distribution of 50 dots over a $10m^2$ area** (dots overlap in example 4-8) and the resulting nearest neighbour distance (NND), coefficient of inter-individual distance (CVIID) and coefficient of variation of Dirichlet polygon area (CVDPA). Pictures are sorted in order of increasing CVDPA. Note that CVIID does not increase systematically, but instead is increases when multiple clusters are formed, and increases further when these clusters are further apart.

Creation of expected values of spatial distribution

Expected values were based on simulations, and two different kinds of simulations were carried out. In the first kind (referred to as the "random distribution"), animals were placed at a random location within the pen, thus without accounting for environmental influences on spacing. Each simulation contained the same number of animals as observed for that treatment in that week.

The second kind of simulation (referred to as the "resource-corrected random distribution") was based on a reshuffling of observed coordinates, and thus reflected environmental influences on spacing. More specifically, a resource-corrected random distribution was created by reshuffling the location coordinates of the same animals, scored in the same pen, but at different times.

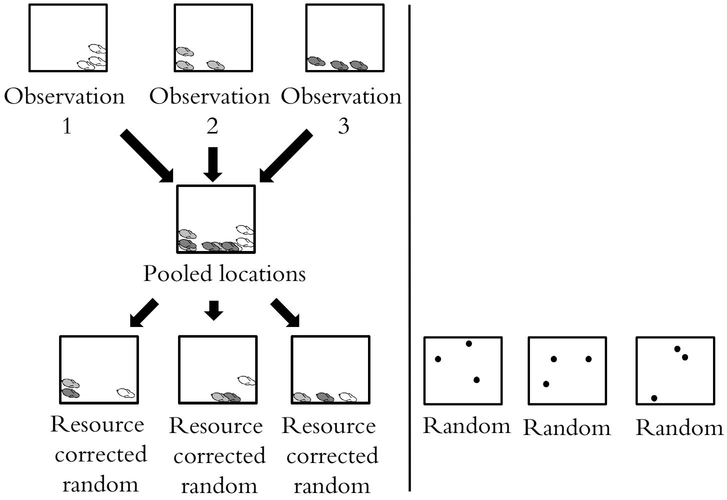

***Figure 7.* Schematic representation of the creation of the resource-corrected random distribution (left) and the random distribution (right)**

The left side of Figure 7 shows a simple example of such a reshuffling for a group of 3 rabbits. The nine animal locations as observed at three different times are pooled, and subsequently three locations are selected from this pool per simulation. Since each simulation is based on locations observed at different times, the location of one simulated "animal" does not influence the location of another (i.e. "animals" are simulated to be indifferent of each other's proximity). However, since all real animals in the three observations are located in the lower half of the pen, all simulated "animals" are located in the lower half in the simulations as well. In contrast, in a random distribution (Figure 7, right side) the chance of finding an "animal" within a certain area is equal for all areas in the pen.

Achievement of stocking densities

As detailed in the introduction, stocking density (or, space allowance per animal) is inevitably confounded with either group size or total space availability (i.e., cage or pen size), because one of these two factors has to change to achieve different stocking densities (Frommen et al. 2009; Leone et al. 2010). When total space availability is varied, this means that either shape or perimeter length is influenced. To create 'the perfect experiment' these variables would need to be set off against each other in a factorial setup, and this for both focal species. In addition, a wide range of different densities would need to be incorporated in order to detect exact cut-offs. Since the size of such an experiment made it unrealistic, choices had to be made in which parameter would be altered in the different experiments included in this thesis.

In the broiler chickens study described in papers I and II, we chose to alter the number of birds, whilst keeping pen size equal, because this is the most common way to alter stocking density in a commercial situation (ordering a different number of day-old chicks, rather than building a differently sized broiler house). Altering group size in an equal area may also correspond more closely with the differences between natural and commercial conditions. The total area of modern commercial broiler houses can be close to the 3 000 m^2 home range mentioned for some groups of wild chickens (Collias and Collias, 1996; Collias et al., 1966), but far more individuals are present in this area in commercial husbandry.

In paper III we chose to alter pen size and shape simultaneously in order to be able to keep group size equal on both sides of the test pen, whilst keeping the difference in perimeter length between the two compartments equal for all treatments. In this way, treatments were created that did not differ in group size. Also, treatments with the greatest difference in shape had the smallest difference in stocking density, thus making it possible to discern between these two factors. Although stocking density co-varied with total space availability, previous studies on layers indicate that it is stocking density rather than total space availability, that motivates chickens' spatial preferences when group size is kept constant. When layer hens were given a choice that only involved total cage size (a preference test between a large and a small empty pen), they showed no significant preference. When this choice was also influenced by density (i.e., when the same number of companion birds were stocked in the large and the small pen), they preferred the larger (less densely stocked) pen (Lindberg and Nicol, 1996).

In the rabbit studies (paper IV and V), cage size was altered instead of group size, since space allowance had not previously been varied in this way in studies of fattening rabbit behaviour. Such a protocol also reflects one of the challenges that commercial husbandry poses to rabbits, as cage size is usually much smaller than the amount of space used under (semi-) natural conditions (Vastrade, 1987).

Paper specific methods

In this section, paper specific methods are discussed in brief. More detailed descriptions can be found in the respective papers.

Paper I

The experiment on which paper I is based consisted of four experimental replicates. Within each experimental replicate 8 different stocking densities were created by placing a different number of day-old chicks in pens of the same size (3.3 m^2). By placing 8, 19, 29, 40, 45, 51, 61 or 72 birds in a pen, stocking densities of 2.4, 5.8, 8.8, 12.1, 13.6, 15.5, 18.5 and 21.8 birds/m^2 were achieved. Dead animals were replaced throughout the first eight days of each replicate, no later replacements were made to avoid changes in behaviour due to the introduction of new group members. Each pen was equipped with 14 feeders and 10 water cups, attached to the outside of the walls so they would not take up floor space. The number of feeders and drinkers was not increased for bigger groups, to minimize environmental differences between the pens. Instead, all pens were fitted with a number of feeders that would allow more than half of the largest flock to feed simultaneously. Eating time, frequency and bout length were not influenced by density, supporting the suggestion that enough feeding space was provided at all densities. Light periods of 21 hours were separated by 3 hour dark periods. To minimize the chance that litter quality would affect spatial distribution, the litter was completely replaced three times within each six week experimental period. Also, occasional wet patches resulting from leaking drinkers were refreshed when discovered.

To assess whether animals had a preference for the wall area at the different stocking densities, the number of animals in four different pen areas (see Figure 8) was determined during the last three experimental replicates (technical errors impeded such a scoring during the first replicate). Distribution was scored 6 times per pen per week, in weeks 4, 5, and 6 of each experimental replicate. At the same moment, the behaviour 8 focal birds per pen was scan sampled, in order to study the association between location and the number of adjustments (changing the sitting or lying posture without standing up fully).

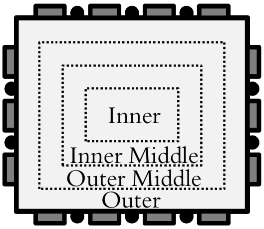

Figure 8. **Top view of the broiler pen used in paper I,** indicating feeder locations (grey squares), drinker locations (black dots) and the four areas in the pen in which space use was compared.

The length of behavioural bouts was determined by performing a continuous sampling of the behaviour of the 8 focal birds, during weeks 2-6 of all four experimental replicates. Each pen was observed 6 times per week, for 5 minutes at a time. In addition to the analysis of bout length, the frequency of adjustments was also scored using continuous sampling of the same material.

Figure 9. **The lowest and highest stocking densities used in papers I and II.** The photograph was taken at an age of 5 weeks. One bird is missing from the pen with the lowest stocking density.

Paper II

Since paper II was based on the same experiment as paper I, all procedures and treatments were the same as described above for paper I. However, for paper II, the frequency and total time spent on each behaviour were analysed instead of bout lengths. Spatial distribution was not analysed in terms of animals' location within the pen, but in terms of their proximity to each other. XY coordinates and the resulting NND, CVIID and CVDPA were determined as described in the general methods, and compared to expected values based on a random and a resource-corrected random distribution. In addition, the previously mentioned scan sampling of behaviour was now used to study the association between an individual's behaviour and its NND.

Paper III

The experiment on which paper III is based was carried out using 4-6 week old broiler chickens. The experiment consisted of two sub-experiments: one that assessed feeding motivation and one that assessed the motivation for a lower density. Six experimental groups were used: 2 groups of 8 individuals for the feeding motivation sub-experiment and 4 groups of 104 individuals for the density motivation sub-experiment. Dead animals were replaced throughout the experimental period.

In the feeding motivation sub-experiment, the maximum barrier height an individual would cross to get to feed was determined. Barrier crossing was used as this was considered to be a more natural behaviour for a broiler chicken than pecking a button or pushing a door, and was assumed to require little training. Feeding motivation was determined before and after 6 hours of feed deprivation. Birds were tested individually in the feeding motivation sub-experiment. They had a maximum of 10 minutes to cross a barrier, and reach the food offered in a familiar feeder on the other side. The barrier consisted of a freely rotating PVC pipe with wire netting underneath, and birds were able to see the feeder by looking over the barrier or through the netting. Birds were tested twice per week (once before and once after feed deprivation). Within each test, 4 trials were conducted, and each barrier height (7, 14, 21 and 28 cm) was offered once. Half of the birds were tested with increasing heights and the other half with decreasing heights, and this order was switched between weeks. Within each test, barrier height was either consistently increased over trials or consistently decreased over trials.

***Figure 10.* A broiler chicken crosses the barrier in the feeding motivation sub-experiment.** Photograph by Carmen De Pauw.

For the density experiment a pen was used that consisted of two compartments separated by a barrier. Two different barrier heights were used. The maximum height that 75% of the individuals had crossed in the feeding motivation sub-experiment when not previously deprived was used as the “low barrier treatment” in the density motivation experiment. The “high barrier treatment” was determined similarly, but with broilers that had been deprived for 6 hours. Thus, feeding motivation was used as a yardstick to assess the motivation for achieving a lower density. These barrier heights were determined separately for 4, 5, and 6 week old animals, to account for changes in body size and walking ability with age.

The density motivation sub-experiment was carried out in pens that consisted of two compartments. One compartment was fixed in size and shape, whereas the other was adjustable. By folding the walls of the adjustable compartment, the area within this compartment could be altered without influencing total wall length (Figure 11).

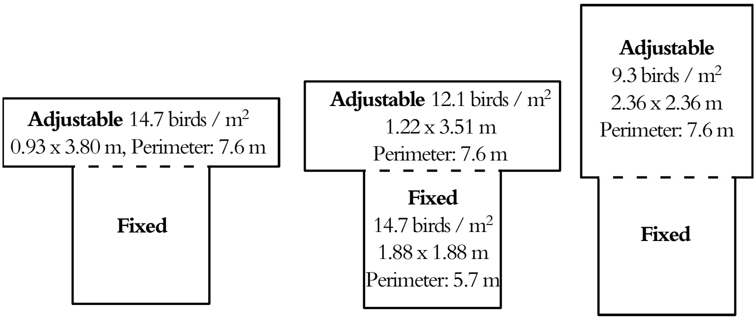

***Figure 11.* The three different settings of the experimental pen** in the density motivation sub-experiment, that could be created by folding the walls of the upper compartment.

At the start of each trial 52 animals were present in each compartment. Thus, stocking density in the fixed compartment was always 14.7 birds/m^2 at the start of the trial, whereas density in the adjustable compartment was either 9.3, 12.1 or 14.7 birds/m^2. Although the perimeter length of the adjustable compartment was equal in all treatments, it differed from the perimeter length of the fixed compartment. However, this was necessary in order distinguish density and shape effects. By doing so, it became possible to create a treatment that maximized density differences but did not differ in shape, and a treatment that maximized shape differences but did not differ in density.

The birds were permanently housed in their test pen from an age of three weeks onward, but could move freely between the two compartments when no tests were run. At the start of each trial the adjustable side was set to the right size and the barrier was set to the correct height. A panel was placed above the barrier to prevent birds from crossing it. The flock was then divided into two predetermined equal halves, which were placed on either side of the barrier. Then birds were left undisturbed for 30 minutes, to allow them to settle down again. Subsequently, the panel placed above the barrier was removed by pulling on a cord from outside the experimental room. Animals could now move between the compartments, and the number of birds moving into the adjustable compartment was determined, as well at the number moving into the fixed compartment. These movements were scored for 17 minutes.

Figure 12. **An experimental pen for the density motivation sub-experiment,** just before testing. The pen is set for a 9.3 vs. 14.7 birds/m^2 treatment. The middle panel keeps the birds from crossing between the two compartments. Birds were colour marked to facilitate the separation of the flock into two equally sized groups, but were housed together outside the time that tests were run. Photograph by Carmen de Pauw.

Paper IV

For the experiment that paper IV was based on, fattening rabbits were housed in groups of 8, in open-top wire cages of either 0.40, 0.46, 0.53, 0.64, 0.80, 1.07 or 1.60 m^2. Each cage was equipped with 4 feeders and 2 drinking nipples. All cages of 0.40 and 0.46 m^2 were barren, but for the five larger cage types half of the cages were enriched with a ⊔ shaped wooden structure (40 × 20 × 20 cm, l × w × h). Divided over 3 experimental replicates, 12 cages were set up for each of the two smallest cage sizes, whereas 6 enriched and 6 barren cages were set up for each of the five larger cage sizes. In total, 684 rabbits were used (12 of which were replacement animals). Video recordings were made during dawn (6 a.m. – 8 a.m., 8 lux), day-time (8 a.m. – 4 p.m., 120 lux) and dusk (4 p.m. – 6 p.m., 8 lux), when animals were 6 and 9 weeks old. The 27 recordings per pen per week were evenly divided over the three light phases (dawn, day, dusk). A single frame was isolated from the middle of each video-recording, and the XY coordinates of all rabbits in the pen were determined. The resulting

NND, CVIID and CVDPA were subsequently compared to expected values (as described in the general methods).

Figure 13. **A 0.80 m^2 rabbit cage with a wooden enrichment structure.**

Paper V

Paper V was based on the same experiment as paper IV, and details on the setup can be found in the previous section. However, the focus of paper V was on the behavioural time budget of the rabbits, and on the association between behaviour and spatial distribution. One scan sample was carried out for each of the video-recordings described above. In this scan sample the proportion of animals performing each behaviour was scored. This scan was performed at the same moment as the determination of the XY coordinates described above. Thus an individual's behaviour and its NND and DPA could be linked.

Statistical analysis

All statistical analyses were carried out using SAS 9, with the exception of the Monte Carlo permutations used to compare spatial distribution to expected values, which were performed in Excel.

For the analyses performed in SAS, non-significant fixed effects and interactions (P > 0.05) were removed from the model stepwise. Generalized linear mixed models were performed using the GLIMMIX procedure. A log link was used, assuming an underlying Poisson distribution. Transformed LSMEANS generated by the GLIMMIX procedure were back-transformed using an inverse link function and are presented in this form. Mixed linear models were performed using the MIXED procedure.

Where pair wise comparisons were made, a sequential Bonferroni correction was applied (Holm, 1979), except in paper III where a full Bonferroni correction was applied.

Paper I

The quadrat scoring described in paper I was analysed using a generalized linear mixed model. The number of birds in the different areas was compared within treatment and week. Area (inner, inner middle, outer middle and outer) was used as a fixed factor. As the four areas were not equal in size, the natural log of the area size was included as an offset, to correct for the larger number of animals that would be expected in the larger areas simply by chance. Samples from the same pen were treated as repeated measures.

The number of adjustments per pen area was compared to expected values (i.e., an equal amount of adjustments per bird in all areas) using a Chi-square test. Because the occurrence of adjustments was low in the scan sampling, data were pooled over densities and replicates, but weeks were analysed separately.

Bout lengths were analysed using mixed linear models. The full models included density, week, sex, and their interactions as fixed effects. Density and week were treated as continuous variables. Time of day was used as a random factor to account for differences in behaviour between the morning, afternoon, and evening observations. Observations on the same pen were treated as repeated measures. The same analysis was used for the frequency of adjustments (as indicated by the continuous scoring of behaviour), except that now a generalized linear mixed model was used.

Paper II

Behaviour was analysed using mixed linear models for total duration and generalized linear mixed models for frequencies. The analysis was carried out in the same way as described for bout length in paper I. When this analysis indicated a significant effect of stocking density on behaviour, a second analysis was run which included density as a categorical rather than as a continuous variable. This second analysis allowed for pair wise comparisons between LSMEANS, to see if there was a specific range of densities in which behaviour was influenced the most.

The association between NND and behaviour was studied using NND as the dependant variable. Two ethograms had been used simultaneously during the scoring of behaviour: "major" and "minor" behaviour. Therefore, both major and minor behaviour, and their interaction, were included as fixed variables. As too few data were available to perform separate analyses for each density and week, these two factors were incorporated as random variables. Observations on the same pen were treated as repeated measures.

For the analysis of spatial distribution, the observed and expected distribution were compared using Monte-Carlo permutations. P-values were calculated as the proportion of the 1000 simulation sets that were equal to, or more extreme than, the observed mean. For comparisons with the random distribution, XY coordinates were generated randomly in R (www.r-project.org), with a minimum distance of 50 mm to the pen walls. Simulations containing a NND or DPA smaller that the smallest observed in the actual data were discarded. As observed means were based on an average of 18 observations, simulations were also grouped in sets of 18 and averaged within these sets. One thousand of such sets were created per density and week. The same procedure was followed for the comparisons with the resource-corrected random distribution, except that XY coordinates were now based on a reshuffling of the observed coordinates (as explained in the section "Creation of expected values of spatial distribution").

Paper III

For the feeding motivation sub-experiment, the maximum height that 75% of the birds crossed was calculated, as well as Spearman correlations between the maximum height crossed and the gait score.

For analysis of the density preference sub-experiment, a mixed linear model was used. The number of extra birds in the adjustable compartment, at the end of the test, was used as the dependant variable (i.e., the number of animals moving into the adjustable compartment – the number of animals moving out of the adjustable compartment). The size of the adjustable compartment, bird age, and barrier height were used as fixed variables, as were their interaction terms. Barrier height had two levels: low (equal to the height crossed by 75% of the birds in the feeding experiment before feed deprivation, at that specific age) and high (equal to the height crossed by 75% of the birds in the feeding experiment after 6 hours of feed deprivation, at that specific age). Tests on the same pen were treated as repeated measures.

Paper IV

The number of animals in the central area and the area near the wall were compared using a generalized linear mixed model. Separate analyses were run for each treatment, within each week. Area (centre or wall) was the only fixed factor. Observations on the same cage were treated as repeated measures. In contrast to the analysis in paper I, no correction for area size was necessary, as both sampled areas were equal in size.

Spatial distribution was analysed as described for paper II, with a few exceptions. A minimum distance of 30 mm to the wall was used in the generation of the random XY values (corresponding with distance to the wall in the observations). As rabbits were often found on top of each other, no minimum NND was used. However, the observations showed a minimum DPA of 8786 mm^2, and simulations containing smaller DPAs were discarded. As observed means were based on 9 photographs, simulations were also grouped in sets of 9 and averaged. This led to 1000 simulations per combination of replicate, pen, week and light phase. Observed data, as well as simulations, were subsequently averaged with those of the same treatment and week. Preliminary analysis showed that light phase had little influence on the results. Therefore, observed data and simulations were averaged over the light phases as well.

Paper V

The results of the scan sampling of behaviour were expressed as the proportion of animals in each scan performing each behaviour. Subsequently, the nine scans made within each combination of replicate, age, cage and light phase were averaged. Averages were analysed using a mixed linear model. Because the set-up was not fully factorial (no enriched cages of 0.40 and 0.46 m^2 were used), the effects of enrichment were analysed using a subset of the data excluding the smallest cages. In this first analysis, enrichment, cage size, week and light phase, and their two-way interaction terms were used as fixed factors. Replicate was included as a random factor and observations on the same cage were treated as repeated measures. Then, a second analysis was performed to study the effects of cage size, age and light phase. The full data set was used for this analysis, but for behaviours that were influenced by enrichment, separate analyses were performed for enriched and unenriched cages. Cage size, week and light phase were used as fixed factors. Replicate was included as a random factor to account for discrepancies between repetitions, and observations on the same cage were treated as repeated measures.

To study the association between distribution and behaviour, average NND and DPA were calculated for animals performing the same behaviour in the same cage. Cages with less than 8 animals were excluded from this analysis. A mixed linear model was used, treating NND as the dependant variable. The effects of posture and activity were studied in separate models. These models included cage size and either posture or activity as fixed factors (and the interaction between the two factors). Replicate was used as a random factor.

Summary of the results

This sections summarizes the main results of papers I-V. More detailed information can be found in the respective papers.

Paper I

In paper I broiler behaviour and spatial distribution over different pen areas was studied.

At an age of 4 and 5 weeks, more birds were present in the inner area of the pen than in the outer area for some of the lower stocking densities. In contrast, at an age of 6 weeks more birds were present in the outer area than in any of the other three areas for stocking densities $\geq$ 12.1 birds/m^2.

Temperature increased slightly with stocking density (20°C at 2.4 and 5.8 birds/m^2, 21°C at 8.8 birds/m^2 and 22°C at densities $\geq$ 12.1 birds/m^2). However, temperature was higher in week 4 than in weeks 5 and 6, whereas the preference for the outer area became apparent in week 6 only. Thus it is unlikely that area preference was caused by a density induced temperature increase. NH_3 and rH did not differ with density, and no difference in litter dry matter was found for the inner and the outer area.

The length of sitting and preening bouts decreased with increasing density, and the length of walking bouts decreased more rapidly with age at higher densities. In contrast, the frequency of adjustments increased with increasing density.

In the fourth week only, birds in the inner area adjusted their sitting or lying posture more often than those in the other three areas.

Paper II

In paper II broiler behaviour and social attraction/avoidance were studied at different stocking densities.

The total time spent preening and ground pecking decreased with increasing density. In contrast, the frequency of sitting and adjusting increased. Pair wise comparisons of treatment means did not show clear cut-off densities above which behaviour changed rapidly.

The expected values of spatial distribution based on the random distribution, and those based on the resource-corrected random distribution, differed significantly from each other in 69 out of 72 cases (3 indices × 3 weeks × 8 densities). For NND the differences between the random distribution and the resource-corrected random distribution did not lead to different conclusions on avoidance/attraction. In contrast, for CVIID and CVDPA the type of expected distribution did affect conclusions on attraction/avoidance (Table 3). Comparisons with the resource-corrected distribution indicated avoidance more often, and attraction less often, than comparisons with the random distribution. Which index of spatial distribution was used also influenced conclusions on avoidance/attraction. NND indicated avoidance more often, and attraction less often, than CVIID and CVDPA did. However, as animals grew older avoidance became more common, and the aforementioned differences caused by the kind of expected distribution and the distribution index disappeared. In the last week of rearing avoidance was indicated for densities $\geq$ 19 birds/pen (5.8 birds/m^2), regardless of which index or expected distribution was used. No evidence of attraction was found at this age.

***Table 3. Overview of the stocking densities at which avoidance and attraction were found for broilers**, as indicated by a divergence of observed values from expected values based on a random or resource-corrected random (R-COR) distribution (P<0.1). Stocking densities are expressed as birds/pen.*

	Expected distribution	Attract Week 4	Avoid Week 4	Attract Week 5	Avoid Week 5	Attract Week 6	Avoid Week 6
NND	Random	8	≥ 29	8	≥ 19	-	≥ 19
	R-COR	8	≥ 29	8	≥ 19	-	≥ 19
CVIID	Random	8, 29, 40, 51, 61	-	-	40, 45, ≥ 61	-	≥ 19
	R-COR	-	-	-	≥ 19	-	≥ 19
CVDPA	Random	≤ 40, 51, 61	72	8	≥ 40	-	≥ 19
	R-COR	-	45, 72	8	≥ 19	-	≥ 19

Longer NNDs were found for eating or drinking birds than for those adjusting their sitting/lying posture, foraging, preening, or showing "other" behaviour.

Paper III

In paper III feeding motivation was used as a yardstick to assess the importance broiler chickens attribute to attaining a stocking density below 15 birds/m^2.

In the feeding motivation sub-experiment, the percentage of birds crossing the lowest barrier when not previously deprived of food was below the predetermined minimum of 75% in all weeks. In contrast, when deprived for 6 hours, at least 75% of the birds crossed the 14 cm barrier in weeks 4 and 5, and the 7 cm barrier in week 6. Thus, the "low barrier" for the density preference sub-experiment was put directly on the floor, whereas the "high barrier" was 14 or 7 cm high, depending on the birds' age.

The extra number of birds on the adjustable side of the pen at the end of the test ($NBIRDS_{extra}$) increased with the size of this compartment, and this effect became more pronounced with age. $NBIRDS_{extra}$ generally exceeded the number than would have been necessary to achieve an equal stocking density on both sides of the pen. Also, $NBIRDS_{extra}$ was greater when the low barrier was used. Although broilers kept crossing the barrier throughout the 17 minute trials, this had only a minimal effect on $NBIRDS_{extra}$ after the first minute, as the number of crossings back and forth cancelled each other out.

The feeding motivation sub-experiment showed a negative correlation between the maximum height crossed and the gait score, but only for deprived animals at four weeks of age. This may have been due to the birds' relatively good walking ability, as more than 80% of the birds had a gait score ≤ 2, and none had a gait score of 4 or 5.

Paper IV

In paper IV the distribution of fattening rabbits was studied at different space allowances in enriched and unenriched cages.

Generally, more animals were present in the area near the wall than in the central area in unenriched cages. Conversely, in enriched cages the central area - which included the enrichment - was used more often than the area near the wall. For the smallest two types of unenriched and enriched cages exceptions to this general trend occurred.

When the observed spatial distribution was compared to the random distribution, avoidance was generally found in smaller cages and attraction in larger cages (Table 4). However, some pattern inconsistencies occurred. For instance, CVDPA indicated that 9-week-old rabbits showed avoidance when housed in cages of 0.53 or 1.07 m^2, but not in cages of 0.64 or 0.80 m^2. In contrast, no such pattern inconsistencies occurred for the comparisons with the resource-corrected random distribution.

Table 4. Overview of the cage sizes at which avoidance and attraction were found for fattening rabbits*, as indicated by a divergence of observed values from expected values based on a random or resource-corrected random (R-COR) distribution (P<0.05). UNE = unenriched, ENR = enriched, Wk = week.*

	Expected distribution	Attract Wk 6 UNE	Avoid Wk 6 UNE	Attract Wk 9 UNE	Avoid Wk 9 UNE	Attract Wk 6 ENR	Avoid Wk 6 ENR	Attract Wk 9 ENR	Avoid Wk 9 ENR
NND	Random	≥1.07	≤0.64	-	≤1.07	≥1.07	≤0.64	-	≤1.07
	R-COR	-	≤0.80	-	All	-	≤1.07	-	All
CVIID	Random	≥0.80	≤0.53	1.60	≤1.07	≥1.07	0.64	-	≤1.07
	R-COR	-	≤0.80	-	≤1.07	-	≤1.07	-	All
CVDPA	Random	≥0.53	-	≥1.07	≤0.64	All	-	1.60	0.53, 1.07
	R-COR	-	≤0.64	-	≤1.07	-	0.53	-	≤1.07

Two different ways were used to identify the optimal density. The first method pinpointed the smallest cage size at which no significant evidence of avoidance was found. The second method determined the two cage sizes between which the switch from avoidance to attraction occurred, regardless of whether this avoidance or attraction was significant. No significant evidence of attraction was found for any of the cage types when comparing with the resource-corrected random distribution. When the rabbits were 9 weeks old, no switch from avoidance to attraction was observed. Instead, all indices indicated avoidance at all cage sizes, although this was not always significant for cages of 1.60 m^2. The one exception to this was CVDPA in enriched cages, which indicated attraction in cages of 1.60 m^2, although this did not reach significance. The switch from avoidance to attraction occurred at larger cage sizes for older rabbits. It also occurred at larger cage sizes for enriched than for unenriched cages. CVDPA indicated avoidance less often than NND and CVIID.

Paper V

Paper V describes the behaviour of fattening rabbits kept in unenriched and enriched cages that differed in size, and the relation between behaviour and spatial distribution.

Rabbits in enriched cages spent 4% of their time interacting with the enrichment structure during the dawn and dusk phase, and 1% during the daytime. In enriched cages less time was spent on social contact, cage manipulation and lateral lying than in unenriched cages. Six-week-old rabbits also drank less when housed in enriched cages.

As cage size increased, sternal lying decreased. In contrast, sitting increased with increasing cage size, but treatment differences were small and pair wise comparisons did not indicate any significant differences. Although social contact, standing and eating were also influenced by cage size, this influence did not lead to a consistent increase or decrease with increasing cage size.

During the daytime rabbits spent less time on hopping, sitting, manipulating their cage, and social contact than during the dawn and dusk phase. Instead, they spent more time lying. During the dawn phase rabbits spent more time grooming than during the daytime and dusk phases. Eating and drinking was observed most during the dusk phase and least during the dawn phase.

NND was influenced by an interactive effect of activity and cage size, as was DPA. In larger cages, grooming occurred at longer NND and greater DPA than several other activities. NND and DPA were also influenced by the posture of the rabbits. Rabbits that were sitting and standing had longer NNDs than those lying sternally or laterally. Sitting and standing rabbits also had greater DPAs than those lying sternally. In contrast to what was found for NND, rabbits that were lying laterally also had greater DPAs than those lying sternally.

General discussion

A detailed discussion of the separate studies can be found in the respective papers, whilst this general discussion focuses mainly on cross-paper comparisons, and on future research that can be recommended based on the results of the papers. The results are also discussed in the light of other welfare parameters that were scored during the experiments, but were not described in papers I-V.

Effects of stocking density on behaviour

The following paragraphs describe the effects of stocking density on resting, which seems to decrease with density for broiler chickens, whilst increasing with density for fattening rabbits. In addition, the lack of a threshold density above which behaviour changed rapidly is discussed.

Papers I and II describe that increased stocking density led broiler chickens to adjust their sitting posture more often, and to perform more, but shorter, sitting bouts. Adjustments usually occurred in reaction to jostling or other physical contact with pen mates. Thus, the increased frequency of adjustments may indicate that the birds' resting behaviour was increasingly disturbed at higher densities. The fragmentation of sitting behaviour also supports this hypothesis. Such disturbances of rest are likely to have a negative impact on broiler chickens' welfare, as rest is especially important for juvenile animals (Malleau et al., 2007). Rest has previously been manipulated by applying continuous (24 h/day) lighting. This increased stress levels in broiler chickens - as measured by fearfulness and heterophil : lymphocyte ratios (Bayram and Ozkan, 2010; Onbasilar et al., 2008). A 6-hour dark period per day is now obligatory when raising broiler chickens in the European Union (2007/43/EG). Therefore it has become more important to assess whether disturbance of rest during the light period affects welfare even when such a 6-hour dark period is applied. Disturbance of rest could be manipulated systematically (for instance by making the floor shake at unpredictable intervals during the light period). By doing so, disturbance of rest could be studied without confounding it with other effects of increased stocking density. In addition to measuring physiological parameters under conditions of increased disturbance, it would also be worthwhile to see if resting shows a rebound effect (Nicol, 1987), i.e., to see if resting is increased when the number of disturbances suddenly decreases after a period with a high number of disturbances.

In fattening rabbits, the only consistent effect of increased stocking density was an increase in sternal lying. Sternal lying in the rabbit can be seen as the analogue of sitting in chickens, as each is the most common posture during inactivity for the respective species, but neither is most the fully recumbent posture (these would be lying for the chicken, and lateral lying for the rabbit). Since sitting was increasingly fragmented at higher densities for broiler chickens, whereas sternal lying increased with increasing density in fattening rabbits, stocking density seems to have an opposite effect on the resting behaviour of the two species. However, it needs to be remarked that behavioural observations were carried out in a different way for the two species. If sternal lying also occurred in shorter, but more frequent bouts, this would have gone unnoticed because of the scan sampling protocol used in the rabbit study. Still, high stocking densities did not seem to promote resting in the chickens, as they did in the rabbits. There are indications that this difference between broilers and rabbits is caused by a different species-specific reaction to crowding, rather than by the fact that stocking density was manipulated in a different way (altering group size in the broiler chicken studies and total cage size in the rabbit studies). A previous rabbit study that increased density by increasing group size also found more resting at the higher density (Morisse and Maurice, 1997), whilst broiler studies that increased density by decreasing pen size do not report such an increase (Andrews et al., 1997). However, the conclusion that the differences between the studies were caused by the different subject species instead of the different protocols is tentative, as for each species only one study was found that altered stocking density in the opposite way of the density manipulations carried out in this thesis (i.e., one broiler study that altered stocking density by changing pen size for broilers, and one fattening rabbit study that altered stocking density by changing group size).

Stocking density may also influence preening/self-grooming in a species specific manner. In this thesis as well as in other studies (Blokhuis and Van der Haar, 1990; Hall, 2001; McLean et al., 2002) increased stocking density decreased the time broilers spent preening. No effects of stocking density on rabbits' grooming behaviour were found in this thesis, and Morisse and Maurice (1997) even report an increase in grooming behaviour at higher density in rabbits. However, in the rabbit experiment included in this thesis, rabbits in large cages used more space when grooming than when performing the majority of other activities. This suggests that grooming was either preferentially performed, or was more frequently elicited, at lower (local) stocking densities. The absence of a stocking density effect on the time rabbits spent grooming may be explained by a strong intrinsic motivation to groom, which led to grooming even when spatial distribution was not optimal for this type of behaviour. Alternatively, growing up at different stocking densities may influence the distance at which certain behaviours are elicited.

One of the goals of this thesis was to see if there were certain cut-offs, or threshold densities, between which behaviour changed rapidly (Figure 14).

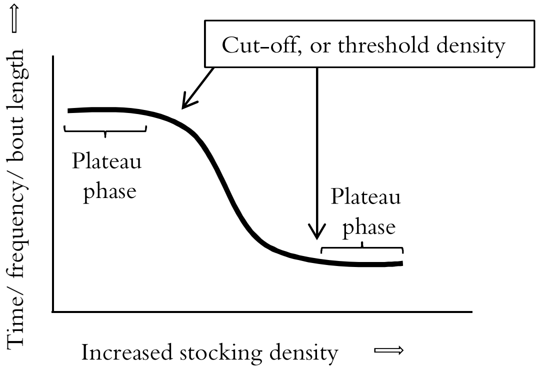

***Figure 14.* An example of a hypothetical relation between behaviour and stocking density.** An opposite relation would occur for 'time fillers', i.e., behaviours that increase in frequency because other types of behaviour are impeded.

For this reason, many different densities were set up for both the broiler and the fattening rabbit experiment. The density range included the highest densities used in commercial practice, as well as densities 9 or 4 times lower (for broilers and fattening rabbits, respectively). Nevertheless, no evidence of a cut-off was found. That is to say, those behaviours that were affected by density changed gradually over the range of densities studied, without clear evidence of a plateau phase at the highest or lowest densities. Theoretically, such a plateau phase is certain to occur both at a high density (as behaviour will become physically impossible at very high densities, and will thus cease to occur completely) and at a low density (as space per animal will no longer be limiting). When one behaviour is decreased in frequency, another has to increase of course. Thus, 'time fillers' will show an opposite relation to density, showing a rapid increase between the cut-off densities. Although the lowest densities observed in this thesis diverged greatly from commercial standards, space allocation was much lower than the amount of space animals chose to use in semi-natural conditions in very spacious enclosures (Keeling and Duncan, 1991; Vastrade, 1987). The plateau phase, in which the behavioural time budget is no longer limited by density, is likely to reside somewhere between the lowest densities used here and those observed in (semi-)natural conditions.

The need to correct for environmental influences when studying social avoidance/attraction

Studies that used spatial distribution to assess social attraction or avoidance often compare observed values to expected values assuming a random distribution. It can be argued from a theoretical point of view that this is not fully correct, as it is unlikely that proximity to conspecifics is the only factor that influences spatial distribution. In a more practical way, this thesis showed that both broiler chickens and rabbits adjusted their spatial distribution to environmental factors: the area near the cage/pen walls was used more frequently than that in the centre of the pen in the majority of the treatments. This is in keeping with previous findings in broiler chickens (Newberry and Hall, 1990). In the fattening rabbit experiment, the use of a centrally placed structure increased the use of the central area of the cage, as had previously been shown for broiler chickens (Cornetto and Estevez, 2001b). In some situations it may be possible to prevent environmental influences by using very homogeneous test areas. For instance, Sibbald et al. (2000) found no differences between comparisons with a random and a resource-corrected random distribution for grazing sheep. However, creating a homogeneous setting may often be difficult as cues less obvious than the position of the walls influence spacing.

Both for broilers and for fattening rabbits, comparisons with the random distribution indicated social attraction more often, and social avoidance less often, than comparisons with the resource-corrected random distribution (which accounted for environmental influences). This shows that the use of a resource-corrected random distribution is not only a theoretical consideration, but has clear implications for the conclusions drawn on social attraction/avoidance. Having said so, comparisons of the observed distribution with the two different types of expected distribution led to different conclusions far more often in the fattening rabbit study than in the broiler study. This is likely to be caused by the fact that in almost all cases more wall area was available per animal in the rabbit study. Thus, more rabbits were able to use the wall area simultaneously and space use is therefore likely to have been more heterogeneous in the rabbit study.

Performance of the different indices of social avoidance/attraction

As detailed in the materials and methods section, it is important to include multiple indices in studies of spacing, as using a single index can lead to an incorrect interpretation of the data. For this thesis NND, CVIID and CVDPA were used. If behaviour influences spacing, this would be a source of variation, since animals are bound not to behave synchronously all the time. Increased variation due to non-synchronous behaviour increases CVIID and CVDPA, thus biasing towards proximity attraction, without affecting the average NND. But such theoretical considerations did not lead to major differences in the conclusions on social avoidance/attraction in the studies included in this thesis. During the last week of rearing, the switch from avoidance to attraction occurred at approximately the same density for all three indices. However, specifically in the earlier weeks of both the broiler and the rabbit experiment, NND indicated social avoidance more often than CVIID and CVDPA, which could mean that non-synchronous behaviour led to increased variation. Alternatively NND may be the most sensitive index to pick up signs of social avoidance because it is most easily influenced by an individual, as it requires interaction with only one conspecific. In contrast, CVIID results from the distances between all animals in the pen, and CVDPA is influenced by the distances between all individuals, as well as by their location in the pen. Even if NND was the most sensitive indicator, this would not mean that only the distance to the nearest neighbour is of importance to the animal. Likely, animals adjust their location to that of several of their more proximate neighbours (Ballerini et al., 2008; Burgess, 1980). By studying the size and shape of the DPA we could learn more about the area around animals that they attempt to keep free from other individuals. In the experiments described in this thesis the coefficient of variation of the DPA was studied, instead of the

DPA itself, because animals were studied in an enclosed space. Thus, average DPA in each pen was simply the total pen area divided by the number of animals in it, regardless of how animals positioned themselves. However, by studying DPA in non-enclosed study areas more may be learned about the area around animals that they attempt to keep free from other individuals. By studying DPA during different behaviours, or near specific resources, more can also be learned about the flexibility of such spatial zones.

Sensitivity of spatial distribution as a welfare indicator

Only spatial distribution and behaviour were described in this thesis, but additional welfare parameters were scored in the same project (Buijs et al., 2009a; Buijs et al., 2009b; Buijs et al., 2010; Van Poucke et al., 2009). Thus, the threshold density as indicated by spacing (i.e., the density around which the switch from social attraction to social avoidance occurred) could be compared with thresholds indicated by these other welfare parameters (i.e., the lower cut-off density, or start of the sensitive range, Figure 15). In this way the relative sensitivity of the different indicators can be compared.

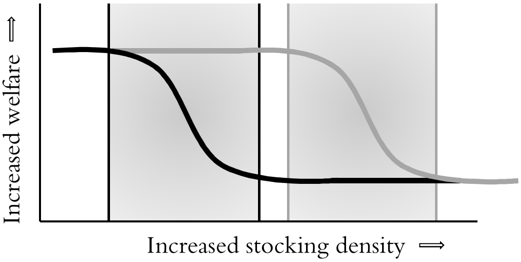

Figure 15. **The hypothetical relation between stocking density and welfare as measured by two different parameters of welfare.** The grey area indicates the sensitive range (i.e., the range in which the indicator responds to changes in stocking density). Although both welfare parameters show an equally great response to density (same angle in the sensitive area), the threshold density (left side of the sensitive area) is much lower for the welfare parameter indicated in black, indicating a greater sensitivity to stocking density.

The threshold densities for the other welfare parameters scored for the broiler chickens have been described previously (Buijs et al., 2009a). In brief, no threshold was found for the weight of the Bursa of Fabricius, mortality, or concentrations of glucocorticoid metabolites in droppings, as these indicators did not differ with density. The threshold density for leg strength may actually occur below densities studied in our experiment, as this indicator showed a steep decrease from the lowest density (2.4 birds/m^2) on. Hock dermatitis showed a threshold density at 13.6 birds/m^2, whilst for footpad dermatitis the threshold occurred at the highest density measured (21.8 birds/m^2). Fearfulness also showed a threshold at the highest density. None of the additional welfare parameters measured in rabbits - fearfulness, faecal glucocorticoid metabolites and bone strength - were affected by density (Buijs et al., 2009b; Buijs et al., 2010; Van Poucke et al., 2009).

The threshold for spatial distribution was found around 2.4 birds/m^2 for broilers, and seemed to occur below 5 animals/m^2 for fattening rabbits. As such, animals showed proximity avoidance at densities at which few other welfare parameters showed a density effect. This could mean that the other welfare indicators used were not sensitive enough. Of course other, possibly more sensitive, indicators could be used. However, these other welfare indicators did show density effects, although at higher densities. And the level of faecal corticosteroids was far lower in enriched than in unenriched cages, but did not differ between cages of different size (Buijs et al., 2010). Such findings suggests that these methods did have the potential to show differences in welfare. The fact that the cut-off of the other welfare indicators occurred at higher densities than the switch from avoidance to attraction shows that the absence of physical or physiological problems does not mean that animals' spatial preferences are satisfied. Conversely, providing animals with enough space to satisfy their spatial preferences seems to safeguard them from most other welfare problems caused by density. Alternatively, providing animals with as much space as preferred may be an unnecessary luxury, as little is known about the importance animals place on such space allocations. This importance is discussed in the next section.

Optimal space allowances as indicated by spacing, and their importance for welfare

For both subject species of this thesis, comparisons with the resource-corrected random distribution (which corrected for environmental influences) showed avoidance at stocking densities far below those used commercially. Broilers were only found to be attracted to each others' proximity at the lowest stocking density (2.4 birds/m^2), whereas no significant evidence of attraction was found for fattening rabbits. Possibly, indications of attraction would have been found for fattening rabbits if even larger cages had been included in the study. As detailed above, the lowest density used in this thesis was very low when compared to husbandry standards, but was still much higher than that observed in natural populations. Alternatively, fattening rabbits may be in a phase in their life in which they are simply not attracted to conspecifics, as in nature most juvenile rabbits disperse to different territories before the age of five months (Künkele and Vonholst, 1996), and a strong attraction to conspecifics may hinder such dispersal.

Although clear evidence of avoidance was found for both species, indicating that the animals preferred to have more space, spatial distribution analysis cannot show how important this preference is to the animal. This is because achieving a certain spacing had a relatively low cost for the animals, as all they had to do was to move to another location. For broiler chickens, the importance of achieving more space was evaluated in the motivation study described in paper III. A considerable motivation for lower stocking densities was found: birds crossed barriers to get to a lower stocking density, which 20-25% of broilers did not cross to get to feed after 6 hours of deprivation. However, all densities were compared to a density of 14.7 birds/m^2, i.e., 40 kg/m^2 at the end of the rearing period. This was close to the 42 kg/m^2 EU maximum for commercial production. The lowest density that could be achieved if the birds spread out equally over the available space was 13.4 birds/m^2, corresponding with 36 kg/m^2 at the end of the rearing period. As such, it is unknown if broilers would also be motivated to work to get from a medium density to the optimal density of 2.4 birds/m^2. Exploring such motivation further may provide additional insight into the validity of the use of spatial distribution to determine suitable space allowances.

For fattening rabbits even less is known about the importance of achieving their preferred space allowance, and it would therefore be interesting to assess this motivation using methodology analogous to that described in paper III. Although cage size motivation studies have been carried out previously (Bessei et al., 2006; Jezierski et al., 2005), the results of these studies are not fully reliable, as animals were first taught to press a button for food, and then to touch the same button for changes in cage space. No learning criteria were set for the tests in which cage size was the reward. As such it was not clear whether all animals actually understood that pressing the button would alter cage size. They may have been contrafreeloading (working for food even though it was now freely available) or pushing the button itself may have become rewarding during the training phase (Inglis et al., 1997). Such suggestions are supported by the finding that, in a subsequent test, the same rabbits worked for decreased cage size. Preferably, a motivation test should be devised in which rabbits learn to perform an operant response in order to get more cage space directly, instead of first teaching them to perform it for food. A previous study in individually housed adult pet rabbits (Dixon and Cooper, 2010) has shown that these rabbits worked harder for access to a larger space. Whether young group housed rabbits have a similar motivation still needs to be elucidated.

Influence of age and behaviour on spatial preferences

In both studied species, indications of social avoidance increased with age. This does not support previous suggestions (EFSA, 2005) that younger fattening rabbits require more space than older ones, in order to show locomotory play behaviour. In fact, in our rabbit experiment locomotion was slightly more common in older animals. As both studied species are slaughtered before reaching adulthood (defined as the onset of fertility), they may be motivated to show play behaviour throughout the rearing period. Older animals will require more space for this, as space is increasingly filled up by their larger bodies, and because stride length increases as animals age (EFSA, 2005). However, even in earlier weeks indications of social avoidance were found for both species studied in this thesis. This is important, because it means that animals are subjected to what seems to be an undesirable situation for a longer period of time than if avoidance occurred only during the last week of rearing.

There were indications of an association between behaviour and spacing in both studied species. Broilers were further from their nearest neighbour when eating or drinking than when adjusting their sitting or lying posture, foraging or preening, although these data need to be interpreted cautiously due to the low occurrence of certain behaviours (paper II). As discussed in more detail in paper V, rabbits were further from their nearest neighbour when sitting than when lying, and had smaller DPAs when lying sternally than when lying laterally, sitting or standing. Neither comparisons with the random distribution nor comparisons with the resource-corrected random distribution accounted for this effect. Possibly, future studies of avoidance/attraction could incorporate such behavioural influences in simulation models by using different minimum NNDs and/or DPAs for animals performing different types of behaviour, and likely also different maximum values. These could then be applied to the simulation model with the same frequency as the observed behavioural frequency. However, to do so a clearer picture of the likelihood of a certain behaviour occurring at a certain distance from other animals would be required. This is because papers II and V indicate that spatial proximity increased or decreased the likelihood of occurrence of a certain behaviour (or the other way around), rather than the occurrence of a clear minimum NND or DPA at which the behaviour suddenly ceased to occur. Ideally, the behaviour of the neighbouring animal would also be taken into account, as an individual might for instance be expected to choose to rest further away from highly active animals than from other resting animals.

Influence of enrichment on behaviour and spatial distribution

The wooden ⊔ shaped enrichment structure decreased lateral lying, social contact and cage manipulation. Six-week-old rabbits also spent less time drinking when housed in an enriched cage. The extra time rabbits in unenriched cages spent manipulating the cage, drinking, or in social contact was approximately equal to that spent manipulating the enrichment in the enriched cages. Thus, it seemed that the rabbits redirected their manipulations towards other objects or animals in their cage when no enrichment was present. Alternatively, the animals to which the manipulations were directed may have used the enrichment structure to avoid such manipulations, by using the structure as a physical or visual barrier. Although social contact is usually interpreted as having a positive influence on welfare, our definition of social contact (sniffing, grooming, gnawing, rubbing or pushing itself underneath another rabbit) potentially included unwanted or even painful interactions like ear chewing and excessive grooming. In fact, it may often be difficult to discern how the target animal interprets social contact, especially in situations in which the animal has little possibility to avoid such contact due to insufficient space or the absence of hiding places. Little is known about the importance of withdrawal areas for fattening rabbits, but adult breeding rabbits frequently use heightened platforms to withdraw from their offspring (Hansen and Berthelsen, 2000), suggesting that such withdrawal is important even in highly social animals.

The enrichment structure usually attracted more rabbits in to the central area, although for the smallest two enriched cage types (0.53 and 0.64 m^2) it increased the use of the peripheral area, probably because the structure was close to the walls in these small cages. In any case, the structure represented an environmental influence on spatial distribution. In addition, it seemed to exert a socio-environmental influence, although spacing was influenced in the opposite direction of what was originally expected. These original expectations were that animals would be more tolerant of each other's proximity when separated visually and/or physically by the (side panels of the) enrichment. If so, the switch from avoidance to attraction (as indicated by divergence of NND from the resource-corrected random distribution) would occur at smaller cage sizes in enriched cages. Since this decreased proximity would only occur for those animals near the enrichment structure, CVIID and CVDPA would be increased. Therefore, the switch from avoidance to attraction would occur at smaller cage sizes in enriched cages for all three indices. However, the opposite seemed to occur. The results on CVDPA aren't fully conclusive (see the discussion in paper IV on the differences between the general trends and the exact switch in significance). But for the other two indices the switch from avoidance to attraction occurred at larger cage sizes in the enriched cages. Thus, animals seemed to show a decreased social attraction in enriched cages. It may be that the rabbits were less focused on their conspecifics when they could interact with the structure, as previously suggested for rats (Abou-Ismail et al., 2010). This suggestion is supported by the decreased occurrence of social contact in enriched cages described in paper V. Alternatively, the rabbits may have used the structure to hide from conspecifics seeking contact, increasing their chances of successful avoidance. Such uncertainties emphasize the importance of combining behaviour and spacing studies. If animals group closer together in order to perform manipulations that are unwanted by the target animal, a clumped distribution should not be taken as a sign of sufficient space provision. In species in which such unwanted manipulations or aggression occur frequently, spatial preferences may be determined most reliably in surroundings that include features that minimize such behaviour. Such features should then also be applied to the housing in practice of course. Alternatively, observations in which aggression on unwanted manipulations occurred could be discarded when analysing spatial distribution. However, since aggression was rare in the experiments described in this thesis, and positive and negative social contact could not be discerned reliably, no such actions were taken.

Methodological considerations

Several methodological considerations arose during the preparation and processing of the experiments described in this thesis. An obvious methodological consideration is that stocking density was varied by altering group size in the broiler experiment (with the exception of the motivation test described in paper III), and by altering cage size in the rabbit experiment. Both experiments would have been improved by altering stocking density by manipulating group size as well as cage/pen size in a factorial setup. However, since we also wanted to study a wide range of densities, this would have led to very large experiments that would have been hard to carry out.

Papers I-II, and to a lesser extent paper III, were based on experiments that were carried out using relatively small groups of animals. This allowed us to study the direct effects of stocking density, whilst minimizing confounding with associated changes in temperature and air quality (or in ventilation rate, to keep temperature and air quality constant). Since broiler chickens are generally housed in groups of tens of thousands of animals (SCAHAW, 2000), behaviour and spatial distribution in our small flocks may differ from what happens in the field. Such effects cannot be excluded fully, since no studies comparing spacing and behaviour of small broiler flocks with commercially sized ones are available. However, a major influence of the experimental scale does not seem likely, since the effects of stocking density on the behavioural time budget described in this thesis correspond roughly to those found when studying commercially sized flocks (Dawkins et al., 2004; Febrer et al., 2006; Hall, 2001), and because group size has previously been shown to have only a limited influence on spatial distribution (Leone et al., 2010). Still, it would be preferable to confirm the results of the broiler spatial distribution study in commercially sized flocks. However, doing so using the methodology presented in this thesis may prove difficult, as locating all animals in a commercially sized flock would be extremely time consuming. Instead, one could choose to score only a certain section of a commercial barn, but this may lead to observations that are valid for this particular area only. In addition, the methods used to assess spatial distribution require the location of every animal in the study area. At high densities it may be hard to locate all animals in a section reliably if the total number of chickens is unknown, as broilers' will often partly block other individuals from view. As an alternative to comparing observed and expected spatial distributions, tests based on the speed at which artificially created empty spaces are filled up could be used. Such spaces would be expected to be filled more quickly if animals avoid each other's proximity more strongly. Preliminary tests on large flocks indicate that broiler chickens do indeed fill up empty spaces more quickly when stocked at higher density (Buijs et al., unpublished data). However these tests can only be used to compare the level of avoidance at different densities, as no model of how quickly such spaces would be filled up if animals were moving randomly is currently available. The preliminary tests indicate that once again, environmental influences, and possibly also for socio-environmental influences, should be accounted to come to valid conclusions.

In paper III, animals could choose between stocking densities by moving between two compartments. However, as more animals moved to what was originally the low density side, the densities on both sides gradually evened out. Such an effect could be prevented by allowing only one individual per flock to move between the high and the low density compartment. However, such a setup is likely to require training of the individual to show it that it can move between the densities. In this thesis, we attempted to avoid training because of the broiler's short active lifespan. But if such training is possible within the limited time frame, this would also allow the use of the same individuals in the density motivation test and the feeding motivation test. By doing so, more exact comparisons could be made between the motivation for food and for decreased density. Additional methodological considerations are discussed in paper III.

In the experiment that resulted in papers IV and V, a wooden enrichment structure was used that had a fully closed, wooden floor. Faeces stuck to this wooden floor, which would have been a hygiene risk if it had not be cleaned regularly. Although this was no problem in our experimental setup, it is unlikely that such structures will be used in practice, and therefore a structure without a fully closed floor would have been preferable in this experiment as well.

In paper V, sternal and lateral lying were defined as resting postures. However, it is unclear if the rabbits were actually resting, that is recuperating from previous activity, or just lying because other behaviours were impeded, or because they were not motivated to show other behaviour. This difference is of importance because rest is of great importance to all animals (Malleau et al., 2007), whereas an increase in time-fillers is unlikely to have a positive influence on welfare. It would have been worthwhile to study the effects on sleep instead of lying, as sleep is truly a resting behaviour, and increased sleep has been suggested to indicate improved welfare (Abou-Ismail et al., 2010). This previous study in rats showed an increase in sleep in the presence of environmental enrichment.

Only scan sampling was used to evaluate behaviour. Although this efficient method allowed analysis of a large number of recordings, it does not provide information on bout lengths. There are indications from literature that the length of locomotory bouts is influenced by cage size (Martrenchar et al., 2001; Postollec et al., 2006), which would have gone unnoticed in the present study. A more detailed analysis of locomotor behaviour will be carried out in the spring of 2011, to determine if density and enrichment influenced the length of locomotory bouts. Simultaneously, social contact will be studied in more detail, in an attempt to distinguish between positive and negative social contact.

Conclusion

This section describes the main findings of this thesis, based on the aims as mentioned on pages 29-30.

No specific threshold could be determined above which the behaviour of broiler chickens changed rapidly, but rest was increasingly disturbed at higher densities. Although the impact of such disturbances on welfare needs to be studied in more detail, broilers seemed to avoid them by increasingly using the wall area at higher stocking densities, indicating that such disturbances were unwanted. Only groups stocked at 2.4 birds/m^2 showed a preference for the central area, instead of the wall area. This was also the only treatment for which no evidence of avoidance was found throughout the rearing period when environmental influences were accounted for, indicating that this is the density at which the broiler chickens' space preferences were satisfied. That achieving a preferred space allowance can be important to broilers was indicated by the considerable motivation these birds showed to achieve densities below 15 birds/m^2, although it is not yet clear if the motivation to move from a moderate density to a density of 2.4 m^2 would be as strong.

In contrast to what was found for broiler chickens, fattening rabbits increased the time spent resting (i.e., lying sternally) as stocking density increased. Comparisons with other studies indicate that such opposite effects are unlikely to have been caused by the different ways in which stocking density was altered. Thus rabbits and broiler chickens seem to show a different species specific response to crowding. Providing the rabbits with a wooden structure decreased the time they spent manipulating the cage and the time they spent on social contact. Possibly, rabbits may have redirected their urge to manipulate their environment towards their conspecific when no enrichment was present. Alternatively, the target rabbits may have used the structure to withdraw from such manipulations. When environmental influences were accounted for, indications of avoidance were found for all treatments, suggesting fattening rabbits' preferred space allowance lies somewhere below 5 animals/m^2. In line with the decreased social contact observed in enriched cages, the enrichment seemed to have a socio-environmental influence on spatial distribution: rabbits seemed less attracted to each other when an enrichment structure was present.

No great difference in the conclusions on attraction or avoidance were found for the three different indices of spatial distribution in the week prior to slaughter (i.e., when animals were the largest and thus stocking densities where maximized). As indices based on absolute distances and those based on the variation in distances ultimately led to the same results, the chance of an incorrect interpretation of the data due to the limitations of each separate index is excluded. In the earlier weeks NND indicated avoidance more often than CVIID and CVDPA did. This may be caused by the fact that since behaviour was not accounted for in the expected distributions, CVIID and CVDPA may have been somewhat biased towards attraction. Alternatively, NND may be a more sensitive indicator of avoidance because it is more easily influenced by an individual than the other two methods. When the expected distribution was corrected for environmental influences, avoidance was indicated more often, and attraction less often, than when a random distribution was used. In addition to biasing towards attraction, comparisons with the random distribution seemed to be less reliable, as they led to a less consistent pattern over densities in both studies, i.e., they sometimes indicated attraction at a higher stocking density, whereas they showed no effect at a lower density.

This thesis shows how spatial distribution can be used to determine the optimal stocking density from the animals' point of view. Correcting for environmental influences, and the use of distance-based as well as variance-based distribution indices, are of great importance when doing so. Optimal densities were 7 and 4 times lower than densities used in commercial practice, for broilers and rabbits respectively. Although the motivation for such optimal densities still needs to be determined, broilers showed a considerable motivation for densities below commercial standards.

Svensk sammanfattning

Tidigare forskning på hur beläggningsgrad påverkar djurvälfärden har huvudsakligen fokuserat på negativa effekter på hälsa och beteende. Frånvaro av sådana effekter betyder inte nödvändigtvis att tillgången till utrymme är optimal från djurets synvinkel. Syftet med denna avhandling var att uppskatta den optimala utrymmestillgången för slaktkycklingar och kaniner, genom att studera deras beteende och utspridning i utrymmet. Dessutom studerades vikten av lägre beläggningsgrad för slaktkycklingar genom att jämföra deras motivation för olika beläggningsgrader med födomotivation. För köttkaniner studerades även hur en berikning påverkade spridning i utrymmet och beteende.

I artikel I studerades beteende och spridning i utrymmet hos slaktkycklingar i olika storlekar av boxar. Fåglarna drog sig mer mot områden nära väggarna när beläggningsgraden ökades. Detta mönster stödjer inte det tidigare antagandet från litteraturen som menar att kycklingar använder väggarna i antipredatoriskt syfte. Det är mer troligt att slaktkycklingarna höll sig nära väggarna för att skydda sig från att störas av sina artfränder. När beläggningsgraden var högre (m a o, när fler kycklingar hölls i boxar av samma storlek) justerade fåglarna sin sitt- eller liggposition oftare, vilket tyder på att de blev mer störda av de andra individerna. Dessutom var fåglarnas episoder av sittande och putsning kortare, och längden på episoder av gående minskade under veckornas gång.

I artikel II studerades beteende och social attraktion/undvikande hos slaktkyckling vid olika beläggningsgrader. När beläggningsgraden ökade sågs en fragmentering av sittbeteendet: fler sittepisoder förekom, trots att den totala tiden av sittande förblev densamma. Mindre tid ägnades åt putsning när beläggningsgraden ökade. Parvisa jämförelser identifierade inte någon brytpunkt i beläggningsgraden varefter beteenden snabbt förändrades, utan effekten kom gradvis vid ökad täthet, även om inte medelvärdet från alla behandlingar överensstämde med denna trend. Fåglarna var längre ifrån sin närmsta granne när de åt och drack än vid födosök, putsning eller justering av sitt- och liggposition. Socialt undvikande (d v s att avståndet till andra djur var längre än vad som är slumpmässigt förväntat) användes som en indikator på att utrymmets storlek tvingade djuren närmare varandra än vad de egentligen skulle föredra, och att tillgången på utrymme därför inte var optimal. Spridning i utrymmet påverkas inte bara av social attraktion/undvikande utan även av faktorer i miljön, varför två olika typer av förväntade värden användes. Det första tog inte hänsyn till miljöns påverkan, medan den andra däremot gjorde det. Den observerade spridningen i utrymmet jämfördes sedan med båda typerna av förväntade värden. När miljöns inverkan togs med i beräkningen upptäcktes socialt undvikande oftare, och social attraktion mindre ofta, jämfört med när inga korrigeringar för faktorer i miljön gjordes. Vilket index som användes för att bestämma spridning över utrymmet påverkade också slutsatserna om undvikande/attraktion: avstånd till närmaste granne pekade på undvikande tidigare i livet än variation i avstånd mellan individer, eller variationer i Dirichlets polygon area (variansen av ledigt utrymme runt ett djur). Emellertid tyder alla tre index över spridningen i utrymmet på att slaktkycklingar i grupper om $\geq$ 19 fåglar per 3.3 m^2 (slutligen motsvarande 15 kg/m^2) började undvika närhet till sina artfränder någon gång under uppfödningens sista 3 veckor.

I artikel III användes födomotivation som en måttstock för att bedöma hur viktigt det är för slaktkycklingar att upprätthålla en täthet mellan individer på mindre än 15 fåglar/m^2. Fåglarna gavs möjlighet att röra sig mellan två avdelningar med olika beläggningsgrad genom att ta sig över ett hinder. Slaktkycklingarna visade en stark motivation för den lägre beläggningsgraden, då fler djur gick från hög till låg beläggningsgrad än tvärtom, till och med när det hinder som användes vid tidigare utvärdering hindrat 20-25% av fåglarna från att lyckas nå mat efter 6 timmars foderberövande.

I artikel IV studerades spridningen hos köttkaniner i olika stora utrymmen i berikade och oberikade burar. Beräkning i kvadrater visade att användningen av utrymme påverkades av faktorer i miljön. I linje med detta visar en jämförelse mellan den observerade utspridningen och de förväntade slumpmässiga värdena om miljömässiga faktorer inte räknades in en antydan till undvikande av artfränder mer sällan och attraktion till artfränder oftare, än när de förväntade värdena inkluderade miljömässig påverkan. Indexet Dirichlets polygon area var speciellt känsligt för ojämnt utrymmesanvändande, troligen på grund av att det påverkas av djurets position i boxen. Jämförelser av förväntade värden som inberäknade miljöns påverkan antydde undvikande av artfränder i alla beläggningsgrader, även om den lägsta tätheten mellan djuren i studien (5 djur/m^2) var 4 gånger lägre än vad som vanligen används i praktiken. Förekomsten av en berikningskonstruktion i trä verkade minska attraktionen mellan kaninerna, vilket tyder på att spridningen i utrymmet påverkas av sociala/miljömässiga influenser.

Artikel V beskriver hur burstorleken hade en överraskande liten effekt på köttkaninernas beteendemässiga tidsbudget när de hölls i grupper om åtta, trots att ett brett spektrum av burstorlekar testades (sju olika storlekar mellan 0,40 och 1,60 m^2). Att ligga på mage var det enda beteende som stadigt ökade med tätheten. Djur som låg på mage hade också mindre utrymme omkring sig än de som befann sig i andra ställningar. Beteendet skulle kunna vara en ”tidsutfyllnad” som utförs när de beteenden som kräver mer plats hindras, eftersom liggande på mage inte tar mer fysisk plats än till exempel sittande och stående. Putsning å andra sidan verkar vara mycket viktigt för köttkaniner eftersom beteendet företrädesvis utfördes när djuren hade mer utrymme omkring sig, men ändå inte minskade i mindre burar. En berikningsstruktur i trä minskade manipulationer av buren och sociala interaktioner mellan kaninerna. Dessa beteenden kan delvis orsakas av en omdirigering av gnagbeteende till burinredning eller artfränder när inget passande material att gnaga på finns tillgängligt.

Sammanfattningsvis ledde högre beläggningsgrader till fragmentering av vilobeteendet hos slaktkycklingar, medan en ökning av vilobeteende sågs hos köttkaniner vid högre tätheter. Förekomsten av en berikningskonstruktion i trä gjorde kaninerna mindre fokuserade på sina artfränder, då social kontakt minskade och bytet från undvikande till attraktion inträffade vid större burstorlekar. Den optimala beläggningsgraden för slaktkycklingar nära åldern för kommersiell slakt var uppskattningsvis 7 gånger lägre än EU:s nya gräns för beläggningsgrad. Slaktkycklingar visade även en betydande motivation att uppnå lägre täthet mellan individer än EU:s satta gräns, vilket visar på att det är viktigt för dessa fåglar att sänka beläggningsgraden. Vad gäller köttkaniner leder en beläggningsgrad som är 4 gånger lägre än den som används i kommersiell praxis fortfarande till socialt undvikande, vilket tyder på att tätheten inte var optimal. Ytterligare resultat från analysen av utrymmesspridning understryker vikten av att räkna in miljömässiga faktorer och att använda ett flertal utrymmesindex vid bedömning av socialt undvikande/attraktion.

Nederlandstalige samenvatting

In het verleden heeft onderzoek naar de effecten van bezettingsdichtheid op dierenwelzijn zich voornamelijk gericht op ongewenste invloeden van hoge dichtheden op gezondheid en gedrag. Echter, de afwezigheid van dergelijke negatieve effecten betekent niet noodzakelijkerwijs dat de beschikbare hoeveelheid ruimte optimaal is, gezien vanuit het standpunt van het dier. Het doel van dit proefschrift was om de optimale hoeveelheid ruimte te bepalen voor vleeskuikens en vleeskonijnen aan de hand van de manier waarop zij zich over de beschikbare ruimte verspreidden. Daarnaast werd het belang dat vleeskuikens aan een lagere bezettingsdichtheid hechtten onderzocht door de motivatie voor een lagere dichtheid te vergelijken met de motivatie voor voer. Voor vleeskonijnen werd tevens de invloed van hokverrijking op de ruimtelijke verdeling en het gedrag bestudeerd.

Artikel I beschrijft hoe vleeskuikens zich gedroegen wanneer zij bij verschillende bezettingsdichtheden gehuisvest waren, en tevens hoe zij zich bij deze verschillende bezettingsdichtheden over de verschillende delen van hun hok verspreidden. Bij een hogere bezettingsdichtheid (wat in deze studie betekende dat er meer dieren werden gehuisvest in een even groot hok) pasten de vleeskuikens hun zit- of lighouding vaker aan, wat impliceert dat zij vaker gestoord werden door hun soortgenoten. Daarnaast bleven de dieren minder lang aan één stuk zitten, en ook minder lang hun veren poetsen, wanneer zij bij een hogere bezettingsdichtheid gehuisvest waren. Oudere dieren liepen minder lang per keer en deze afname werd versterkt door een hogere dichtheid. Naarmate de bezettingsdichtheid toenam, maakten de vleeskuikens meer gebruik van het gebied langs de wanden van hun hok. Dit verspreidingspatroon kon niet verklaard worden vanuit de bestaande literatuur waarin gesuggereerd wordt dat kippen bij voorkeur dicht bij wanden blijven omdat deze bescherming bieden tegen predatoren. In plaats daarvan leken de vleeskuikens het gebied langs de wanden te prefereren omdat zij hier minder kans liepen om gestoord te worden door hun soortgenoten.

In artikel II werd het gedrag van vleeskuikens gehuisvest bij verschillende bezettingsdichtheden bestudeerd. Tevens werd gekeken bij welke dichtheden de vleeskuikens hun soortgenoten opzochten, en bij welke zij hun soortgenoten ontweken. Hogere dichtheden leidden tot een fragmentatie van het gedrag. Er werd vaker gezeten, maar minder lang per keer, waardoor in totaal even veel tijd zittend doorgebracht werd. Tevens werd er bij een hogere bezettingsdichtheid minder tijd besteed aan het poetsen van het verenkleed. Uit paarsgewijze vergelijkingen van de behandelingsgemiddelden kwam geen duidelijke drempelwaarde naar voren, waarboven het gedrag een plotselinge verandering onderging. In plaats daarvan had dichtheid een gradueel effect op het gedrag, ook al weken sommige behandelingsgemiddelden af van de algemene trend. De vleeskippen positioneerden zich verder van hun dichtstbijzijnde soortgenoot wanneer zij aan het drinken of eten waren, dan wanneer zij aan het scharrelen of poetsen waren, of wanneer zij hun zit- of lighouding aanpasten. Wanneer dieren door ruimtebeperking gedwongen worden zich dichter bij elkaar te positioneren dan gewenst, zullen zij proberen om de afstand tot hun soortgenoten te maximaliseren. Daarom werd een ruimtelijke verdeling waarbij dieren zich verder van hun soortgenoten positioneerden dan zou worden verwacht als zij soortgenoten opzochten noch ontweken, gebruikt als een indicator voor een tekort aan ruimte. Omdat de ruimtelijke verdeling waarschijnlijk niet alleen beïnvloed wordt door ontwijking van en aantrekking tot soortgenoten, maar ook door omgevingsinvloeden (bijvoorbeeld de nabijheid van een muur of een voerbak), werden twee soorten verwachte waarden gebruikt. De eerste soort werd niet gecorrigeerd voor omgevingsinvloeden, de tweede wel. Vervolgens werd de waargenomen ruimtelijke verdeling vergeleken met beide soorten verwachte waarden. Wanneer er gecorrigeerd werd voor omgevingsinvloeden leidde dit tot andere conclusies omtrent de optimale dichtheid dan wanneer deze correctie niet doorgevoerd werd. Tevens was de keuze van de index waarmee de ruimtelijke verdeling bepaald werd van belang. De afstand tot de dichtstbijzijnde soortgenoot (NND) toonde reeds op een jongere leeftijd dat de vleeskuikens elkaar ontweken dan de variatie in inter-individuele afstand (CVIID) en de variatie in de oppervlakte van Dirichlet polygonen (CVDPA). Echter, alle drie indices van ruimtelijke verdeling toonden dat vleeskippen gehuisvest bij een dichtheid ≥ 19 dieren per 3.3 m^2 (ofwel 15 kg/m^2) elkaars nabijheid begonnen te ontwijken ergens gedurende de laatste 3 weken van hun leven.

In artikel III werd de motivatie voor voedsel afgezet tegen de motivatie voor bezettingsdichtheden lager dan 15 dieren/m^2. De vleeskuikens konden zich verplaatsen tussen twee compartimenten met een verschillende bezettingsdichtheid door over een barrière te klimmen of springen. De vleeskuikens toonden een aanzienlijke motivatie voor lagere dichtheden: er was een netto instroom naar het compartiment met de lagere dichtheid, zelfs wanneer de compartimenten gescheiden werden door een barrière die zo hoog was dat deze 20-25% van de dieren ervan weerhield om bij hun voer te komen nadat zij 6 uur niet hadden kunnen eten.

In artikel IV werd de ruimtelijke verdeling van vleeskonijnen gehuisvest in verrijkte en onverrijkte kooien met verschillende afmetingen bestudeerd. Het ruimtegebruik bleek beïnvloed te worden door omgevingsfactoren. Overeenkomstig met dit resultaat toonden vergelijkingen die niet gecorrigeerd waren voor omgevingsinvloeden vaker aantrekking tot soortgenoten, en minder vaak ontwijking van soortgenoten, dan wanneer er wel een correctie voor omgevingsfactoren werd uitgevoerd. Met name CVDPA was gevoelig voor omgevingsfactoren, waarschijnlijk omdat deze index beïnvloed wordt door de positie van de dieren ten opzichte van hun hok. Vergelijkingen tussen de waargenomen ruimtelijke verdeling en waarden die wel gecorrigeerd waren voor omgevingsfactoren, toonden aan dat de vleeskonijnen van 9 weken oud de nabijheid van hun soortgenoten ontweken in alle kooitypes, ook al was de bezettingsdichtheid in de grootste kooien vier keer zo laag als in de gangbare praktijk. Tevens leken de dieren zich minder aangetrokken te voelen tot elkaar wanneer er een houten verrijkingsstructuur in de kooi aanwezig was.

Artikel V beschrijft hoe kooigrootte verrassend weinig invloed had op het gedragsrepertoire van vleeskonijnen gehuisvest in groepen van acht, ondanks dat er een brede spreiding in dichtheden bestudeerd werd (zeven verschillende dichtheden tussen 0.40 and 1.60 m^2). De enige gedraging die continu toenam bij een stijgende bezettingsdichtheid was sternaal liggen (liggen op de buik). Dieren die sternaal lagen hadden ook minder vrije ruimte om zich heen dan dieren in een andere houding. Waarschijnlijk nam sternaal liggen niet toe met toenemende dichtheid omdat de behoefte aan deze gedraging toenam, maar omdat andere gedragingen die meer ruimte vereisten moeilijker werden bij een hogere dichtheid. Het poetsen van de vacht leek echter wel een gedraging waar de vleeskonijnen veel belang aan hechtten. Hoewel er in grote kooien bij voorkeur gepoetst werd op momenten dat de dieren meer vrije ruimte om zich heen hadden, werd er toch evenveel gepoetst in kleinere kooien. De houten verrijkingsstructuur verminderde de tijd die besteed werd aan kooimanipulatie en sociale interacties. Dit zou er op kunnen duiden dat deze gedragingen deels veroorzaakt werden doordat de konijnen hun knaagbehoefte naar de kooi en naar hun kooigenoten richtten wanneer er geen verrijking aanwezig was om aan te knagen.

Samenvattend leidde een hogere bezettingsdichtheid tot een fragmentatie van het rustgedrag van vleeskuikens, terwijl vleeskonijnen juist meer rustten bij een stijgende bezettingsdichtheid. Konijnen leken minder gericht op hun soortgenoten wanneer er een houten verrijkingsstructuur aanwezig was in hun kooi, aangezien de tijd die besteed werd aan sociale interacties verminderde, en aangezien er al bij lagere dichtheden ontwijking van de nabijheid van de soortgenoten waargenomen werd. De optimale dichtheid voor vleeskuikens nabij slachtleeftijd was ongeveer 7 keer zo laag als de dichtheid die wordt opgelegd door de recente Europese richtlijn. Vleeskuikens toonden tevens een aanzienlijke motivatie voor lagere dichtheden, wat suggereert dat lagere dichtheden belangrijk zijn voor het welzijn van deze dieren. Vleeskonijnen ontweken elkaar zelfs nog in de grootste kooien, hetgeen impliceerde dat ook deze kooigrootte nog sub-optimaal was, ook al bood deze 4 keer zoveel ruimte per konijn als gangbaar is in de praktijk. De resultaten van de analyse van de ruimtelijke verdeling benadrukken tevens het belang van de correctie voor omgevingsfactoren, en van het gebruik van meerdere verspreidingsindices, wanneer de afstoting dan wel aantrekking tussen dieren geanalyseerd wordt.

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