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The relevance of different group sizes to performance, health and welfare in poultry and emotional state in pigs

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

SUMMARY

The objectives of this project were:

a. To review the influence of group size on poultry welfare, social behavior and productivity.
b. To assess the influence of group size on broilers' welfare, productivity and leg disorders.
c. To assess pigs' preferences for living in very large versus small groups.
d. To assess pigs' emotional experience when living in very large vs. small groups by using cognitive bias tests.

First, impact of group size on performance parameters, damaging behaviors and welfare in poultry was reviewed in chapter 2. Current industry guidelines and standards for commercial poultry space requirements and group size vary, and they are different from those that have been examined scientifically. Chapter 2 reviews the current industry guidelines and standards as well as scientific literature pertaining group sizing on behavior, welfare, and productivity of poultry including chicken, turkey and quail. We suggested that current industry guidelines and standards for commercial poultry space requirements and group size vary, and they are different from those that have been examined scientifically. On the other hand, scientific research into group size effects on poultry behavior, welfare, and productivity has been conducted in experimental settings with flock sizes that are much different from those in the commercial poultry industry. There is currently little information available regarding how much space commercial poultry require for particular behavioral activities. This paper reviews the current industry guidelines and standards as well as scientific literature pertaining group sizing on behavior, welfare, and productivity of poultry including chicken, turkey, and quail. Furthermore, future researches should include the application of different group size in different systems (e.g., in different housing systems, same-sex or mixed-sex groups in turkeys, free range, organic and higher welfare indoor).

In chapter 3, we present a report of our experiment in broiler chickens. The aim of the present study was to assess welfare and performance of broiler chickens housed in different group sizes. 6400 mixed-sex day-old broiler chicks were housed in four types of rectangular enclosures which provided $10m^2$ [small], $30m^2$ [medium], $100m^2$ [large] and $500m^2$ [very large] floor space. Per pen there were 100, 300, 1000 and 5000 birds, respectively and therefore constant density for all groups (10 birds/m^2). Welfare and health parameters were assessed using scoring systems on a scale from 0 (indicating non-affected birds) to 2, 3 or 5 (indicating severely affected birds). Results for gait scores, hock burn and plumage cleanliness indicated better welfare of birds in small (except hock burn, $P>0.05$) and medium sized groups, compared to very large groups ($P<0.05$), and TD was more severe in very large compared to large groups. Results of this study show that the general assumption of detrimental effects of large group sizes needs to be reassessed, especially for new commercial broilers, but further research is needed with regard to commercially relevant group sizes.

In chapter 4, we present a report of our experiment on cognitive bias and group preference when housing fattening pigs in a small vs very large group. It has been suggested that pigs are unable to individually recognize all animals when living in groups > ca. 50 individuals, raising the question, if this potentially unnatural social situation negatively affects animal welfare. Therefore, the aim of the present study was to investigate the emotional state of fattening pigs when housed in a small vs very large group. After six weeks of training, 91 pigs originally housed together in one large group were split into a small (11 pigs) and a large (80 pigs) group with equal densities (0.8 m^2/pig) and 7 trained pigs per group. Cognitive bias and preference tests were conducted with the trained pigs at -1, 1, 3,7,10 and 13 days in relation to splitting the original group. Results of this experiment suggest that pigs' welfare in small groups is negatively affected, likely due to the reduced total space availability.

Keywords: behavior, cognitive bias, group size, pig, poultry, welfare.

1st Chapter

General Introduction

1-1- **Animal welfare**

Successful animal production must be based on sound biological principles. Animal biology and behavior must be taken fully into consideration in the design and management of husbandry systems, in the production process and solving of problems. Efficient husbandry methods that combine suitable indicators are needed to understand and assess environmental impacts on animal welfare and behavior.

The general concept of animal welfare is comprised of mental and health physical (Dawkins, 2004; Webster, 2004) and that includes several aspects such as physical comfort, the absence of hunger, pain, fear and disease, possibilities to perform a motivated behavior, etc. (Farm Animal Welfare Council, 1992). Some scientists suggested that animal welfare depends on how an animal is coping with the conditions in which it lives (Dawkins, 2004). Providing stable animal welfare situation needs prevention of disease and veterinary treatment, proper shelter, nutrition, management, humane slaughter, and humane handling. Animal welfare refers to the physical and mental state of the animal; the treatment that an animal receives is covered by other terms such as animal husbandry, animal care, and humane treatment (Britain and Brambell, 1965).

The scientific advisory council for agricultural policy of the federal ministry of food and agriculture in Germany (2015) considers the on-farm self-assessment an important tool for aligning societal expectations of livestock production and the situation on farms. Experts have developed sets of largely animal-based indicators for routine on-farm self-assessment in dairy and beef cattle, rearing calves, sows, piglets and finishing pigs, turkeys and broilers, as well as pullets and laying hens. Using these indicators, it should be possible to identify the most important animal welfare problems (Zapf et al., 2015).

1-2- **Effect of group size on welfare and behavior**

Formation of artificial groups of domestic animals occurs frequently in modern husbandry systems. These groups are kept in a closed space where the individuals are not able to withdraw from the group and because of commonly limited resources in the environment, they will engage in competitions which result in violent fights, aggression and social stress (Chase, 1974). Some factors including group size and species of animal can affect the frequency of aggressive interactions for an initial period ranging from hours to weeks. For many species of animals, groups of three or four members usually settle their relations in a day or so; for larger groups, around 10 members, several weeks may be required (Chase, 1974).

A decrease in aggression with increasing group size has been observed in species from various taxa, such as domestic fowl (Lindberg and Nicol, 1996; Estevez et al., 1997; Guo et al., 2012) pigs (Turner and Edwards; Meyer-Hamme et al., 2016) and fish (Syarifuddin and Kramer, 1996), but some results are not clear, and few, if any, studies have provided an explanation for why this is so. The potential for resource monopolization will be reduced in larger groups since a high rate of intrusions reduces the effectiveness of aggression in controlling a resource (Davies and Houston, 1981) and also increases the costs in terms of time spent, energy expended and injury. As group size increases, more individuals will benefit from not getting involved in fights and will use alternative nonaggressive strategies to acquire resources. The result will be that more individuals will avoid each other or that more individuals will fight less and less intensively. According to Pagel and Dawkins (1997), the probability of encountering the same individual will decline sharply with increasing group size, and at a certain group size, it may not be worth paying the cost of establishing a dominance relationship, e.g. because the animals may not be capable of recognizing and memorizing all individuals of their group. Larger groups imply a more complex social environment. An example of that is that the probability of linear hierarchies forming is relatively low even for moderate group sizes of seven to eight individuals (Mesterton-Gibbons and Dugatkin, 1995). It is only the individuals with high resource-holding potential that can afford to get involved in serious fights. It is therefore expected to find large individual differences in aggression within groups which has been confirmed in pigs (Mendl et al., 1992; Hessing et al., 1993; Andersen et al., 2004).

Highly intensive housing is a common practice in commercialized farm animals, especially in pigs and poultry. Crowding is one of the main concerns for welfare because there is a direct correlation between overcrowding, behavioral abnormalities, and discomfort. It also endangers the other freedoms, being likely to cause hunger, thirst, pain, injury or disease. Insufficient provision of space or facilities causes crowding (Appleby, 2004). Some factors including enclosure size and configuration have a strong influence on movement and use of space patterns (Leone et al., 2010). Although these factors interact with each other, they may all have separate effects. Concerning space, these components are the number of individuals, total area and area per individual. In the case of facilities, the three components are the number of individuals, total provision (for example, length of food distribution time) and provision per individual.

On the other hand, animal husbandry in large groups is viewed with scepticism by the general public, although scientific studies yield contradictory results regarding the effect of keeping animals in very large groups on their welfare in general and in particular aspects such as social structures. Social dominance is one of the most important elements of life in animal groups. Higher social status confers numerous advantages to higher-ranked individuals, such as access to better food or territories (Collias, 1970; Gagneux, 1991; Lahti et al., 1998). In addition to access to resources, dominant individuals are often able to gain more matings and produce and effectively rear more offspring (Lill, 1966; Cheng and Burns, 1988; Jones and Mench, 1991), although the former aspect usually no longer is the case with livestock in the situation of controlled breeding. In feral chicken (*Gallus gallus*), most hens mate with the dominant territorial males (McBride et al., 1969). In domestic chickens, males low in peck order mate infrequently or not at all, whereas the dominant male sired about 65% of the progeny produced by the flock (Guhl and Warren 1946, Craig and Bhagwat 1974). Recommendations on minimum space requirements cannot be based on the simplistic view of mere units of space per animal. Features such as the structural characteristics of the environment or social aspects of the group must also be considered to establish meaningful recommendations.

1-3- Innovative method for welfare and behavior assessment

During last years, different welfare monitoring systems have been introduced in European countries, such as the TGI[1]35 in Austria (Bartussek, 2001), the TGI200 in Germany (Hörning, 2001) and a decision support system in the Netherlands (Bracke et al., 2002). These systems largely depend on observations of the environment (i.e. design of houses or equipment supposed to affect animal welfare) and on selected observations of the animals (i.e. production performance or behavioral parameters that are assumed to expose the animals' internal state). However, the correlation between specific principles and the animals' welfare status are not always straightforward to evaluate. Considering reasons mentioned above, the general belief is that integration of the most relevant specialist research in Europe is necessary to standardize, refine, and develop welfare assessment systems and to classify and authorize practical strategies for improving welfare (Blokhuis et al., 2003).

Emotion evaluation methods from human psychology are the main source of information about the assessment of welfare and emotional states in animals. The "cognitive bias test" is one of the adapted methods form human phycology that has been used for animal behavioral test during the last years. Cognition in definition referring to "the mechanisms by which animals acquire, process, store and act on information from the environment" (Shettleworth, 2001). Since 2004 which the first study on cognitive bias in animals was published (Harding et al., 2004) many studies carried out on cognitive bias in animals including pigs and poultry.

In human psychology, it has been proven that cognitive processes can be influenced by affective state (Bar-Haim et al., 2007; Mitte, 2008; Eldar et al., 2010). When animals live in

[1] TGI: Animal need index (Tiergerechtheitsindex)

firm social groups, this may involve in major cognitive demands. For instance, individuals may benefit from identifying appropriate supportive partners, checking other animals in group, reacting to the behavior of others and allocating value to particular relationships in order to maximize the advantages of living in group (Dunbar, 1998; Melis et al., 2006; Brosnan et al., 2010). Cognition is about the storage and information organizing and its application in learning, memory, decision-making and problem-solving and may, therefore, make individuals enable to solve and cope with possible social tasks or challenges (Shettleworth, 2009; Elizabeth Bolhuis et al., 2013; Grimberg-Henrici et al., 2016).

There is a variety of direct method to measure biological function such as growth rate, productivity, reproductive and immune function but mental or subjective experiences are not easy to determine, as methods for measuring the incidence of different emotions and feelings have not been completely established (Bennett et al., 2004; Klasing, 2007; Dawkins, 2008). The successful experience of affectively induced cognitive biases in humans has inspired animal researchers to use cognitive bias as a novel method for objectively measuring both positive and negative affective states in non-human animals (Paul et al., 2005; Mendl et al., 2009).

Physiological stress measurements (e.g. hormone releasing, autonomic nervous system, etc.) are often interpreted either as indicators of biological functioning or as indicators of negative emotional states, However current interpretations of physiological indicators do not provide opportunities for the comprehensive assessment of positive affect (Yeates and Main, 2008).

There are different defentions and explanation for "happiness" in animal. Sometimes *happiness* is used equal to ecstasy, contentment, delight but also health and strength or state of mind and body that is precarious and contingent (Miele, 2011). Recently powerful interpretations of animals' happiness were provided in the context of farming, and it does so by providing a particular translation of the `natural' in the domesticated environment of farming. Happiness in animals is triggered by an event evaluated as slightly sudden, quite predictable, very pleasant and consistent with expectations (Veissier et al., 2009). Providing of opportunities for happiness or pleasure of animals is one of the main goals for animal production managers as they focus on reduction of suffering in order to achieve a more comprehensive improvement of animal welfare (Mench, 1998). Utilizing cognitive bias methods to present animals with appropriate challenges is a potentially effective method for achieving this goal. It can be assumed that there is a gradient of emotional responses, from the mere expression of rather automatic responses to the experience of emotional feelings and the consciousness of self-emotional experiences, depending on the level of the cognitive processes used to appraise the situation (Veissier et al., 2009). Also, animals in different social groups may have distinctive cognitive biases. For instance, cooperative partners can have beneficial or harmful effects in individuals and suitable behavioral interaction may result in specific relationships and maximizes the benefits of living in group. Previous research has found that cognitive performance has higher rate in species with regular social interactions in comparison to species with rarely social interact (Mirville et al., 2016).

Moreover, it has been proven that housing quality alters cognitive bias in pigs and affective state, but it is not proved conclusively that pigs have subjective feelings of happiness or satisfaction associated with their state (Douglas et al., 2012).

However considering that intraspecific variation in performance among individuals in social groups of varying size has not been investigated exhaustively by researchers, it must be more explored whether it is beneficial for welfare and behavior to increase group size along with total space and facilities available to the individuals. Reflecting current concern about intensive methods of farming, this dissertation considers some aspects of biology and behavior in pigs and poultry using different parameters for welfare assessment and how they are influenced by the group size. Pigs and poultry are by far the most omnivorous of the domesticated farm animals and it is in their nature to be highly explorative. In the case of pigs, there are several commonly observed abnormal behaviors and of those directed to pen mates, belly nosing, and biting and chewing on the ears or tail of group mates are the most commonly observed. Also, both pigs and poultry frequently show harmful behavior (i.e. tail biting, feather pecking), which in part may be caused by stress-induced by problems with the social structure in a given group. Consideration of two very different classes with very different social behavior and cognitive abilities might help to better understand the importance of social structure in a given group to animal welfare. In both pigs and laying hens, the injurious behaviors directed toward other individuals may escalate and develop into abnormal and aggressive behaviors (Brunberg et al, 2016). Also the topic of group size is most relevant for poultry and pigs from a practical point of view as groups considerably larger than group sizes found in nature are very common in practice. Therefore, the aim of the present study was to evaluate the effects of group size in poultry using production, behavioral and welfare indicators and using innovative method (cognitive bias test) to assess effects of group size in fattening pigs.

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2nd Chapter

A review of the effects of group sizing on behavior, welfare, and productivity of poultry

2-1- **Abstract**

The aim of this review is to discuss the impact of group size on performance parameters, damaging behaviors, and welfare in poultry. Group size has been found to affect damaging behavior, such as injurious pecking in laying hens; production, such as growth rates and feed efficiency. Domesticated poultry is changed in many natural behaviors and they had modified their food search strategies, hierarchy, and aggressiveness. Consequently problematic behaviors in poultry must be evaluated in commercialized conditions in large groups. Current industry guidelines and standards for commercial poultry space requirements and group size vary, and they are different from those that have been examined scientifically. On the other hand, scientific research into group size effects on poultry behavior, welfare, and productivity has been conducted in experimental settings with flock sizes that are much different from those in the commercial poultry industry. There is currently little information available regarding how much space commercial poultry require for particular behavioral activities. This paper reviews the current industry guidelines and standards as well as scientific literature pertaining group sizing on behavior, welfare, and productivity of poultry including chicken, turkey, and quail.

Key Words: chicken, quail, turkey,

2-2- **Introduction**

Due to growing population and more demand for animal protein, particularly for meat and egg, poultry has been considered as best and most efficient alternatives to red meat to sustain the food production (Speedy, 2003). This approach caused more intensive farming on poultry than other animals in any other sector of animal agriculture and has arisen severe change in poultry husbandry which resulted in some problematic alteration in behavior and welfare (Wolfson, 1996). Group size and density as a common practice have been using by poultry industry in order to adjust behavior, welfare and performance of birds in intensive commercial condition (Estevez et al., 2003).

In addition to chicken, quails and turkeys have been widely used as commercial poultry over the past years. As with other poultry, the welfare of turkey can be affected by different husbandry management systems. Whereas using at low light intensities or beak trimming as common practice to control against cannibalism raised welfare questions, more studies on other husbandry strategies including optimal group size are required. There are few studies on evaluation of group size and density on welfare parameters in turkey and findings are confounded between density and group size (Martrenchar, 1999).

Regarding quails, small body size and easy handling, hardiness, rapid generation turn over, high laying turn over and being oviparous animal are advantages of quail to be used in genetic studies and embryological experiments (Tsudzuki, 1994). On the other hand, the general concept of being more healthy and natural for quail´s product causes increasing use of this bird both in industry and science, consequently it seems studying the welfare of the Japanese quail is critical (Minvielle, 2007). Although there are many similarities in the breeding conditions of this commercial poultry, physiological differences including body size, thermal comfort zones and moving ability, variances in the breeding objective (meat or egg), regional priorities in the prevalence of different poultry products consumption have resulted different strategies in husbandry.

Several studies have proven that group size can play an important role regulating social dynamics aggression, mating system, feather pecking, laying performance, blood parameters and etc. in poultry (Campo and Davila, 2002; Estevez et al., 2007; Bovera et al., 2014). Therefore altering these factors by changing in group size, can severely affect the main welfare parameters in poultry including skeletal and metabolic disorders, lesions in the hip joint and painful leg disorders, such as tibial dyschondroplasia, angular bone deformity and contact dermatitis, i.e., breast burns, hock burns, and foot pad dermatitis (Vits et al., 2005).

In natural life conditions, poultry typically form different size of social groups; one male with several females in chickens, same-sex or mixed-sex groups in turkeys, and mixed-sex groups in ostriches. In order to commercialize of poultry production, there have been constant modifications by purposeful breeding program to improve performance of physiological organisms in commercial strains, including respiratory system, digestive system, nutritional requirements and skeletal system (Rauw et al., 1998). These differences resulted in alteration in many husbandry strategies like density, ventilation and feeding systems. Consequently it seems that other commercial rearing conditions, including group size, must be justified accordingly.

Although there has been legislation specifying group size for different commercial poultry, particularly caged poultry, by some countries which focus on behavioral requirements as indicated by the increase in legislation regarding housing and maximum group size for poultry birds (e.g.: in the United States, Proposition 2 of 2008 in California and Public Act 117 of 2009 in Michigan; in Europe, Council Directive 1999/74/EC of 19 July 1999, Article 4, laying down minimum standards for the protection of laying hens: at least one nest for every seven hens. If group nests are used, there must be at least $1m^2$ of nest space for a maximum of 120 hens). For example, the national veterinarian institute of Sweden guidelines state that maximum group size for laying hens must not exceed 16 hens in each cage (National veterinarian institute of Sweden, 2018, Sweden's most well-known sustainability label for food (www.krav.se)). Also, conventional cages have been banned since 1999 in Sweden (SFS[2], 1988: 534, 539) because of lack of possibilities for hens to perform natural behavior and furnished cages (i.e., in cages furnished with nests, litter, and perches) have been replaced instead of conventional cages (Wall and Tauson, 2007).

Furthermore, in recent years, the poultry husbandry systems for laying hens in Germany have undergone fundamental changes with significant overall improvements. This is primarily due to the fact that housing in conventional battery cages has been banned in Germany since 1 January 2010. Since then, it has only been permitted to keep laying hens in small-group housing systems, barn and free-range systems or in organic production systems (federal ministry of food and agriculture, Germany, www.bmel.de). Moreover, German animal welfare ordinance stipulates that no more than 6,000 laying hens may be kept without spatial separation (KAT[3] 2013; Council Regulation 834/2007 for animal production). In addition to more legislation, the number of animal products labeling programs according to welfare assessments protocols has increased in recent years. For instance European commission directorate general for health and consumers (2010) assigned that reexamination of the overall risk assessment strategies is necessary in order to ensure the continued protection of consumers, animal welfare and the environment.

While group size has been identified as one of the main factors affecting poultry welfare (Wechsler and Schmid, 1998; Estevez et al., 2003; Buchwalder and Huber-Eicher, 2005), specific group size recommendations for poultry vary widely among industry guidelines and

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[2] SFS: Swedish ministry of agriculture

[3] KAT: Association for controlled alternative animal husbandry in Germany (Verein für kontrollierte alternative Tierhaltungsformen)

certifying programs. Also, since group size is commonly associated with behavioral parameters and welfare, a better knowledge of the factors that affect group size or affected by group size can increase our understanding of the welfare and behavioral parameter in poultry. This paper reviews the scientific literature pertaining to the effects of group sizing on behavior, welfare, and productivity of poultry including chicken, quail and turkey. An understanding of the effects and importance of group size can help to optimize flock size recommendations for commercially housed poultry.

2-3- **Chicken**

The ancestors of the poultry species that have been domesticated display a variety of different forms of social organization (Mench and Keeling, 2001). Some, such as jungle fowl, live in small relatively stable groups. The most common groupings seen in jungle fowl are of several females with one male, with other males being solitary or in small groups (Collias and Collias, 1996). Each group has a regular roosting site and an area in which it usually forages. The same situation is found in feral domestic fowl, which form distinct social groups, each with a home range (Wood-Gush et al., 1978). Conditions are also similar for small farmyard flocks, and social behavior in these groups is probably very like that of wild birds but many changes happened for commercial poultry during domestication and further breeding programs. Domesticated animals became less fearful and they had modified their food search strategies, hierarchy reduced the aggressiveness when several birds were kept at the same house, birds with high egg production were selected for breeding purposes, which in turn leads to a higher food and energy requirements consequently more time to search for more food sources (Eklund and Jensen, 2011). Schütz et al. (2001) showed that frequency of energy-demanding behaviors such as foraging and exploratory behaviors and social interaction decreased in selected leghorns hens for high production. They concluded that genetic selection for production traits may lead to less social behaviors. Also, Pagel and Dawkins (1997) indicated that in small groups hens can pay and expect to recoup large costs of establishing dominance relations and increasingly so as group size diminishes. Moreover it has been shown that hierarchy formation breaks down at larger group sizes not because animals are unable to recognize all the individuals in their group but because they do not benefit from using recognition in this way (Pagel and Dawkins, 1997).

Commercial chickens can largely be split into two types, broiler and laying hens.

2-3-1- Broilers

Because of large economic and husbandry effect of reducing group size in broiler farms, it is very important to determine the relationship between group size and welfare as precisely as possible. When more accurate evaluation on interaction between group size and welfare parameters is provided, decisions can be taken on what group size is acceptable from an animal welfare point of view. Unlike the relationship between group size and farm profitability, however, the relation between group size and welfare is much more complex and so more difficult to determine precisely.

The effects of group size and stocking density on broiler behavior and welfare have been investigated by some researchers (Reiter and Bessei, 2000; Shahani, 2003; Leone et al., 2007; Leone et al., 2010; Kiani and von Borstel, 2019), and center on damaging behaviors including feather pecking and cannibalism, aggression, stress, fear and behavioral disturbances.

Studies which examine the effects of group size involve some degree of confounding with density, and enclosure size (Christman and Leone, 2007). Some researchers tried to separate effect of group size and density and interactions between group size and density. For example Reiter and Bessei (2000) combined four different group sizes (10, 20, 40 and 60 birds) and three different stocking densities (5, 10, and 20 birds/m or 9, 18, and 36 kg/m^2 floor area) in a two-factorial design and measuring performance and behavioral parameters for 5 weeks of fattening period. They reported that feeding activity increased with increasing group size in the 2nd week of age and in the 5th-week scratching was significantly increased by group size. They also showed that Feeding activity was highest at group size of 20 birds. There was a short - time periodicity of activity and resting with a cycle length of 20 minutes and this rhythm disappeared under high group size and stocking density. But they concluded that both group size and stocking density in the tested range of their experiment had an only little impact on the performance and behavior of the birds. They explained the effects of both factors on scratching and walking behavior by ambient temperature, conditions of the litter and social stimulation rather than the physical space restriction. They also concluded that lacking synchronization of the behavior among group mates may leads to the disappearance of short-time rhythms in activity and resting.

While many authors have studied effect of group size in broiler, different results have been reported. Some of these may have been affected by differences in study design (under controlled circumstances vs. on-farm, varying group size by changing density or pen size, and so on) and others by the use of different indicators of welfare. This makes it hard to compare these studies. As a consequence, no specific range in which group size affects welfare has been identified yet. It has been suggested that when relevant aspects of the multidimensional concept of welfare respond simultaneously at the same factor increase, for example group size or density, the determination of such a critical factor for broiler welfare as a whole would be straightforward. For example, Buijs et al. (2009) evaluated the welfare of 4 replicates of birds stocked at 8, 19, 29, 40, 45, 51, 61, and 72 broilers per pen (or 6, 15, 23, 33, 35, 41, 47, and 56 kg actually achieved BW/m2) using physiological (i.e. leg health and postmortem measurements) and psychological welfare indicators (i.e. tonic immobility and corticosteroid metabolites. They showed that the groups of 72 birds had longer tonic immobility duration than those of 8, 19, 29, 45, and 51 and tended to differ from 61. Also, there was a significant difference in latency-to-lie between the groups of 8 and all groups ≥40 birds per pen. These researchers mentioned that disadvantage with their method was that a low score on one indicator, representing a real welfare problem, could be masked by a high score on another indicator. In another research by Kiani and von Borstel (2019) effects of different group sizes [small(100 birds), medium (300 birds), large (1000 birds) and very large (5000 birds)] with constant density on leg disorders and plumage cleanliness in broiler chickens were investigated. Results of this sresearch showed that gait scores, hock burn and plumage cleanliness indicated better welfare in small groups. Kiani and von Borstel (2019) concluded that the general assumption of detrimental effects of large group sizes needs to be reassessed, especially for new commercial broilers, but further research is needed with regard to commercially relevant group sizes.

Perching is a highly motivated and natural behavior for chickens and sleeping undisturbed is important for the welfare of the individual. It has been proven that there is relationship between group size and welfare indicators including perching behaviors in laying hens (Abrahamsson and Tauson, 1997; Wall and Tauson, 2007) but few authors have studied this possibility in broiler. Martrenchar et al. (2000) compared perching behavior of broilers between 2 different group sizes (1020 vs 4590 birds, 17 birds/m^2 with no replicates). They showed that perching behavior of broilers was slightly lower during weeks 5 and 6 in the large group size compared to the small group size (6.8% vs 7.9% respectively in week 6). They concluded that group size does not have an important effect because the difference of absolute value of percentage of perching birds between the 1020 and the 4590 group sizes was 1.1% at week 6. They also mentioned that there was no replication in their experiment and large pens were used, therefore results of their experiment should be considered as a preliminary result.

There are several reports about the impact of group size, density, as well as the shape and dimensions of the pens, which have been reported by various researchers. In addition to different experimental conditions, such as age, breed and husbandry conditions in these experiments, some researchers tried to mix effect of group sizes with other factors to investigate interaction effects (Christman and Leone, 2007; Leone et al., 2010). Therefore, distinguishing and dividing of outcome of each effective factor, especially group size, in these studies might be difficult.

Some researchers tried to employ different experimental design which enables them to control one factor at a time, and control significant effects through the use of multiple contrasts. For example, Leone et al. (2010) included different factors including group size, density and enclosure size in their study and hypothesized that these factors would have a distinctive effect on space use and movement in broiler chickens. They constructed square enclosures: small (S.,1.5m^2), medium (M.,3.0m^2), and large (L.,4.5m^2), and three group sizes of 10, 20, and 30 birds. Their reported that there is no differences in movement activity between enclosures of differing size at constant group size (10S, 10M, 10L) but they found differences between enclosure sizes when density was held constant (10S, 20M, 30L) and when comparisons were made across constant enclosure sizes (10M, 20M, 10L, 30L). They concluded that there are distinctive effects among group size, enclosure size, and density on movement and space use in broilers. Moreover, it has been shown that broiler chickens in small pen size restrict the amount of space use in compare to the large pen size, this restriction is probably because birds could move less distance before contacting an end wall and being reflected back towards areas they had already spent time in (Newberry and Hall, 1990). Therefore when changing in group size, which is associated with changing in pen size, can affect alter used space and movement in chickens.

Specifically, it is not possible to determine the size of the group, regardless of the structure of the social hierarchy in each flock. It has been shown that hierarchy formation might be affected by many factors including breeds, sex, environmental factors and husbandry practices (Siegel and Hurst, 1962; Hocking, 1993). Also, it has been proven in several studies that hierarchy in poultry flocks can be affected by age (Newberry and Hall, 1990; Hocking, 1993; Anderson et al., 2004). Newberry and Hall (1990) tried to investigate the effects of age and pen size on the use of pen space by male broiler chickens. Broilers were assigned in two groups, large group with 203.5 m^2 space and 1520 birds vs small pens with 203.5 m^2 space and 1520 birds. The locations of 18 marked chickens in large group and 10 in each of two small groups were recorded at hourly intervals. Results of their experiment showed that amount of space used by broilers over a 6- week period in small groups were lower than large group. They hypothesized that birds in small groups move less distance before contacting an end wall and being reflected back towards areas they had already spent time in. They also discussed that chicken's tendency for staying close to the walls is why larger proportion of the available pen area is not used by broilers. These researchers concluded that distance travelled by male broilers at a commercial stocking density can be affected by group size and age. Newberry and Hall (1990) reported that birds in the large pen tended to spent more time near their home brooder while birds in the small pens did not.

Selection for production traits resulted in reducing slaughter age in broilers and also has affected the behavior of the broilers (SchuÈtz and Jensen, 2001). Several researchers concluded that with increasing age, chickens limit their movements because of social pressure (McBride and Foenander, 1962; Craig et al., 1969). On the other hand some researchers have shown that pecking and threatening behavior in broilers fed ad libitum remained extremely low between 4 and 9 weeks of age (Mench, 1988). Finding from broods of young domestic fowl living in the wild have shown that movements of young fowl can be influenced by the availability of food, movements of the hen, and strategies to avoid predators during the night (McBride et al., 1969; Wood-Gush et al., 1978). Decline in walking time and distance moved per hour with increasing age, which is usually associated with increased difficulty in walking, result in decline in home range size (Newberry et al., 1986).

Developing and maintaining social relationships with more than 100 flock mates is unlikely in domestic fowl (Guhl, 1953). Therefore broiler chickens kept in flocks of several thousand birds are continually encountering strangers during their movements within the pen over rearing period. Encountering stranger flock mates results in adrenal hypertrophy and increase the likelihood of aggressive behaviors and adverse effect on broiler welfare (Siegel and Siegel, 1961). On the other hand, some researchers suggested that in commercial poultry husbandry with large group poultry would restrict their movements to small areas, thus becoming acquainted with the other birds in the vicinity and to avoid encounters with strangers will be possible for individuals (McBride and Foenander, 1962).

2-3-2- Laying Hens

Existing housing systems for laying hens are classified between conventional cages, furnished cages, and barn systems with and without outdoor access. Parallel to the intensification of laying hen husbandry and egg production in the 1960s, especially the use of cage batteries, the protests of scientists, animal welfare groups and political parties against this form of keeping farm animals began in Europe and later spread to Northern America. Housing systems for hens differ in the possibilities for hens to show species specific behaviors such as foraging, dust-bathing, perching and building or selecting a suitable nest. If hens cannot perform such high priority behaviors, this may result in significant frustration, or deprivation or injury, which is detrimental to their welfare.

On the other hand, relatively little is known about how hens respond to different numbers of birds in a cage as cage-mates, and also these responses are not assessed in different housing systems under different group sizes. Many behavioral interactions within group are associated with reproduction which affects group size. In those wild birds that defend territories during the non-breeding season but do not breed in them, do not defend their territory and mates in units larger than two birds (Davies and Houston, 1981; Faaborg and Arendt, 1984).Two main parameters are involved in reproduction behaviors which based on increased available energy profits at the season of reproduction: first, the increased availability of energy for aggressive behaviors in the population during the reproductive season and second, a range of behavior including production and care of young appears in the breeding season. Social interaction within group member during reproduction activities is not vastly studied in poultry (Brown and Brown, 1981; Brown, 1982).

Preliminary researches on evaluation effects of group size on welfare in laying hens recommended that hens should neither be housed singly, nor in groups of 4 or more, but implied that the welfare of hens was best served in groups of 2 or 3 (Britain and Brambell, 1965). However, these recommendations were changed according to further research and adequate evidence to substantiate the relationship between welfare and group size, mostly based on two parameters: mortality and egg production (Dawkins, 1982).

Guo et al. (2012) conducted an experiment to evaluate the effect of group size and stocking density on the welfare and performance of hens housed in furnished cage systems during summer. They designed three housing systems: a standard battery cage system (control, 4 hens per cage and 398 cm^2 per hen), two furnished systems (including perches and nest); one with a small (SFC, 21 hens per cage; 586 cm^2 per hen) and one with a large group size (48 hens per cage; 543 cm^2 per hen). The results showed that furnished cage with small group size hens had a higher level of egg breakage compared to the control group. Also, hens kept in the furnished cage with small group size cage tended to have a lower rectal temperature than that of controls. They also concluded that furnished cage systems with small group sizes (around 20 hens) were favorable for the thermal balance during summer. The result suggests that furnished cage systems with small group sizes are favorable for hens' welfare without affecting performance. In another research by Vits et al. (2005), classification of furnished cages under practical conditions was evaluated. In their experiment there were three different furnished cage systems (Aviplus, Eurovent 625a, Eurovent 625A) and each system consisted of 4 tiers of double-decker cages. Hens were housed in groups of 10 and 20 per cage in the Aviplus and Eurovent 625A systems and in groups of 40 and 60 per cage in the Eurovent 625a system. Results of their experiment showed that group size within housing system had a significant influence on all production traits and Haugh units (The Haugh unit is the measure of albumen quality used by the poultry industry). The highest egg production per average hen housed (89.4%) was found in the groups of 20 hens in the Aviplus system whereas the proportion of cracked eggs was higher (0.7%) in groups of 60 hens than in the other group sizes. The strongest humerus bones (198.2 N) were found in the groups with 10 hens in the Aviplus system, and tibia strength was greater (146.7 N) in the groups of 20 hens in the Eurovent 625A system. These researchers concluded that more cracked eggs in larger group size (60 hens) might be because of more eggs in the nest box and/or on the conveyor belt at the same time.

Injurious pecking is another serious problem in laying hens production systems and is especially difficult to control in large group furnished cages and in non-cage systems. The problem can be minimized by appropriate housing and management as well as genetic selection. With increasing group size, there is a greater potential for birds to be disturbed by others in the group and increase of aggressive behavior in caged birds. Although the incidence of feather pecking is much higher in birds housed in cages rather than on deep litter in pens (Hughes and Duncan, 1972), it has been shown that feather pecking and aggressive pecking are two quite distinct behavior patterns (Hughes, 1973; Blokhuis and Arkes, 1984).

Savory et al. (1999) conducted a series of experiments with groups of 10 to 20 growing bantams in multi-unit brooders and investigated effects of certain environmental and dietary factors on development of feather pecking damage to 6 weeks of age. In their experiment they had two group sizes (10, 20 birds) and stocking density (744, 372, 186 cm^2/bird). Results of their experiments showed mean pecking damage score was significantly higher with the larger group (20 birds) and highest density (186 cm^2 floor space per bird) than with lower group size/density treatments. Also there was thus a significant effect attributable to stocking density with the larger but not the smaller group and there was no effect attributable to group size with the same (intermediate) density. These researchers concluded that in large groups, as in alternative layer housing systems, the number of birds present may be less important than stocking density. In another experiment, Bilčík and Keeling (2000) conducted an experiment with 4 different group sizes of 15, 30, 60 and 120 birds, at four different ages and focused on the number of feather pecks and aggressive pecks, both given and received. Results of this study showed that for severe pecks, groups 120 differed from groups 15 and 60. For gentle feather pecks received, group 120 differed significantly from groups 15, 30 and 60. In the number of severe feather pecks received, groups 120 differed significantly from all other groups. They concluded that increasing group size causes increasing frequency of aggressive pecks. Hughes and Wood-Gush (1977) showed that the level of agonistic behavior in both strains was much lower in crowded cages than in more spacious pens. This confirmed earlier findings in which much larger group sizes were studied (Craig and Bhagwat, 1974; Polley et al., 1974).

In addition to behavior, a group size that is too large can affect the performance of laying hens including feed intake, feed efficiency and laying rate. Wider freedom of movement and behavioral opportunities in modern poultry production systems may also increase the incidence of undesirable behaviors and causes negative impacts in terms of animal health, welfare, and production performance of laying hens (Sossidou and Elson, 2009). Marin et al. (2014) conducted a study to evaluate whether differences or changes in the phenotypic appearance of Hy-line Brown laying hens may affect their body weight and egg production, and if so, whether these effects are dependent on the group size and previous experience. Groups were of 10, 20, or 40 individuals (8 hens/m^2). In their study, they manipulated the phenotypic appearance to constant proportions of birds along the different group size treatments. Therefore, in a small group, the 30% consisted of 3 whereas for group size of 20 and 40 the 30% consisted of 6 and 12 birds, respectively. The results in egg production showed no effects of initial phenotypic appearance or group size on first egg laid, cumulative 25% egg production, or cumulative 50% egg production, and no effects on the cumulative hen-day egg production were detected at the end of the first phase of the study (34 wk of age). These findings suggest that, even though behavioral and body weight changes can be induced by the particular social conditions used herein (group sizes and phenotypic appearance combination), when these conditions are imposed from a very early age, they do not appear to significantly affect the group egg production.

Tonic immobility by definition is a state of motor inhibition and reduced responsiveness to external stimuli induced by a brief period of physical restraint (Gallup, 1977; Jones, 1990). There are some researches that used tonic immobility as a parameter to fearfulness evaluation. For example Bilčík and Keeling (2000) used tonic immobility to evaluate effect of group size on fearfulness in in group sizes of 15, 30, 60 and 120 of laying hens in floor pens. They reported that group size had a significant effect on tonic immobility duration when birds were tested in their home pens. The results of this study do not support the hypothesis that birds are more fearful in the smaller groups than the larger groups due to a theoretically higher risk of predation.

Results of conducted experiments on flocks with small group size cannot necessarily be adapted to the commercial flocks where birds are kept in flocks of many thousands, because of scaling effects. For example, aggressive behaviors like feather pecking are relatively high in small flocks in compare to large flocks because birds can adopt themselves to avoid negative social interactions (Hughes et al., 1997; Nicol et al., 1999). Zimmerman et al. (2006) studied the behavior of laying hen under commercial stocking densities (low: 7 birds m^2, medium: 9 birds m^2, high: 12 birds m^2), flock sizes (small: 2450/3150 birds, large: 4200 birds) and management conditions (standard and modified). They showed that feather pecking and level of aggression was affected by the interaction between flock size and age and concluded that relationships between stocking density and feather pecking alter as flock sizes increase. Also, Nicol et al. (2006) assessed physical and physiological indicators of hens in commercial non-cage systems. These researchers indicated that there were no clear effects of flock size on the welfare indicators. A summary of the available literature for laying hens is provided in Table 2-1.

Table 2-1. Summary of published literature examining group size effects on laying hens welfare and productivity.

Reference	Age (week)	Experimental design	Evaluated parameters	Group size	Density	Results
Lindberg and Nicol (1996)						
Experiment 1	-	T-maze preference tests	Preference for being in a larger or a smaller group of familiar flockmates	5, 120	Constant for both groups	When space was constant and large, a strong preference for the smaller group in a large space emerged.
Experiment 2	-	5 different group size/space options were tested using a T-maze	Pecking behaviors in different group sizes	4, 70		There were preferences for a larger group (70 over 4 or 0 hens), a larger space (9 m^2 over 1 m^2) and 4 hens rather than an empty space.
Hughes (1977)						
Experiment 1	1-18	Battery brooder subdivided into groups of 50 at 2 weeks, 25 at 6 weeks and 12 at 10 weeks of age	Selection of cages containing different numbers of birds	1, 2, 3, 4, 5	-	Cages became progressively less attractive as the number of birds in them increased.
Experiment 2	-	Large battery was divided into 8 cages with a central runway	Selection of empty cage versus occupied cage	1, 2, 3, 4, 5	-	Hens reared singly chose empty cages rather than cages occupied by one other unfamiliar bird, whereas group reared hens selected the occupied cages.
Hughes and Wood-Gush (1977)	1-72	Factorial design with two housing methods (battery cage and deep-litter pen)	Aggressive Head Pecking, Threats, Pecks and Pulls	3, 6	0.76, 0.81 m^2/bird	Aggressive head pecking occurred more often in groups of six than in groups of three
Abrahamsson and Tauson (1997)	1-79	Two housing systems were used, a modified furnished cage in two blocks and a conventional battery cage in one block	performance, health and space usage	5, 6, 7, 8	600 cm^2/bird	The rolling out efficiency from nests was best in the larger group sizes but hens in the larger group sizes had the dirtiest feet
Bilčík et al. (1998)	1-40	Randomized block design	Tonic immobility	15, 30, 60, 120	5 m^2/bird	Duration of tonic immobility increased with group size
Nicol et al. (1999)	14 -30	Four identical percheries, each perchery was treated as one independent unit	Production performance, feather pecking and aggression	72, 168, 264, 368	6, 14, 22, 30 m^2/bird	Aggressive pecking was most common in the smaller flocks at the lowest stocking densities.
Bilčík and Keeling (2000)	1-37	Randomized block design	Feather pecking and ground pecking	15, 30, 60, 120	5 m^2/bird	Higher rate of feather pecking in the largest group size

Table 2-1. Continue

Campo and Davila (2002)		Different mating ratios with two group sizes	Blood indicators of Fearfulness and Stress	12, 60, 120, 240		The heterophil to lymphocyte ratio was significantly higher when the group size was 60 birds than when it was 12 birds
Estevez et al. (2003)	3-18	4 groups (12 focal birds per pen were used for tests)	Aggressive behaviors	5, 30, 60, 120	5 m^2/bird	Linear reduction in the frequency of pecks and threats given per focal bird with increasing group size but the frequency of pecks and threats received per focal bird was higher in larger than smaller groups
D'Eath and Keeling (2003)		Two large pens and four small pens	Social discrimination and aggression	10, 120	6.67 m^2/bird	Hens in small groups discriminated between familiar and unfamiliar subjects by more aggression towards unfamiliar hens. In large groups, the overall level of aggression towards subjects was reduced in that attempted fights were rare
Vits et al. (2005)		Three different furnished cage systems with different group sizes	Production, performance, Bone and egg parameters	10, 20, 40, 60	Constant for all groups	The highest egg production was found in the groups of 20 hens in the Aviplus system and highest proportion of dirty eggs was found in the groups of 10 hens in the Eurovent 625A system.
Guo et al. (2012)	1-36	Three housing systems: a standard battery cage system two furnished systems	Performance, Nesting, perching and walking behavior, blood parameters	4, 21, 48	398, 543 and 586 cm^2/bird	The furnished cage systems with small group sizes were favorable for hen welfare without markedly affecting performance
Bovera et al. (2014)	20 - 36	2 groups	Performance and egg quality	25, 40	749 Cm^2/bird	Hens raised from group of 40 hens had lower percentage of egg production and higher feed conversion ratio than group of 25 hens
Marin et al. (2014)	1-44	Randomly assigned to 45 pens provided with nests and perching	Production performance, first egg laid, morphometric measures of the eggs	10, 20, 40	8 m^2/bird	Groups of 40 individuals showed a reduction in BW gain and weekly hen-day-egg production after 30% phenotypic appearance changes

2-4- **Turkey**

Similarly to chicken, wild turkeys may live in small mixed-sex groups during the non-breeding season, but in commercial condition all-female or all-male flocks are more common (Schorger, 1966; Brant, 2007). Due to different growth rate and nutrient requirements, male and female flocks are separated. In mixed sex production, during the breeding season sometimes commercial turkeys are found in groupings like those of wild turkeys, several females with one male, but more frequently male sibling groups stay together (Appleby et al., 2004).

Fast-growing strains of turkey broilers are usually housed in large buildings, which may contain 1000–25,000 birds at stocking densities of up to 60 kg/m^2 (Farm Animal Welfare Council [FAWC] 1995) that is approximately 3 adult $males/m^2$. Turkeys reared under commercial conditions demonstrate intense aggressive behavior. Some researchers suggested that this intense behavior might be related to factors such as housing, management and feeding or endogenous factors such as genetic disposition (Sherwin and Kelland, 1998; Hafez, 1999). As long as there are different fighting behavior pattern for wild and commercial fattening breed, increase of aggressive behavior in turkeys might be domestication-related factors (Healy, 1992).

Unfortunately, few studies have been conducted on effect of group size on welfare or behavioral parameters in commercial turkey and published studies do not represent the condition in commercial flock sizes. Likewise mentioned for laying hens, there are no straightforward methods to separate effect of group size and density for poultry flock. Different housing systems in different climate with diverse husbandry strategies make it more problematic to have a conclusion on few published research on effect of group size but there are some researches that address the main point more direct than others which are mixed with density effect. For example Buchwalder and Huber-Eicher (2005) conducted a study to evaluate the effect of introducing individual birds into small or large test groups of turkey toms on the subsequent incidence of aggressive interactions. They hypothesized that reaction to an introduced bird would be more aggressive in small groups than in large groups. They used six groups of 6 (small groups) and six groups of 30 (large groups) animals and measured aggressive behavior including fights, pecks and leaps for numbers and duration between residents and introduced or reintroduced birds. Results of their study showed that turkeys in small groups did more aggressive behavior with the introduced bird than members of the large groups did. More pecks toward newly introduce unfamiliar toms in small (2 × 3 m) group were received more pecks compared with large group (6 × 13 m). Some other researchers reported the same behaviors when unfamiliar conspecifics are introduced to wild turkeys flocks in order to drive them out of the group (Watts and Stokes, 1971; Williams, 1981). Buchwalder and Huber-Eicher (2005) concluded that turkeys in large groups barely are able of differentiating between resident group members and introduced bird but domesticated turkeys as well as wild turkeys are able to distinguish between group members and non-group members, and aggressive behaviors will raise among non-group members markedly more than group members, at least when in groups of four.

The welfare of turkeys broilers kept at high stocking densities has been evaluated by several researchers, especially during the final rearing period when the body weight of birds per space is high (Coleman and Leighton Jr, 1969; Zuidhof et al., 1993; Martrenchar et al., 1999). In some researches because experimental pen size was not altered between treatments, there was confounding between group size and density. For example Martrenchar et al. (1999) provided floor space of 24 dm^2, 18.5 dm^2 and 15 dm^2 until week 12 and 40 dm^2, 31 dm^2 and 25 dm^2 from week 12 for the males and 16 dm^2, 12.3 dm^2 and 10 dm^2 for the females but because the size of the pens was same in the experimental design, treatments differed in both stocking density and group size; Consequently determination of respective effect of each variable is not possible. These researchers mentioned that this option was chosen deliberately in their experiment, because despite new regulation concerning stocking density it is not likely that farmers would change the size of their houses and pens; they prefer house fewer birds and changing stocking density and group size at the same time.

In another study by Sherwin and Kelland (1998) frequency of comfort behaviors and incidence of injurious pecking was measured for different group size and stocking density when the male turkeys were housed as pairs in pens. In contrast to Buchwalder and Huber-Eicher (2005), they reported that severity of injuries and frequency of fighting were lower in small group and suggested that small group size and/or low stocking density might moderate or reduce the effects of injurious pecking amongst turkeys.

Although the hypotheses of lowering aggressive behaviors with increasing group size are supported by observations of relatively little aggression in large groups of domestic fowl (Hughes and Wood-Gush, 1977; Carmichael et al., 1999; Nicol et al., 1999; Estevez et al., 2002), patterns of aggression in different age, commercial strains and husbandry systems have not been investigated systematically over a wide range of group sizes. Estevez et al. (2003) hypothesized that whereas domestic fowl in small groups establish a dominance hierarchy through aggressive interactions those in large groups adopt a low-aggression (tolerant) social strategy. These researchers conducted an experiment with groups of 15, 30, 60 and 120 female White Leghorn chickens housed in a constant density and assessed aggressive interactions among group members as group size increases. They concluded that while the majority of birds may adopt a tolerant strategy in larger groups, a minority may be despotic, directing aggression indiscriminately towards other birds. Optimal group size for a dominant member of a certain group may differ from that for new or junior members. Dominance can influence many aspects of behavior within social interactions. When food resources are not enough or not properly distributed within group a dominant have less problematic access to food resources and even often take food discovered by subordinates (Baker et al., 1981; Rohwer and Ewald, 1981) therefore effects of resource depletion may be less for a dominant than for a subordinate (Brown, 1982; Lacher Jr et al., 1982). Also, Craig et al. (1969) revealed that strangeness and crowding in female chicken are associated with higher frequencies of social interaction in compare to socially undisturbed and uncrowned flocks. Different social interaction within group members like competing for resources and space can effect on accepting new member (Brown, 1982).

Classifying appropriate group size for commercial conditions is not an easy task, most behavioral experiments and observations are implemented on group sizes much smaller than practical group size under commercial conditions. In behavioral studies large pens are divided into smaller ones and each small pen is considered as an independent replicate. Because of technical difficulties in most researches replicates are not enough (usually less than 6), consequently the extrapolation of results of these experiments to commercial flocks is challenging (Denbow et al., 1984; Cunningham, 1992; Classen et al., 1994).

2-5- **Quail**

Quail farming is a quite recent addition to the commercialized poultry farming in compare to chicken and turkey. 131 species of wild quail are found all over the world (MacHenry et al., 1987) but only Bobwhite quail (Colinus virginianus) and Japanese quail have been domesticated for commercial purposes. Northern bobwhite, *Colinus virginianus*, forms social units (covey), averaging about a dozen birds of mixed ages and sexes during the nonbreeding season (Johnsgard and Jones, 1988). Northern bobwhite forms coveys of no more than 30 individuals, with an average group size of 12 (Wing, 1941). In spring when males and females pair for breeding, the composition of these coveys change and new coveys are formed. Social interaction in Japanese quail depends on migration rate and group composition changes as a consequence of migration. Overall, group size and Social organization in the wild depend on several factors such as the current resources availability in the environment and the predation risk, and consequently can change when these conditions vary (Wilson and Bermant, 1972).

Stocking density and group size are effective environmental parameters that can affect performance parameters and welfare indicators in cage breeding of quails (Seker et al., 2009). Seker et al. (2009) investigated effects of group sizes of 3 and 10 with constant density of 125 cm^2/bird on perofromanec, mortality and cracass characteristics of Japaneese quails. Findings of this research showed that with constant density of 125 cm^2/bird, group size of 10 when compared to group size of 3 might yield better results in term of live weight, feed intake and feed conversion ratio. In another research, Waheda et al. (1999) reared 90 Japanese quails in 2 group sizes (6 and 9 birds/cage) and 3 stocking densities (150, 175 and 200 cm^2/bird) from 50 to 125 days of age and reported that egg production was higher in the smaller group size and in the intermediate stocking density than in the larger stocking density and other stocking density. Group size where birds are housed can play a significant role on the relationships between domesticated fowl group mates, social interactions and modifying their adaptation to new situations (Jones, 1996; Bilčík et al., 1998; Estevez et al., 2003) therefore the quality of these relationships between groupmated can affect production performance, health, and welfare indicators.

Although Japanese quail have been used widely as model animal to investigate social behavior in the management of large groups of domestic birds (Schweitzer et al., 2009), there are not many studies related to social relationships in Japanese quail.

Social motivation is likely to influence several aspects of social interactions including cohesion, affiliation, and aggression (Launay et al., 1991; François et al., 2000; Williams et al., 2003).

In industrial cage production, breeding groups typically consist of 15 to 20 birds kept in battery cages with a floor area of 1.0 m× 0.5 m and a height of 16 to 20 cm (Gerken, 1993).

In industrial condition quail are frequently distress with several welfare problems such as aggressive pecking, head-banging as a consequence of escape responses, feather damage, leg weakness and foot problems. Aggressive pecking can cause head injuries, skin or eyelid lesions

and eye loss in quail which is an important welfare problem in quail farming. The influence of breeding groups on the aggressive behavior of Japanese quail was investigated by Wechsler and Schmid (1998) who designed an experiment with 4 groups of 5 males and 15 hens and 4 groups of 5 males and 35 hens in a 2 × 2 factorial design and introduced two groups of each composition into the pens at the age of 4 and 6 weeks, respectively. The authors reported that there was no significant effect of group size and age of introduction into the experimental pen on pecking rate and also the interaction between the 2 factors was not significant but there was a trend to lower pecking rates in the newly introduced groups (0.8± 0.5, mean ± SD[4] compared to 3.5 ± 1.9).
Since environmental enrichment or environmental modifications can enhances behavioral opportunities and leads to improvement in biological functioning they can be used to improve animal welfare, reduce fear and aggression and change social behavior in poultry husbandry (Gvaryahu et al., 1994; Newberry, 1995). It has been shown that provision of enrichments can increase aggressive interactions among caged laying hens (Reed et al., 1993) therefore it is expected that environmental enrichment may affect social behaviors including grouping behaviors in poultry. Japanese quail as a common laboratory and production species has frequently been the subject of environmental enrichment and social behavior studies. Miller and Mench (2005) indicated that housing type, social housing compare to singleton, affected social proximity preference in Japanese quail.

It has proven by many studies that selected quail for high social motivation preferentially approached conspecifics and stay more time close to them (Launay et al., 1991; Carmichael et al., 1998; Formanek et al., 2008) and were more disturbed by social isolation than quail selected for low social motivation (Launay et al., 1993; Mills et al., 1993). In quail chicks housed in pairs, low social motivation favors social bonding between cage mates, whereas chicks with high social motivation express a social attraction for any conspecific, whether they are familiar or not

[4]SD: Standard deviation

(Schweitzer et al., 2010). Schweitzer et al. (2011) conducted an experiment in order to determine how group size affects the strength of social bonds between familiar conspecifics in Japanese quail with different levels of social motivation. The behavior of quail were selected for high or low social reinstatement behavior and housed in different group sizes of 6, 15 and 30 were measured. Increasing group size increased the calming index only in high social reinstatement quail chicks which indicates that a lower calming effect of the return of a conspecific with increasing group size. The increase in group size caused a decrease in the number of nonaggressive pecks and the time spent in contact in both lines. Results of this study show that there is social bond in high social reinstatement and low social reinstatement quail chicks, which is in contrast to finding by Schweitzer et al. (2010) which showed that social bonds between familiar conspecifics in low social reinstatement but not in high social reinstatement quail chicks housed in pairs. They conclude that high social reinstatement and low social reinstatement quail chicks living in groups of more than two individuals are able to form social bonds with their group mates and to recognize one individual from a group of 30 birds as a familiar conspecific.

Maternal behavior is one of the influencing chicks' behavioral progress and precocial bird mothers can alter their chicks' emotional and social behaviors through non-genetic postnatal mechanisms inducing both long term and transgenerational consequences (Houdelier et al., 2013). Quail chicks spent their first 10 days after hatching with a mobile robot incorporating a heat source and their behavior was compared to that of chicks confronting the same robot but with its locomotors programme deactivated. The few studies that address the effects of the size of precocial birds' broods focus on offspring survival more than on maternal care. The influence of brood size on Japanese quail's maternal behavior and on mothers' interactions with chicks was investigated by Aigueperse et al. (2017) who compared two types of broods: small broods of three chicks (N = 9) and large broods of six chicks (N = 9). They also used two methods to assess maternal behavior (Pittet et al., 2014). The authors observed one day after maternal induction mothers in large group emitted more maternal vocalization (cooing and food calls) than mothers in small group. They also showed that brood size did not affect allocated time to warming chicks (GLM, $P > 0.05$) and at the end of the mothering period, mothers in large group expressed less covering postures than mothers in small group. Authors conclude that maternal behaviors including vocalization, warming, huddling can be affected by brood size. Vocalization behavior in chickens is considered as a welfare indicator in chickens (Manteuffel et al., 2004). Distress vocalization in chickens usually occur when chicks are exposed to conflict situation or loss of group contact to call assistance (Andrew, 1964). Also, it is raising question regarding whether or not the birds are aware that when companions are being removed from the herd in commercial situation until numbers were drastically reduced (Jones and Harvey, 1987). Although there is no study exploring effects of social interaction on vocalization behaviors in commercial scale in chickens, some researchers have shown that vocalization behaviors changed under different group size in chickens (Andrew, 1964; Jones and Harvey, 1987; Marx et al., 2001).

Parasites are one of main problems in poultry industry (chicken, turkey and quail) and they are almost where ever poultry are raised and can have severe effects on economic and production parameters. It has been proven that combination of interrelated factors such as management, stress, nutrition lead to exposure to parasites (Lynch Ianniello et al., 2014). Moore et al. (1988) assumed that parasite transmission in stable social groups occurs easier and larger and to evaluate their hypothesis conducted an experiment with different covey size in bobwhite quail for different season. Results of their study showed that number of monoxenous parasites is associated with group size. They also reported that intensities of T. tenuis and Rail- lietina cesticillus were higher large coveys than small covey. Also, authors mentioned that the immune system in host animal, variation introduced by the biology of intermediate hosts or a longer generation time must be considered for parasitism evaluation.

2-6- **Conclusion**

Many of recent literature shows that optimal group size in industrial scale need to be further studied, preferably in commercial trials, with respect to recent specified legalization in poultry industry in different countries. Still it is obvious that result from studies focusing on ideal group size have some degree of confounding and interactions between density and enclosure size. However, many of knowledge in the scientific literature can be applied in industry. We suggest that future research in welfare and behaviors in poultry should focus on the effect of group size on more specific responses and separation the effect of group size from other correlated factors. Also, more specific parameters, i.e. parameters with more economical application like leg disorders, growth performance and laying rate, should be studied in commercial scale group sizes in comparison to small group sizes. Furthermore, future researches should include the application of different group size in different systems (e.g., in different housing systems, same-sex or mixed-sex groups in turkeys, free range, organic and higher welfare indoor).

2-7- **References**

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3rd Chapter

Impact of different group sizes on performance, plumage cleanliness and leg disorders in broilers

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3-1- Abstract

The aim of the present study was to assess welfare and performance of broiler chickens housed in different group sizes. 6400 mixed-sex day-old broiler chicks were housed in four types of rectangular enclosures which provided 10m2 [small], 30m2 [medium], 100m2 [large] and 500m2 [very large] floor space. Per pen there were 100, 300, 1000 and 5000 birds, respectively and therefore constant density for all groups (10 birds/m2). Fifty birds per group were randomly selected as focus animals, and these birds were individually tagged to ease identification. Performance parameters [average daily weight gain (ADG) and body weight (BW) gain] as well as mortality and health and welfare parameters [gait score, hock burn, tibial dyschondroplasia (TD), foot pad dermatitis, plumage cleanliness] were measured at the end of starter (0-10d), grower (11-24d) and finisher (25-38d) period. Welfare and health parameters were assessed using scoring systems on a scale from 0 (indicating non-affected birds) to 2, 3 or 5 (indicating severely affected birds). Broilers in small and medium group sizes had higher BW and ADG in the three rearing phases compared to large and very large groups ($P<0.05$). Results for gait scores, hock burn and plumage cleanliness indicated better welfare of birds in small (except hock burn, $P>0.05$) and medium sized groups, compared to very large groups ($P<0.05$), and TD was more severe in very large compared to large groups. However, foot pad dermatitis was less severe in very large (0.27± 0.04) compared to medium sized groups (0.44± 0.05; $P<0.05$), and there were no significant differences for mortality. Results of this study show that the general assumption of detrimental effects of large group sizes needs to be reassessed, especially for new commercial broilers, but further research is needed with regard to commercially relevant group sizes.

Key word: Foot pad dermatitis, health, slaughter, tibial dyschondroplasia, welfare

3-2- **Introduction**

Optimum group sizes and densities in which farm animals should be maintained to assure acceptable welfare levels continues to be subject of frequent debate (Estevez et al., 2007). During last decades, many reasons including price instability, reduction in capital cost per hen housed, and competitiveness in market goals caused a noticeable increase in group size of poultry flocks. Consequently, the intensive broiler farming sector has been growing which involves the increasing use of large farms and high capacity houses. Due to a number of both advantages and disadvantages of large groups and according to new demands of market, group size has continued to be of interest to so many researchers and economists (Shahani, 2003; Christman and Leone, 2007; Estevez et al., 2007). For example, large group sizes have been suggested to lead to poor welfare brought about by overcrowding and various diseases (Meluzzi et al., 2008). Also, increasing the number of chicken housed together results in a higher risk for detrimental behaviors such as aggressive interactions and cannibalism, as well as leg disorders (Fiks-van Niekerk, 2001; Rodenburg and Koene, 2007; Buijs et al., 2009).

However, the relation between stocking density and welfare is not a simple one. Sufficient welfare comprises consideration of many factors including group size, quality of available space, the litter and housing system, hygiene condition and many others (Buijs et al., 2009; Delezie et al., 2015). Increased group size is expected to increase conflicts between the birds, leading to an increase in stress (Estevez et al., 2007). While group size has been identified as one of the main factors affecting poultry welfare (Estevez et al., 2003; Leone et al., 2007), specific group size recommendations for poultry vary widely among industry guidelines and certifying programs.

Further adding to the welfare problem, broiler chickens are, due to a strong focus on selection for weight gain (WG), amongst the fastest growing farmed species. Fast-growing muscles outpace bone development during the early life of chickens, stressing bones, joints, and ligaments, leading to problems with skeletal weakness (Julian, 1984; Sanotra et al., 2003). This higher susceptibility to skeletal problems in turn raises the question how present-day birds cope with different group-sizes and densities. On the other hand, it has been proven that there are substantial differences among broiler strains in the prevalence of leg disorders. Due to genetic selection strategies in breeding companies, significant decreases in the prevalence of leg disorders have been achieved by a strong focus on accurately scoring selection candidates and a stringent culling policy of discarding any selection candidate with clinical leg defects. Main focus of most breeding program was on deformities of the long bones (LD), crooked toes (CT), TD, and two forms of contact dermatitis including hock burn and foot pad dermatitis (Kapell et al., 2012). Although the heritability of leg health traits were low and their genetic correlations with BW unfavorable but low to moderate, breeding strategies for simultaneous selection for performance and leg health have been, and continue to be, effective (Ask, 2010; Kapell et al., 2012).

In addition to legislation, according to international agreement standards (Grandin, 2010; OIE, 2010) labeling programs in slaughterhouse have been planned to discriminate products according to welfare standards (Martelli, 2009). Currently, different animal welfare guarantee systems are being used to boost the adoption of animal welfare standards in food industry (Fraser, 2006; Sørensen and Fraser, 2010). Most of these assessment programs use parameters that are easier to evaluate in a slaughterhouse (Allain et al., 2009).

To the best of our knowledge, there are no studies in the published literature on effect of group size in broilers with comparable populations to commercial scale. Therefore, the aim of the study was to assess welfare and production parameters of broiler chickens housed in different group sizes (100, 300, 1000 and 5000 birds per group) with focus on welfare parameters that can be measured at the slaughterhouse.

3-3- **Material And Methods**

3-3-1- **Bird husbandry**

This study was conducted in private broiler house (ABIDI), located in City of Amol, North of Iran (36.4676° N, 52.3507° E, at an altitude of 76 m above sea). A total of 6400 mixed-sex day-old broiler chicks (Ross 308) were obtained from a commercial hatchery. Birds were housed in a separate holding enclosure and four types of rectangular enclosures where constructed which provided $10m^2$ (small, 10×1 m), 30 m2 (medium, 10×3 m), 100 m2 (large, 10×10 m) and 500 m2 (very large, 10×50 m) with 100, 300, 1000 and 5000 birds in each pen, respectively. Thus, the density was constant for all groups (10 birds/m2). Design of enclosures and distribution within the barn is shown in figure 3.1.

Fifty randomly selected birds per group were chosen as focus animals, and these birds were individually tagged to ease identification. Identification tags were made of white paper disks 5 cm in diameter with a unique two digit black number printed on both sides.

During the first 3d, additional feed was spread on lengths of paper placed underneath the drinking nipples to ensure that all chicks learned to associate the pellets with food, and from the 4^{th} day feed was available ad libitum via pan feeders (50 birds per pen, filled automatically) from a central tubular hopper for the barn and separate hoppers and scale for each enclosure to control feed intake for each group. Pan feeders have the advantage that all are filled simultaneously, making feed available to the birds immediately. Water was supplied with lines of nipple drinkers, with 10 birds per nipple, by a separate water tank for each group. The feeding program consisted of a standard three phase commercial diet (starter: 1-10 days, grower: 11-24 days and finisher: 25-slaughter). Because we wanted to have more uniform diet, both in chemical composition (Table 3-1) and size of components, we used a small feed mill and mixer to mill and mix the experimental feed by ourselves rather than a commercial feed production factory. 200 grams of *diclazuril* (Elcano® Netherland) per ton were added in feed as a coccidiostats.

Table 3-1. Ingredients and calculated composition of experimental diets fed to broilers

Ingredients (g/kg):	**Starter (1-10d)**	**Grower (11-24d)**	**Finisher (25-38d)**
Corn	53.87	56.22	60.49
Soybean meal-48	39.43	37.45	32.38
Soybean oil	3.63	4.02	4.83
DCP	0.18	0.19	0.25
Limestone	0.52	0.52	0.52
DL- Met	0.35	0.32	0.29
L-Lysine	0.17	0.16	0.16
L-Threonine	0.8	0.70	0.05
Sodium Carbonate	0.24	0.23	0.23
Mineral premix[1]	0.25	0.25	0.25
Vitamin premix[1]	0.25	0.25	0.25
Salt	0.31	0.31	0.31
Total	100	100	100
Calculated composition:			
AEMn (kcal/kg)	3000	3100	3200
Crude protein (%)	23.8	21.6	19.65
Calcium (%)	0.96	0.87	0.81
Available P (%)	0.48	0.43	0.40
Total P (%)	0.74	0.70	0.66
Sodium	0.20	0.20	0.20
Potassium	1.02	0.92	0.83
Lys (%)	1.44	1.29	1.16
Met + Cys (%)	1.08	0.99	0.91

[1] Supplied by Razak Co., Tehran, Iran. Vitamin premix provided per kilogram of diet: vitamin A, 11,000.0 IU; vitamin D3, 2,000.0 IU; vitamin E, 18.0 IU; vitamin K, 4.0 mg; vitamin B12, 0.015 mg; thiamine, 1.8 mg; riboflavin, 6.6 mg; calcium pantothenic acid, 12.0 mg; niacin, 30.0 mg; pyridoxine, 2.9 mg; folic acid, 1.0 mg; choline, 260.0 mg. Mineral premix provided per kilogram of diet: manganese, 64.5 mg; zinc, 33.8 mg; iron, 100.0 mg; copper, 8.0 mg; iodine, 1.9 mg; selenium, 0.25 mg.

During brooding chicks were exposed to 24 h of light for the first three days of life with 40 lux of light intensity and thereafter were maintained on a 14 L:10 D light schedule with 15 lux, uniformly distributed through the house. This was done with the intention to slow birds' growth and to promote leg health. The room was heated before the beginning of the experiment so that the floor had reached 27°C before wood chip litter was applied in a 5-cm thick layer (2 kg/m^2). The temperature was maintained at 35 ◦ C from d 0 to d 4 posthatch, and was progressively reduced to 25 ◦ C on d 14 and was maintained at this level until the conclusion of the study at 38 days.

In order to provide more equal environmental conditions, we conducted the experiment in a house with side ventilation system, with curtain windows for air intake in one side wall and exhaust fans on another side wall (figure 3.1). Temperature, relative humidity, and ventilation rate were constantly controlled by digital sensors in several sites of each enclosure to keep them in range with recommendation of commercial strain (ROSS 308) catalog. Ventilation (air exchange) rates were based on the requirements for 6400 broilers and were provided 0.074 m^3/hr per bird from the first day and then on a daily-basis increased gradually to 1.35 m^3/hr per bird at 38 days of age. Ventilation rates were increased equally in all pens over the course of the study. Relative air humidity was maintained at 70% for d 0 to d 7 posthatch, and was decreased approximately 4% per week to reach 50% in 38d. The thermometers and equipment to control relative humidity were distributed in several places in barn to control uniformity of environmental conditions within pens. For first days of rearing period, litter moisture was raised to 20% and thereafter litter moisture was measured at the end of every week during rearing period and was maintained in range of 20-25% (average of litter moisture of whole barn) for d 0 to day 21 and 30-35% for d 22 to 38.

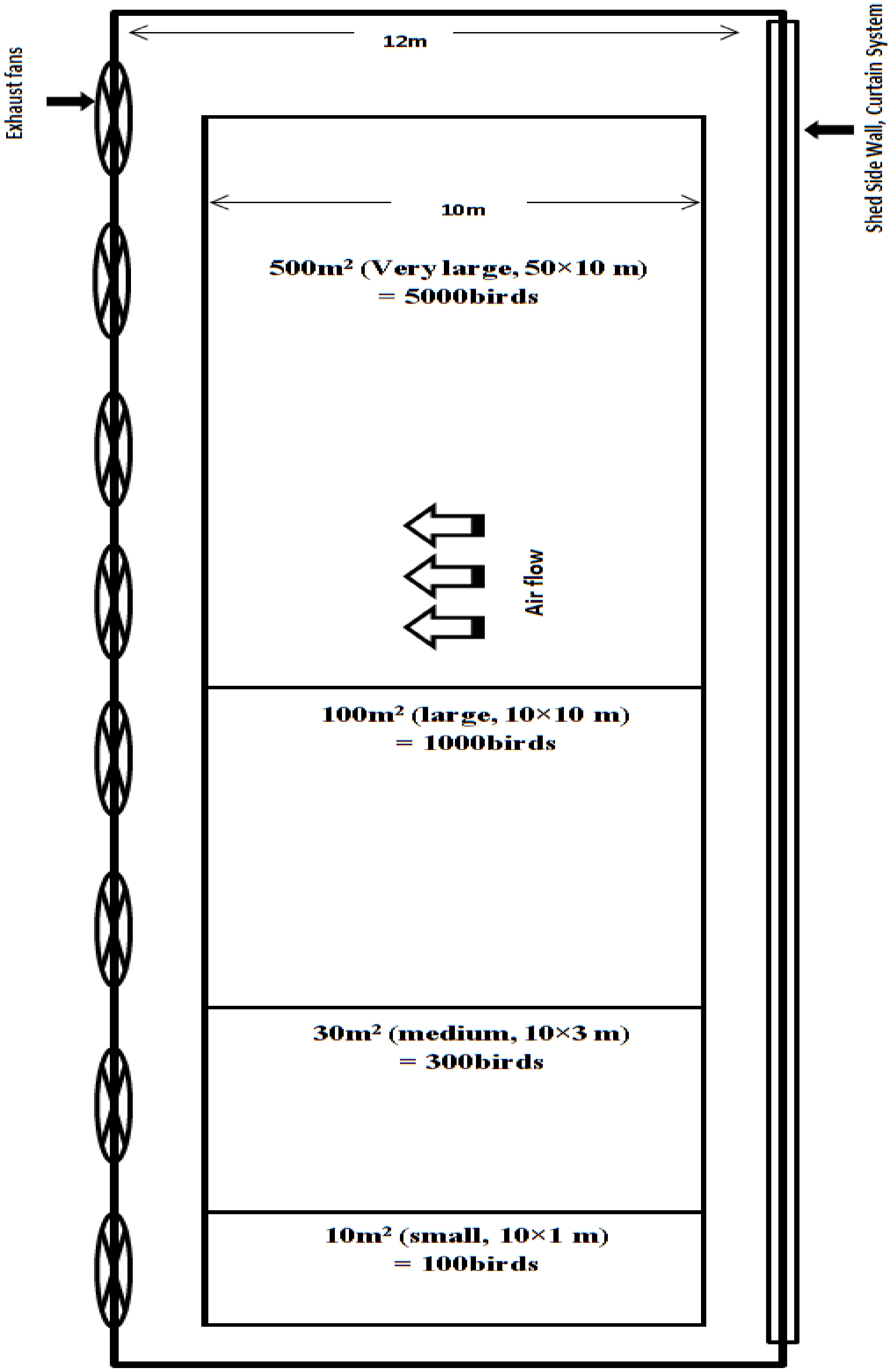

Figure 3-1. Distribution of pens within the barn and direction of air flow.

3-3-1- Parameters measured

Performance:

All 50 focal animals in each group were individually weighed at the end of each diet phase to calculate body weight at the end of each diet phase.

Feed intake (FI) and body weight (BW) was likewise recorded at the end of starter (0-10d), grower (11-24d) and finisher (25-38d) period, and average daily gain (ADG) was calculated. Feed per group was weighed daily and fresh mesh feed were supplied.

Gait score:

All focal birds were gait scored three times before slaughter (age 10, 24 and 38 days). Gait scoring followed the protocol described in the Welfare Quality Protocol applied for broiler chicken (Welfare Quality, 2009). The scoring scale ranged from 0=normal gait to 5=unable to walk (Table 3-2). For these procedures, birds were confined in a catching pen at five to seven different locations in dimmed light. The assessment locations always represented at least a central part of the house, the wall side and the front and near to end of the house. During the scoring, which took approximately three hours light intensity was increased back to normal.

Table 3-2. Description of the scoring system for broiler gait using a six-point scale (following the Welfare Quality® protocol applied for broiler chicken) (Welfare Quality, 2009)

Score	Description
0	Normal gait: even steps, toes furled while foot is in the air.
1	Uneven gait at times, slight defect not easily defined, toes may furl in the air.
2	Uneven gait, mild but definite defect, foot flat in the air, gait abnormality does not compromise bird's maneuverability.
3	Obvious, moderate gait abnormality, impaired ability to move around, chooses to sit when not forced to walk.
4	Severe walking difficulties, takes only few steps if forced and sits readily at every opportunity, bird's maneuverability severely compromised.
5	Unable to walk, uses wings or crawls when forced to move, growth often seriously reduced.

Hock burn

Fifty selected broilers within each group were evaluated three times before slaughter (age 10, 24 and 38 days). Both left and right hocks of individual birds were analyzed for hock redness. For classification of the measurements the following score was applied: 1= not affected; 2= color changes or minor lesions and 3= severe lesions, as proposed by Thomas et al. (2004).

Plumage cleanliness

Immediately after electrical stunning, the 50 focal birds per group were scored for cleanliness of the plumage. The classification of the measures was completed according to the following description: 0=clean feathers (white feathers with absence of dirt); 1=moderately dirty feathers (soiling feathers localized in the breast and abdominal areas without caked dirt) and 2=very dirty feathers (generalized dirty brown feathers sometimes with dirt adhered or caked to feathers) (adapted from Welfare Quality®, 2009).

Footpad dermatitis:

The development of footpad lesions for the 50 focus birds per group was followed over the rearing period, three times before slaughter (age 10, 24 and 38 days). The severity of foot pad dermatitis was scored as follows: 0=no lesions (no visible lesions: smooth epidermis, no discoloration), 1=mild lesions (papillae only with hyperkeratosis and/or mild/superficial lesions with discoloration or erosions in the epidermal layer up to 5 mm) and 2=severe lesions (severe papillae and ulcerations: discoloration, hyperkeratosis, ulcers and signs of inflammatory reactions up to 5 mm) (Dawkins, 2008; Shepherd and Fairchild, 2010).

Tibial dyschondroplasia:

The severity of tibial dyschondroplasia was determined at the slaughterhouse in 50 randomly collected birds per each group. The condition of the proximal growth plate of both tibias was assessed with a four-point scale (Table 3-3).

Table 3-3. Description of the four-point scoring system for tibial dyschondroplasia in broilers

Score	Description
0	Normal growth plate (including slight, uniform thickening of growth plate)
1	Mild lesion with cartilage development ⩽0.5 cm
2	Moderate lesion with abnormal cartilage developed >0.5 to 0.75 cm
3	Severe lesion with cartilage extended >0.75 cm

Mortality:

Total number of animals which died and were found dead during the flock cycle in each group was recorded daily and mortality was calculated.

All of the measurements were carried out by experienced official veterinarian. Before starting the experiment, two veterinarians were trained using the corresponding method for each parameter. If veterinarians gave different scores to the same chick, the average of these scores was recorded.

3-3-2- Statistical analysis

Statistical analyses were conducted in SAS (SAS Institute, 2008). The performance data (ADG and BW) were analyzed via the GLM procedure. Since recording of individual FI for tagged birds without depriving them from their normal daily activity was not possible, only total FI of each treatment were measured and therefore not compared statistically. The GLIMMIX procedure assuming a Poisson distribution was used for the traits gait score, hock burn, plumage cleanness, tibial dyschondroplasia and foot pad dermatitis. In these models, group size (4 levels) was considered a fix effect, while accounting for repeated measurements (d 10, 24, and 38). Mortality was tested by FREQ procedures (X^2 test). All statements of significance were based on $P<0.05$. To increase comparability of our study with other studies, additionally the proportion of birds affected with the most severe class for each parameter was calculated.

The following equation was used as a model in data analysis:

$$y_{ijk} = \mu + a_i + b_j + e_{ijk}$$

Where, μ is the mean, a_i is the effect of i^{th} group size (i=1:4), b_j is the effect of j^{th} measurements of each individual (j=1:3) and e is the residual effect.

3-4- **Results**

Broilers' growth performance differed between group sizes of broiler and feed intake in three rearing periods (Table 3-4). Broilers in small and medium group sizes had higher BW and ADG in the three rearing phases compared to large and very large group sizes (P<0.05). ADG for birds that were raised in medium size group was higher than other groups (P<0.05). The effect of group size on mortality was not significant (P>0.05).

Table 3-4. Effect of different group size on growth performance and mortality of broiler chickens in three rearing periods (1-10, 11-24 and 25-38d)[1]

	1-10d		**11-24d**		**24-38d**		
Group size	ADG[6] (g)	BW[7] (g)	ADG (g)	BW (g)	ADG (g)	BW (g)	**Mortality (%)**
Small[2]	25.03[a]	292.66[a]	55.91[ab]	1075.38[a]	90.59[ab]	2343[a]	4.00
Medium[3]	24.54[a]	287.89[a]	56.95[a]	1085.25[a]	93.20[a]	2390[a]	3.33
Large[4]	21.22[b]	255.09[b]	54.67[b]	1020.65[b]	85.42[bc]	2216[b]	4.10
Very large[5]	20.99[b]	252.43[b]	55.42[ab]	1028.43[b]	83.29[c]	2194[b]	4.76
SEM	0.48	4.80	0.65	8.11	2.04	27.55	0.09

[1]Means with different letters are significantly different (P<0.05)
[2]Small = 100 birds in pen
[3]Medium = 300 birds in pen
[4]Large = 1000 birds in pen
[5]Very large= 5000 birds in pen
[6]ADG = average daily gain
[7]BW = body weight

Gait score was affected by group size, and in comparison with small and medium groups, the walking difficulties were more severe in the large and very large groups (P<0.05; Figure 3-2).

Increases in group size also caused more severe hock burns (P<0.05; Figure 3-3 with more severe burns in very large and large groups, compared to medium group sizes.

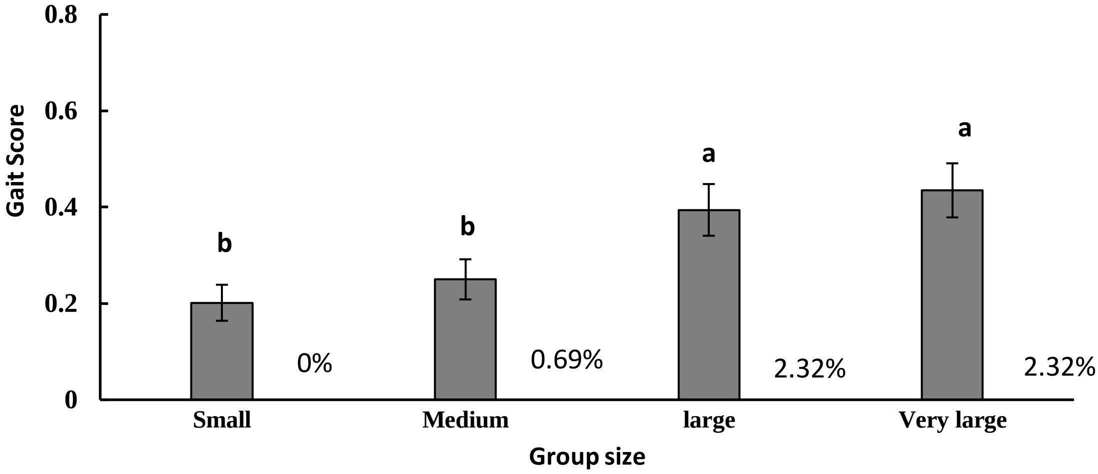

Figure 3-2. Effect of group size (small: n= 100 birds, medium: n=300, large: n=1000, very large=5000) on gait score (re-transformed least square mean ± SE, higher values indicate higher abnormalities). Different letters (a,b) indicate statically significant differences at P<0.05. Numbers beside each bar show the prevalence of the most severe class (score 5) of gait score per group size.

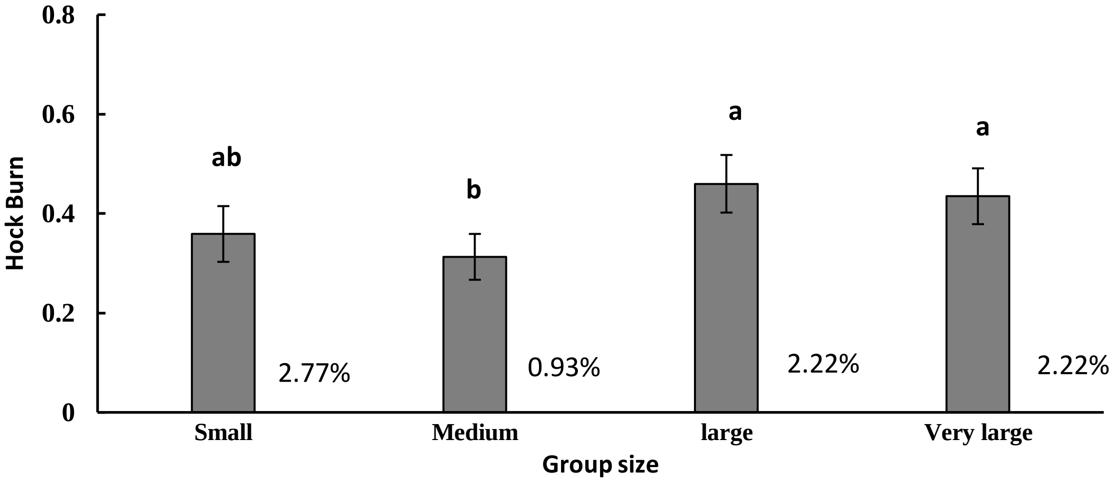

Figure 3-3. Effect of group size (small: n= 100 birds, medium: n=300, large: n=1000, very large=5000) on hock burn (re-transformed least square mean ± SE, higher values indicate higher abnormalities). Different letters (a,b) indicate statistically significant differences at P<0.05. Numbers beside each bar show the prevalence of the most severe class (score 3) of hock burn per group size.

Tibial dyschondroplasia was more severe very large compared to large groups (P<0.05), with small and medium groups ranging in the middle (Figure 3-4).

In contrast, foot pad dermatitis was more severe in medium compared to very large groups (P<0.05) but neither of these two groups differed significantly from small and large groups (Figure 3-5).

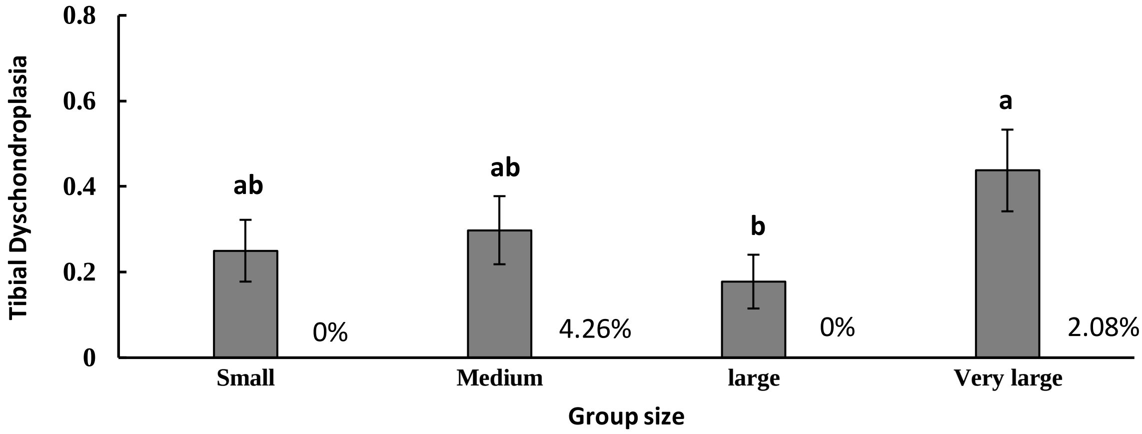

Figure 3-4. Effect of group size (small: n= 100 birds, medium: n=300, large: n=1000, very large=5000) on tibial dyschondroplasia (re-transformed least square mean ± SE, higher values indicate higher abnormalities). Different letters (a,b) indicate statistically significant differences at P<0.05. Numbers beside each bar show the prevalence of the most severe class (score 3) of tibial dyschondroplasia per group size.

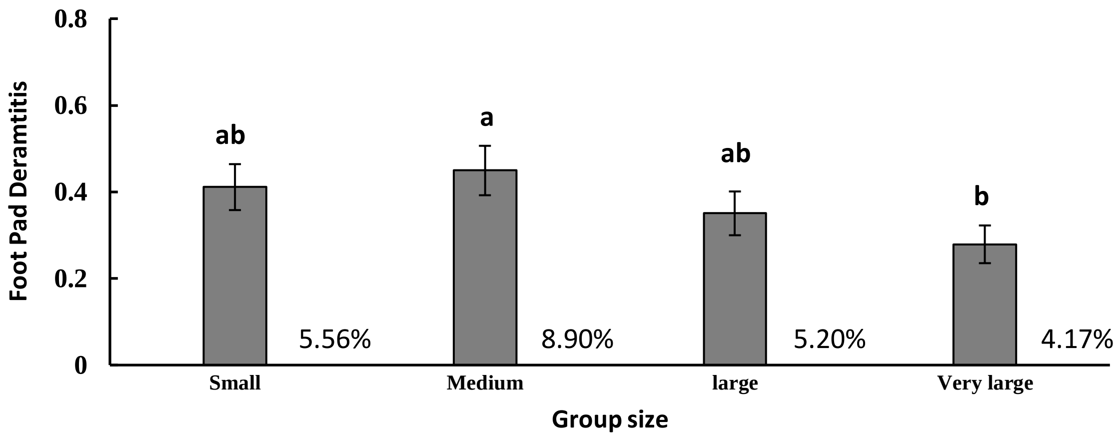

Figure 3-5. Effect of group size (small: n= 100 birds, medium: n=300, large: n=1000, very large=5000) on foot pad dermatitis (re-transformed least square mean ± SE, higher values indicate higher abnormalities). Different letters (a,b) indicate statistically significant differences at P<0.05. Numbers beside each bar show the prevalence of the most severe class (score 2) of foot pad dermatitis per group size.

Means of scores for plumage cleanliness assessed at the slaughterhouse are shown in Figure 3-6. Dirtiness of birds' feathers was more severe in the very large group, compared to the medium group (P<0.05).

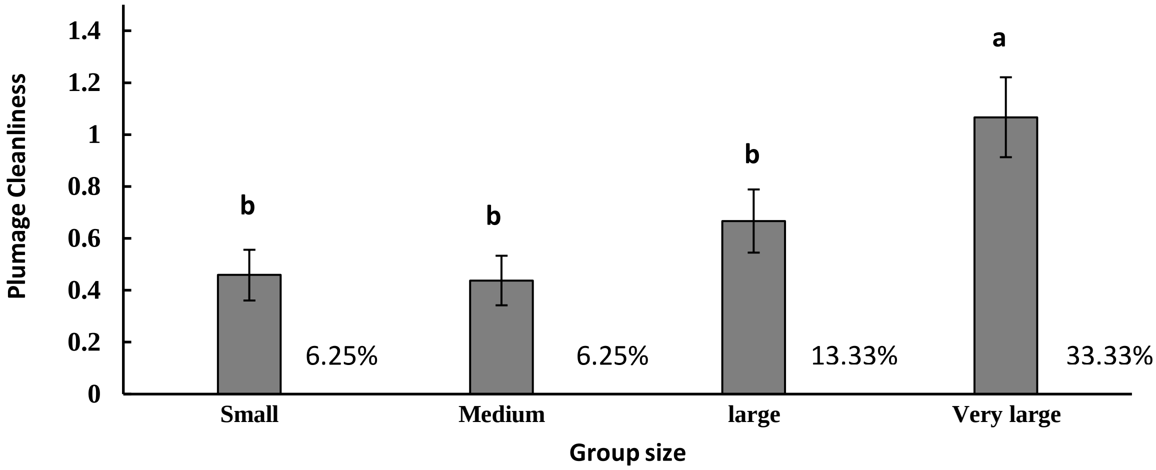

Figure 3-6. Effect of group size (small: n= 100 birds, medium: n=300, large: n=1000, very large=5000) on plumage cleanliness (re-transformed least square mean ± SE, higher values indicate higher abnormalities). Different letters (a,b) indicate statistically significant differences at P<0.05. Numbers beside each bar show the prevalence of the most severe class (score 2) of plumage cleanness per group size.

3-5- **Discussion**

The objective of this study was to evaluate effects of group size on performance and welfare indicators by focusing on leg disorders. The effects of group size, while significant, were not as pronounced as we expected, but there were some noticeable effects on leg disorders. Overall, welfare and performance of birds in groups with group size of 300 or 1000 birds/pen was slightly superior to birds in the group with 5000 birds/pen. Given that the major environmental factors such as air flow, light density and air humidity were the same for the different pens, we assume that location effects, if any, were of minor importance, and instead the observed differences were indeed caused by the different number of birds per group and e.g., resulting differences in birds' behavior. Nevertheless, it is possible that additional environmental factors not measured in the present experiment may have influenced our results, and they should thus be seen as an indication only, and not as definite result.

3-5-1- Performance

There are few experiments on effect of group size on broiler performance but the results of the present experiment are in agreement with other research, which suggests that the poultry production environment may induce stress and discomfort and thus reduced performance in broilers as a result of large group sizes and high densities (Feddes et al., 2002; McLean and Savory, 2002; Dawkins et al., 2004a).

Studies which evaluate the effects of group size and density, involve some degree of confounding regarding to movement patterns and behavioral parameters (Christman and Leone, 2007; Leone et al., 2007). While some other researchers indicated that variations in bird group size and density can influence movement and space use (Estevez et al., 1997; Le Neindre et al., 2004), other authors suggested that group size can create social restrictions to movement (Banks et al., 1979; Pagel and Dawkins, 1997). Also most of experiments have used laying hens to determine the effect of group size in which their movement patterns are relatively different from broiler movement pattern particularly at feeding distribution time (Pagel and Dawkins, 1997; Leone et al., 2007; Averós et al., 2010).

3-5-1- Gait score

Our finding of better walking ability in birds in smaller groups indicates that broilers benefited from the locomotion facilitated by the decrease of the group size. In fact, walking ability has been shown to be improved by any measures that increase the mobility of broilers such as exercise equipment (Bizeray et al., 2002), lower stocking density (Knowles et al., 2008) or increased walking distances (Kaukonen et al., 2017).

In this study, the number of birds with gait scores 0 (83.12%) and 1 (10.19%) was remarkably high, as also indicated by the low mean scores. In previous field studies, only 10% (Kestin et al., 1992) to 25% (Sanotra et al., 2003) or 29% (Knowles et al., 2008) of the tested birds demonstrated normal gait. The large number of birds with scores 0 and 1 in the present study could be due to the subjectivity of the gait scoring method. A high welfare standard is considered to be achieved when 95% - 99% of the broilers present gait score below 1, and welfare is considered acceptable when 70% of the birds walk soundly (Grandin, 2007). In the present study, broilers probably walked longer distances in smaller groups, offering them additional exercise compared to larger groups. Usually fast-growing broilers spend a considerable proportion of their time-budget lying and even slightly increased activity may be sufficient to improve agility. Also, exposure to different temperature and relative humidity, age, number, breed, and size of the animals in each enclosure, different ventilation methods of enclosures and bedding materials in different experiments can cause behavioral fluctuations and morphologic alterations (Leone et al., 2010; Dunlop et al., 2015), which might negatively affect animal well-being and research performance as well as outcomes of research results. Finally, the breeding company's efforts to increase leg and skeletal health through genetic selection also may have been effective (Kapell et al., 2012) and in part be responsible for the fairly high proportion of unaffected birds.

3-5-2- Hock burn and tibial dyschondroplasia

When broilers present hock-burn lesions, the damaged skin exposes nocireceptors, which are the sensorial receptors related to pain (Gentle, 2011). Therefore, hock burn presents a considerable welfare problem. Furthermore, the lesions may serve as a point of entry of pathogenic bacteria in the body (Kyvsgaard et al., 2013) and may cause performance losses and carcass discard in the meat processing plant. In most severe cases, the lesions become ulcerated, with inflammation of the subcutaneous tissue, leading to pain and compromised welfare (Bassler et al., 2013). Higher incidence of hock burn in large and very large group sizes in this experiment may be related to different litter quality in these groups. Although the density was constant for all groups in our experiment, group sizes may have changed some other factors including litter exploring and colonizing behaviors and leading to better possibility for the litter to dry off. Also, one of the difficulties in assessment of hock burn incidence is the variety of outcome explanations used (Allain et al., 2009), for example some researchers following method described by Su et al. (1999) and using a methods including a combined score for both legs given by four scores. While other researches, including this study, followed methods described by Thomas, et al. (2004) or Dawkins et al. (2004b) which includes three scores. This variety causes difficulties for comparisons between studies.

Dyschondroplasia is the most common lesion seen in broiler leg bones. It is recognizable by the build-up in the bone growth plate of an avascular mass of prehypertrophic chondrocytes (Cook, 2000). These are chondrocytes, which have not fully matured and hence do not allow the normal process of bone calcification to occur. It develops usually between two and five weeks of age (Lynch et al., 1992) before regressing. Incidences of tibial dyschondroplasia as high as 47.5% have been reported in one commercial line (Rauw et al., 1998) whereas this problem was far less frequent in most of the commercial crossbreds.

In the current experiment, the effect of group size was significant on tibial dyschondroplasia severity between birds in large and very large groups. The focus of most previous research was on evaluation of tibial dyschondroplasia in relation to different flock density. It has been shown that stocking density has no effect on prevalence of tibial dyschondroplasia which indicates that stocking density is not important in regulating the expression of this condition (Sørensen et al., 2000). This lack of importance is probably because tibial dyschondroplasia lesions first develop very early in life before stocking density becomes important. In the current experiment, the density was the same for all four groups, i.e.10 birds/m^2, which is not a high density for closed housing systems, and it seems unlikely to have an effect on tibial dyschondroplasia incidence. Bizeray et al. (2000) concluded that if the relationship between early and late activity will be confirmed, manipulation of activity by environmental factors during the first week of life may prevent leg disorders at slaughter. Higher severity of tibial dyschondroplasia in very large group may be related to more stress induced by competitive access to feeder and drinkers in large groups in early age of chickens and some competitive exclusion or late access to feeders and nutrient deficiency which causes some skeletal abnormalities including tibial dyschondroplasia.

3-5-3- Foot pad dermatitis

The occurrence and severity of pododermatitis in broiler chicken is one of great concerns to the broiler industry, both from the animal welfare perspectives and product quality (Ekstrand et al., 1997). The welfare of broilers affected with this condition is impaired not only the pain caused by this condition, but also because their walking is compromised, delaying their feed and water intakes, as they are not able to reach the feeders and drinkers (Haslam et al., 2007; Shepherd and Fairchild, 2010). Some researchers showed that increased bodyweight has been associated with an increase in leg disorder (Kjaer et al., 2006). Higher body weight in smaller groups may increase the prevalence and severity of pododermatitis due to their abrasive action (Bilgili et al., 2009). On the other hand, feed composition may negatively affect excreta degradation, increasing litter moisture and leading to the occurrence of lesions (McIlroy et al., 1987) and the difference in diet composition in experiments can cause diverse findings.

3-5-4- Plumage cleanness

Feather cleanliness is essential for thermoregulation and when the feathers are wet or soiled by litter, feces or dirt they may lose their protective properties, having adverse effects on welfare of birds (Greene et al., 1985). In our study, dirty feathers were more prevalent in the very large group, which might be explained by prolonged contact with poor quality litter. Since population density, ventilation rate, temperature, relative humidity and light density were the same for the different pens, we assume that differences in moisture of litter in some area within the pens may happened during rearing which might be result of differences in group size and associated differences in birds’ behavior, litter exploring and colonizing behaviors, rather than from differences in environmental conditions.

Unfortunately, litter moisture was measured for whole barn only for the purpose of controlling rearing conditions. Since the main focus of this study was on evaluation of welfare parameters in slaughterhouse, some aspects of barn monitoring parameters including litter moisture and water: feed-ratio in different pens as well as behavioral parameters including social interaction and flocking behaviour of broilers were not assessed. Therefore further analysis and measurements including these parameters to find possible patterns in behavior associated with group size and their effects on environmental parameters in similar studies are needed.

3-6- **Conclusion**

Behavioral reactions considered as social interactions rarely take place in broilers because there are few locomotor activities such as walking or exploring in fast-growing broilers. Consequently, the majority of interactions take place during feed distribution when broilers compete to access the nearest feeder. Therefore, the assumption of increasing population sizes leading to decreasing harmful social interaction needs to be reassessed, especially for new genetic commercial broiler lines. However, with regard to the additional welfare benefits on leg strength detected at the low density in this experiment, more information would be needed. Obviously, to confirm the conclusions from this experimental study, further research with stable stocking densities with increasing group sizes or stable group sizes at different stocking densities reflecting commercial conditions with much larger group sizes should be performed.

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4th Chapter

Effects of group size on pigs’ cognitive bias and group preferences

4-1- Abstract

It has been suggested that pigs are unable to individually recognize all animals of their group when living in groups containing > ca. 50 individuals, raising the question, if this potentially unnatural social situation negatively affects animal welfare. Therefore, the aim of the present study was to investigate the emotional state of fattening pigs when housed in a small versus very large group. Fourteen pigs were trained on a go/no-go task to discriminate two visual stimuli, a positive cue that predicted a food reward, if the pig approached a bucket, and a negative cue that predicted no reward. After six weeks of training, 91 pigs originally housed together in one large group were split into a small (11 pigs) and a large (80 pigs) group with equal densities (0.8 m^2/pig) and seven trained pigs per group. Cognitive bias and preference tests were conducted with the trained pigs at -1, 1, 3,7,10 and 13 days in relation to splitting the original group. For all control comparisons, according to mixed model analysis results were as expected, i.e. pigs of both groups approached the positive cue equally fast and more rapidly than the negative cue, and before splitting the group there were no significant differences ($P>0.1$) in latency to approach the ambiguous cues between pigs that were later housed in the small vs. large group. However, on day 3, 7 (both $P<0.05$) and 10 ($P<0.1$) after splitting, pigs of the small group showed greater latencies to approach the ambiguous cue, compared to pigs of the large group, indicating a more pessimistic bias in pigs housed in the small group. The preference test revealed no clear preference ($P>0.05$) for the small or large group for either pigs from the small or large group, but pigs were generally very reluctant to return to the group housing (and instead seemed to strongly prefer to return to the test arena), indicating that no active choice was made. Although results may not be generally valid for all groups and the change to an altered environment rather than the group size per se might also play a role, results suggest that

pigs' welfare in small groups is negatively affected, likely due to the reduced total space availability.

Key words: Cognitive bias, Group size, group preference, pig.

4-2- Introduction

During the last decades, due to reduced profit margins, average size of pig production systems has increased considerably (Tilman et al., 2002; FAWC, 2012; Eurostat, 2013). According to Eurostat (2014), 77.8 % of pigs are reared in large fattening farm (at least 400 pigs). Along with the change in farm size, changes in group size are observed. Common group sizes for growing pigs range between 10 and 30 animals per pen, but larger groups of 40 to 100 and more animals are found in some farms as a managing approach to improve overall productivity (Schmolke and Gonyou, 2000; Schmolke et al., 2003). The majority of consumers consider such large herd and large group sizes to be a threat to animal welfare (Verbeke et al., 2010), and in fact certification procedures for animal welfare labels often include upper limits for herd sizes (Kapitel, 2013; Denver et al., 2017). However, scientific evidence for clear benefits of smaller group sizes to pig welfare is scarce, and studies investigating welfare indicators yield contradictory results.

Pigs belong to relatively stable social hierarchies, which play an important role in maintaining group harmony. Whereas unfamiliar pigs who are intensively confined and crowded in industrial operations will engage in aggressive, agonistic behavior, in the wild, pigs are naturally gregarious animals and group members maintain close contact, often synchronizing their behavior (Stolba and Wood-Gush, 1989; Ekesbo and Gunnarsson, 2018). Some studies revealed, for example, a negative impact on welfare indicators when group sizes are larger than those found under natural conditions, which are typically comprised of two to four sows with their recent litters and young offspring of previous litters (Gonyou, 1997). Some researchers (e.g., Keuling et al., 2008) differentiate mother-families further between single mothers and their piglets, two females and their piglets or more than 2 females with piglets. According to Bieber et al. (2019), free-ranging wild boar populations can be differentiated into three grouping formations: 1) mother-families, 2) matinggroups, 3) yearling-groups (Briedermann, 2009). Also, Bieber et al. (2019) reported that in their study on social structure between individuals in wild boars, all observed group sizes in wild boars (yearlings 11-16 months) ranged consistently between 11 to 20 females and concluded that

the optimal female group size at this age varied between 11-20 individuals independent from the size of enclosures. Moreover, Briedermann (2009) indicated that in northern Europe, a group size of 6 - 10 individuals is the most frequently observed (40%) in winter, and a group size more than 10 is the most frequent group size in summer (more than 45%). McGlone and Newby (1994) equally found increased morbidity and prevalence of injuries in pigs housed in groups of 40 animals compared to groups of 10 or 20 animals. In contrast however, Samarakone and Gonyou (2008) did not find differences for mortality, morbidity or behavioural vices, such as tail biting, when comparing groups of around 20 and 100 pigs.

Group size was shown to influence behaviour, productivity and overall welfare of pigs (Rabaste et al., 2007; Meyer-Hamme et al., 2016). Meyer-Hamme et al. (2016) concluded that in pens with more than 30 animals the presence of wounded and dirty pigs and of negative social behaviour was greater. They also showed that there is a better human–animal relationship in these large groups. However, social instability is a concern in large groups of pigs and can affect overall productivity and welfare (Stricklin and Mench, 1987). The social stability of a small group relies primarily on the development of a social hierarchy (Meese and Ewbank, 1973), whereas the mechanism of social stability and behavior of pigs in large groups is unclear. Some argue that with increasing group sizes the number of dyadic relationships that have to be verified increases as well, leading to greater social tension within large groups (Stricklin and Mench, 1987).

Results regarding performance of pigs kept in large versus small groups are contradictory. Some early literature discussed negative relationships between group size and growth rates as well as higher incidences of behavioural vices such as tail biting in conjunction with larger group sizes for pigs (Kornegay and Notter, 1984; Brouns et al., 1994). In a more recent study, Wolter et al. (2000) compared the performance of weaned pigs (20 vs. 100 pigs per pen) and found reduced average daily weight gain (ADG) and average daily feed intake (ADFI) in the larger group of 100 pigs. Turner and Edwards (2000) found a reduction in ADG when grower pigs were kept in groups of 80 compared to 20 pigs per pen, whereas Schmolke et al. (2003) reported similar levels of ADG in grower-finisher pigs when keeping 10, 20, 40 or 80 pigs per pen. The variation in results suggests that, rather than an inherent problem with large groups of pigs, many factors such as age or body weight (BW) at mixing and availability of resources, may contribute to the differences observed in performance among group sizes.

Cognitive bias in animals is a pattern of deviation in judgment, whereby perception of other animals and situations may be affected by unrelated information or emotional states (Mendl et al., 2009). By analogy, cognitive biases or biases in judgment are used as a prevalent method to assess affective states in non-human animals (Harding, 2004). Animals in a more positive affective state show 'optimistic' biases, characterized by responding to ambiguous stimuli as though they predicted a positive outcome. In contrast, animals in a negative mood show 'pessimistic' biases by responding to ambiguous stimuli as if expecting a negative consequence. If such mechanisms operate equally in human and non-human animals, then mood is predicted to interrelate with personality to determine cognitive bias. Several studies of cognitive bias have been carried out in pigs so far but there were many differences in test design and huge variability in number of training sessions among these studies. Also most of these studies compared two treatment groups; some of these studies failed to find difference between treatment groups (e.g. Düpjan et al., 2013; Scollo et al., 2014) while some others found differences between treatment groups (e.g. Douglas et al., 2012; Murphy et al., 2015). However, it has been reported that pigs can perceive the treatment differently due to individual differences and respond consistently to specific challenges, at least over the short term (Spoolder et al., 1996). The aim of the present study was to investigate the emotional state of fattening pigs when kept in a small versus a very large group. Based on the assumption that small groups better reflect pigs' natural social conditions, we hypothesized that pigs of the large group would show a more pessimistic bias and that pigs would prefer to return to the small rather than the large group in a free choice test, regardless of where they were last housed.

4-3- **Materials, animals and methods**

4-3-1- Animals and housing environment

This experiment was conducted in experimental farm Relliehausen of the University of Goettingen (51°46´55″ N, 9°42´13 ″ E, at an altitude of 230 m above sea). In this study, 91 pigs (breed: BHZP Victoria x Piétrain), approximately 12 weeks of age and weighing 40 kg at entry to the experiment, were involved in the experiment. 14 pigs (female pigs, named experimental animals) out of these 91 were chosen for participation in the behaviour tests, and an extra ear tag was used for identification. Ratio of male –to-female pigs were constant in both group. Initially, after moving from the rearing quarters to the fattening unit, pigs were housed together in one pen as a large group (Figure 4-1, in accordance with the minimum legal provision for pigs housed intensively under EC Directive 2008/120/EC) providing 0.8 m^2 of space per pig and fully-slatted concrete flooring. The barn provided a controlled-environment, with automatically controlled fan ventilation set to maintain a room temperature of 24-28°C and lighting from 0700–1600. The pen contained feeders providing four feeding places with water freely available from two bite drinkers adjacent to the feeders and positioned 0.5 m above the slatted floor. A commercial pig diet was available *ad libitum* throughout the experiment. The pigs were health checked daily and showed no health issues during the study. This type of non-invasive research does not require a study-specific approval and is approved under the general German animal welfare regulations.

Training phase

For the first five weeks of the experiment, the training phase (see Figure 4-2), pigs were housed together in one large group of 91 pigs. On training days of the first week, pigs were mildly food deprived by changing feed distribution time (four times instead of six times per day) to ensure that pigs would show enough exploratory and feeding behaviour for searching and consuming the reward. The pigs were trained individually in a single test arena (3.25 length ×2.7 m width) in a go/no-go discrimination task similar to set ups used in previous cognitive bias experiments with pigs and other species (Bateson and Matheson, 2007; Brydges et al., 2011; Harding et al., 2016). In order to check for potential differences in baseline cognitive bias, the first cognitive bias test was performed at the end of the five-week training phase while the pigs were still housed in their initial environment. Thus, eleven out of the total of 91 pigs were moved to a small pen of 8.8 m^2 that was partitioned from the large pen. The experimental animals were split equally between this small and the remaining large group, such that both the small and the large group included seven experimental animals each. Subsequently, cognitive bias and preference tests were conducted at -1, 1, 3,7,10 and 13 days after splitting the group. The timeline of the experiment is summarized in Figure 4-2.

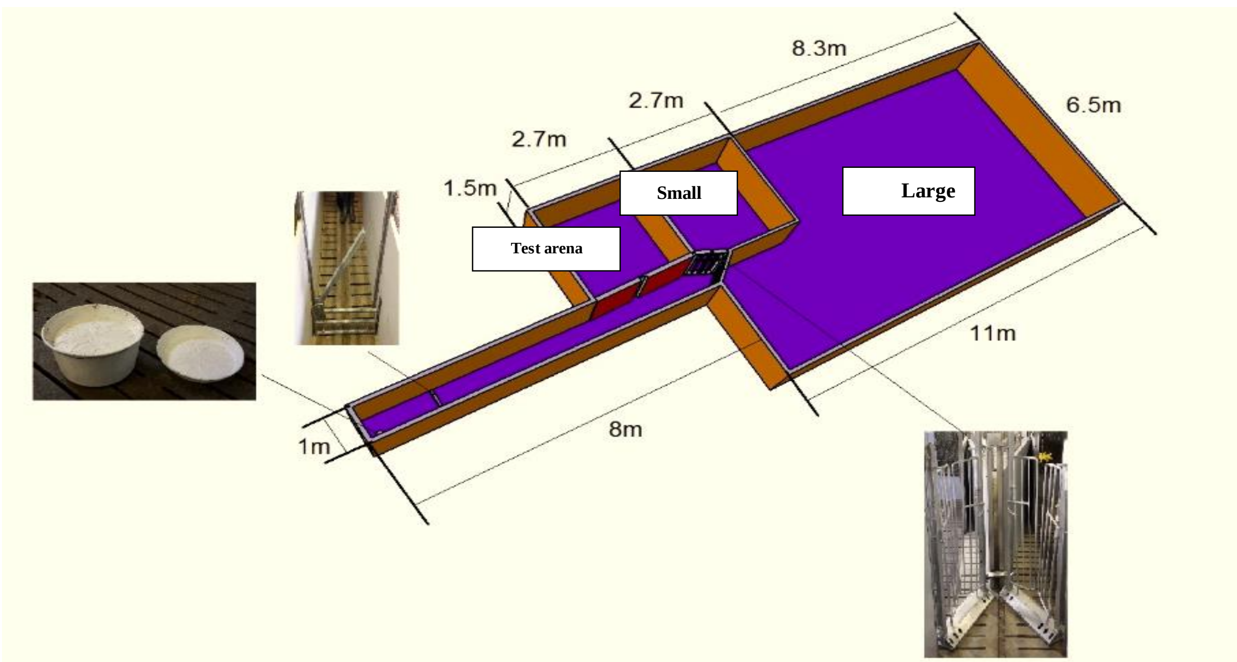

Figure 4-1. Design of experimental apparatus used for both the small and large group, test arena, bucket, obstacle and sheep door.

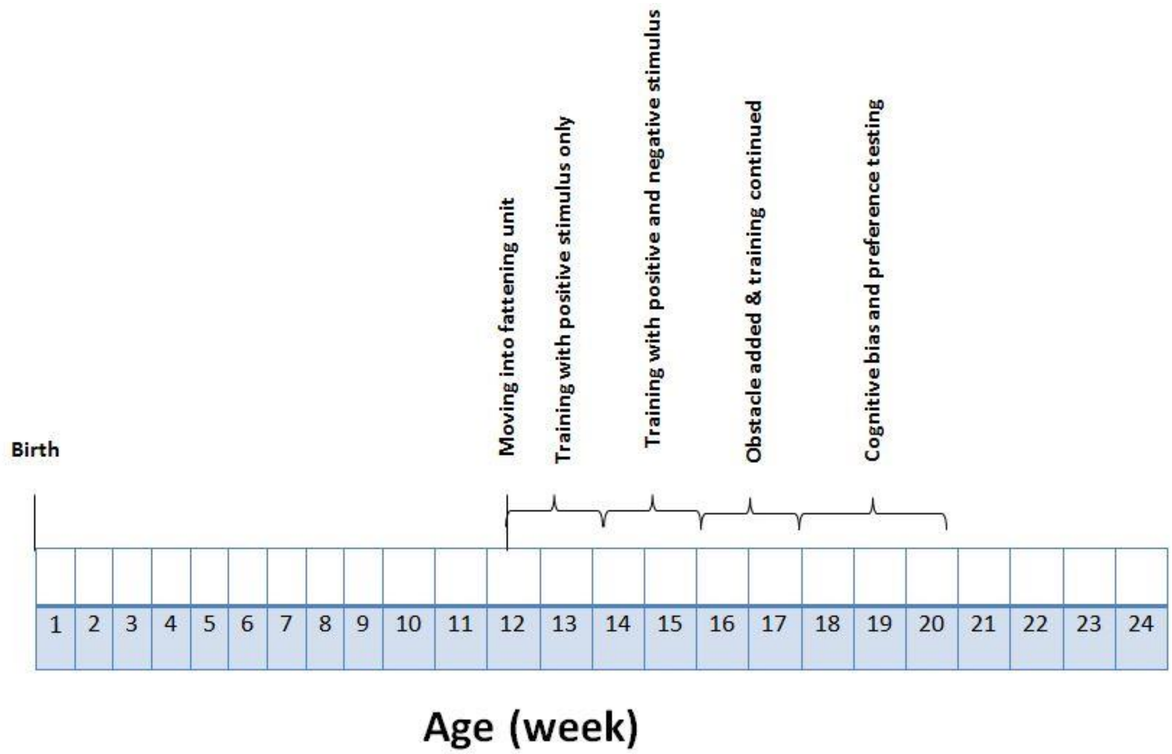

Figure 4-2. Experimental timeline.

4-3-2- Behaviour tests (cognitive bias and preference test)

Cognitive bias training and testing

For the cognitive bias test, plastic buckets (17 cm high and 29 cm in diameter) coloured either black or white were used as positive and negative stimuli, respectively. The pigs were first classically conditioned to associate the positive visual cue (black bucket) with pieces of apple delivered in the bucket covered by a thin cap which pigs could easily open with the nose. During training, pigs were exposed to the test arena once per day to allow them to explore the arena and habituate to it. In order to avoid that the stress of social isolation reduced the pigs' motivation to work for food (Pedersen et al., 2010), individual training was gradual. Each animal received one session per day starting on average with four trials per session which was then gradually increased to 12 trials per session, comprising equal numbers of positive and negative cues in a pseudorandom sequence (the trial type was determined by flipping a coin until 6 trials of either one of the two trial types had been given). When starting a trial, the pig was held behind a pig board from where it was released into the corridor leading to the arena where it could approach the buckets and eat the pieces of apple. Each pig was allotted a maximum of 30 s per trial to approach the bucket before being returned to the holding area for starting the next trial. A correct response was defined as approaching the hatch (i.e. a "go" response) within 30 s following a positive cue and not approaching the hatch (i.e. a "no-go" response) within 30 s following a negative cue. This phase of training continued until all experimental animals successfully completed at least 10 out of the 12 trials presented on a given training day (= learning criterion: > 80% correct trials). During this training period, the holding time in the start box was also gradually increased up to 30 s to allow the experimenter to replace the rewards and arrange for the next trial. The next phase in training involved introducing the negative cue, an otherwise equal, but white-coloured bucket that did not contain any food rewards. The pre-cue procedure was the same as for the positive cue.

In the following training phase, an obstacle was introduced, which was located 1.5m in front of the bucket (Figure 4-1). This was done to increase the effort to reach the buckets, so that costs in terms of energy expenditure would outweigh potential benefits for the pigs to open the non-rewarded bucket out of curiosity or as play behaviour.

For cognitive bias test, pigs were individually moved from the housing pen to an 8.7m^2 (3.25 length ×2.7 m width) test arena. After bringing each pig to test arena, pigs were released to a corridor (8 length ×1 width m) to record the latency to approach the stimuli. The cognitive bias tests were identical to the discrimination training with the exception that instead of twelve trials including six positive and six negative cues, we conducted 15 trials including five positive, five negative and five ambiguous cues in pseudo-random order (maximum of two consecutive trials of the same type). The order was determined by rolling a 6-sided die with two faces each allocated to represent one of the three visual cues. If more than two trials of one cue type in a row had been elected, or if five trials of a given trial type had already been performed, the die was rolled again until one of the remaining trial types turned up. Once five trials each of two cue types had been performed the remaining trials were all of the remaining incomplete cue type.

During the test, the positive and negative cues were reinforced and not reinforced, respectively, as in the discrimination training. Trials in which the ambiguous cue was presented, were never reinforced and were terminated either when the pig approached the hatch or after 30 s, which ever occurred first.

Directly before each morning or afternoon test session, the previously learned discrimination was reinforced. Each pig was presented with the positive cue and simultaneously rewarded with apple. It was then presented with the negative cue, i.e. the empty white bucket. These "reminders" were not counted in the subsequent 15 trials of the cognitive bias test. Pigs received a total of five cognitive bias tests spread over the course of the experiment. Also, the cognitive bias test was started and finished at same time for all test days (around 8:00 AM to 15:00 PM)

During the cognitive bias test, the following variables were assessed per trial: latency between entering the arena with the forelegs and opening the bucket's cap; time needed to finish the trial (i.e. either opening buckets cap or the maximum time of 30 s); number of correct and incorrect reactions.

Preference test

Each time, when pigs returned from the test arena to the pen, they were given the choice between entering the small group to the left or the large group to the right. For this purpose, one-way gates were designed and placed as the main door for entering the pens for the small and the large group (Figure 4-1). After pigs made a choice, regardless of which group they originated from, they remained for two hours in the chosen group to ensure they would associate the consequence of staying in the chosen group with the choice they made.

4-3-3- Statistical analyses

All statistical analyses were conducted using the statistical analysis software (SAS 9.3). Latency for cognitive bias test data was analysed using mixed model (PROC MIXED). The animal effect was considered as a random effect, and group size (large or small group) and cue type (positive, negative, ambiguous) and their interaction was included as a fixed effect. Additionally, the average latencies for positive (black) and negative (white) cues were considered as 0 and 100%, respectively and accordingly, the relative latencies for the ambiguous cue (grey) was calculated. Frequency procedure (PROC FREQ) was used to analyse group preference test data. The statistical significance level of 0.05 ($P < 0.05$) was used to compare differences between groups. Data are presented in the text as LSMeans ± standard error (SE).

4-4- Results

4-4-1- Cognitive bias training

It took ten days training (60 trials) for all pigs to reach learning criterion to approach to the positive cue (white bucket) for obtaining the reward. It took a further ten days of training (i.e. 120

trials) for all the pigs to reach criterion on the go/no-go task for discriminating between the positive and negative cue. Also, it took another ten days to introduce and train all pigs with the obstacle. Thus, all 14 pigs acquired a significant discrimination of the positive and negative cues. In the last four days of discrimination training, there was no significant difference in the proportion of correct responses between small and large groups.

4-4-2- Cognitive bias

On all test-days, pigs in both groups took significantly longer to approach the negative cue compared to the positive cue ($p<0.05$, Figure 4-3), and pigs of the large versus small group differed neither in their responses to the negative nor the positive cue (both $P>0.1$; Figure 4-3).

Pigs in the large group showed no significant difference in latency time to approach the black and grey buckets, representing the optimistic and ambiguous choice respectively, in the test before dividing the groups; however, latency times for all tests conducted after the separation differed significantly from each other ($P < 0.05$, Fig.4-3a).

For pigs in the small group, the difference in latency was significant for all tests after division of pigs into the small and large group ($P < 0.05$, Fig.4-3b).

A

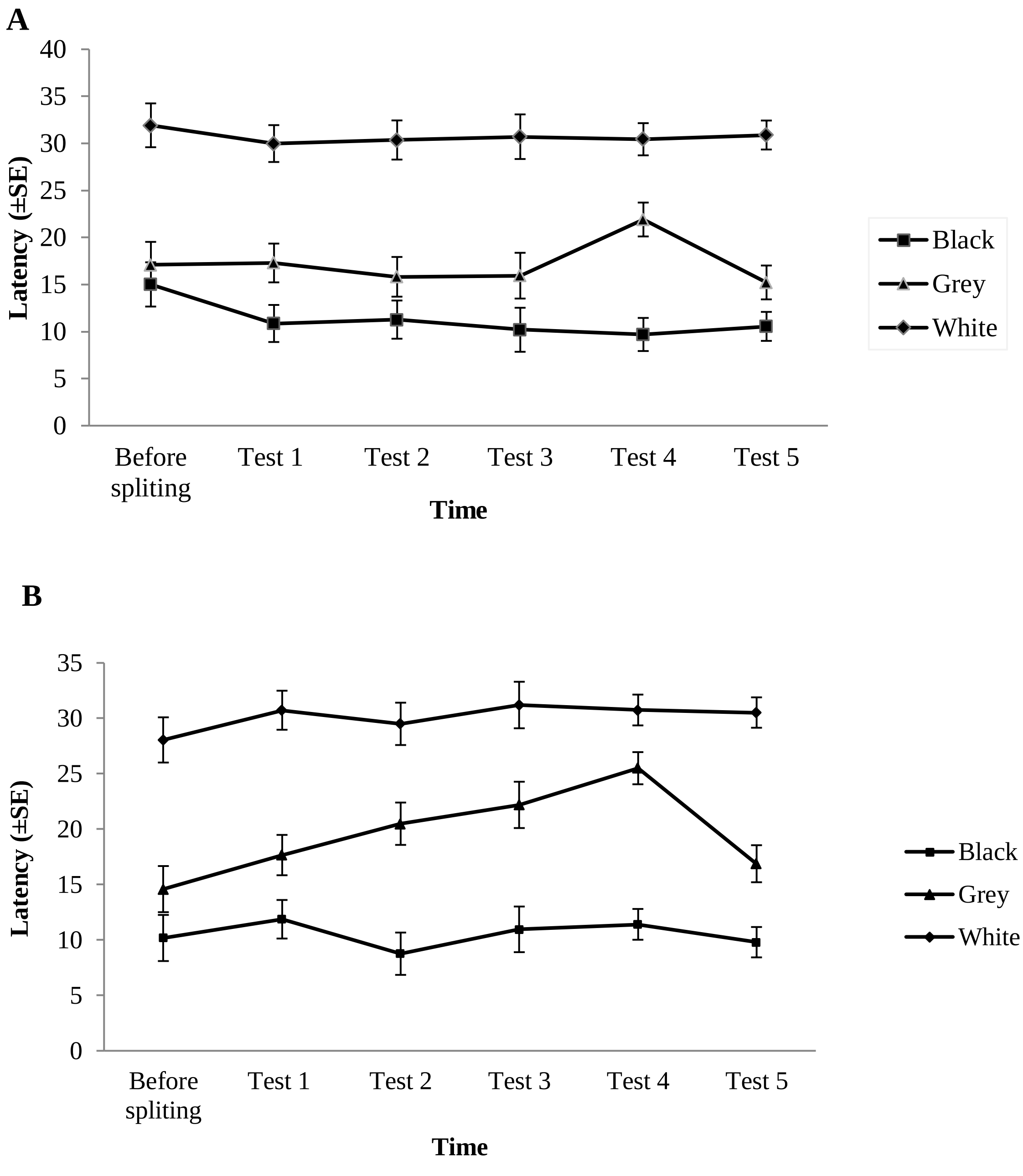

Figure 4-3. Average latency time (± standard error) to approach the positive cue (black bucket), negative cue (white bucket) and ambiguous cue (grey bucket) shown by pigs kept in a large group (80 pigs, A) and in a small group (11 pigs, B). Time refers to testing of the pigs at -1, 1, 3,7,10 and 13 days in relation to splitting.

On the test-days before and immediately after splitting pigs into the small and large group, there were no significant differences (P>0.1) in latency to approach the bucket with the ambiguous cue (Figure 4-4), indicating no difference in the emotional state of pigs kept in the large versus small group at the initial phase of the experiment. However, latency time to approach the ambiguous cue differed significantly between the two groups at 3 and 7 days (test 2 and 3, both P < 0.05) and 10 days (test 4, P<0.1) after splitting, with pigs of the small group showing greater latencies to approach the ambiguous cue (Fig.4-4).

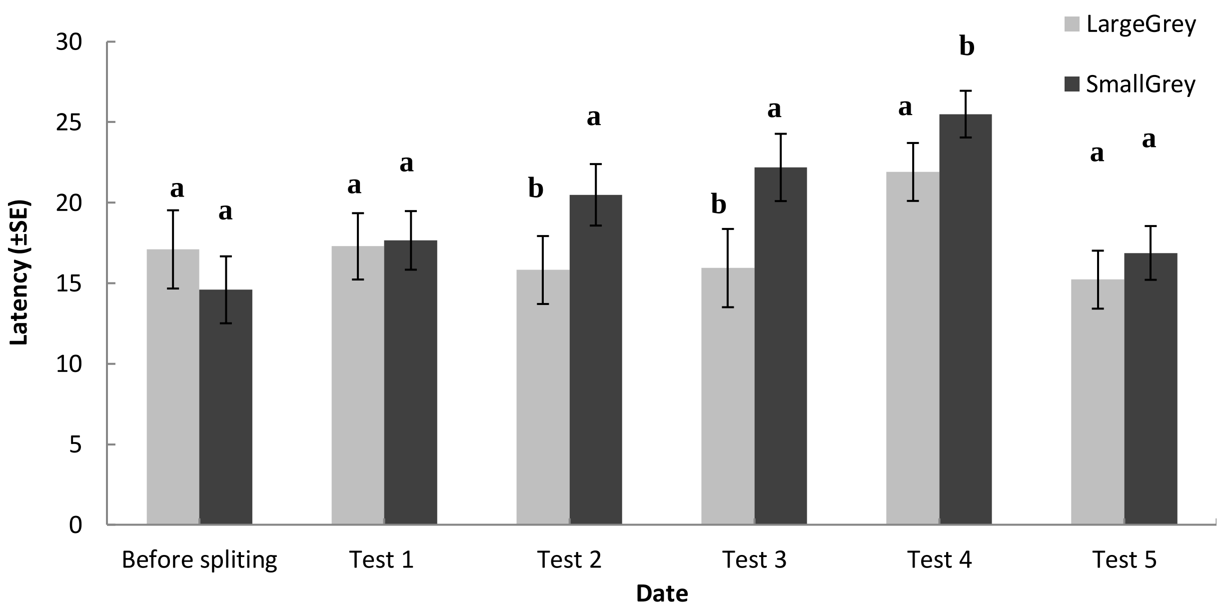

Figure 4-4. Average latency time (± standard error) to approach the ambiguous cue (grey bucket) shown by pigs kept in a large group (80 pigs, light grey bars) and in a small group (11 pigs, dark grey bars). Date refers to testing of the pigs at -1, 1, 3,7,10 and 13 days in relation to splitting. Different letters (a,b) indicate statistically significant differences at P < 0.05.

The relative latencies for the ambiguous cue as a proportion of differences between black and white cues were calculated (data for relative latency time are not presented). Pigs in small group spent more time than those assigned in the large group to reach the grey buckets, except in test 1. In tests 2 and 3, as a value of 56% was calculated for small group, the spent time was quite higher than that for the large group and was near to the time that spent for negative stimuli (white bucket). But in the mentioned testing time, the relative latencies of 24% and 28% were obtained for the large group which imply that spent time for grey buckets were near to time spent to reach the black buckets. In Test 4, higher relative latencies (>70%) was obtained for small groups which indicate that the spent time for grey buckets was similar to white buckets rather than to black one. In Test 5 as relative latencies were low (<40%) for both groups, the spent time for grey buckets were near to black buckets.

4-4-3- Preference test

Neither at the individual nor group level, pigs of the small or large group showed a significant preference for returning to one of the groups (Table 4-1). However, casual observations suggested that pigs did not actively choose one of the groups, but instead they were very reluctant to reenter the group pens and rather attempted to return to the testing arena.

Table 4-1. Proportions of pig choices in preference test for different group size[1] (large or small)

	Small group	**Large Group**
Choosing small group	52.76[a]	42.11[a]
Choosing large group	47.24[a]	57.89[a]

[1]Proportions without common letters are statistically different ($P < 0.05$).

Numbers in Table 4-1 compare the proportions of pig choices in small and large groups in group preference test. For both groups, there was no significant difference in the proportion of pig priorities for being in small or large groups.

4-5- **Discussion**

4-5-1- Cognitive bias

The lack of significant differences between pigs of the small and large group in latencies to approach the positive cue, the negative cue and the ambiguous cue before splitting the group (i.e. the control situation) shows that there were neither any inherent differences in motivation and ability to obtain the reward nor in emotional state before group-splitting between experimental animals that were later housed in the small versus large group. Therefore, it can be assumed that the observed change in judgement bias of pigs kept in the smaller group towards a more pessimistic state was caused by the change in social and/or housing environment.

Pigs are highly social animals inclined to live in stable groups consisting of sows and their piglets or young boars (Ellegaard et al., 2010) and will work to improve access to each other (Matthews and Ladewig, 1994). Regrouping of pigs causes stress to the animals and provokes dominance fights, which normally last for 24-48 hours after introduction (Stolba and Wood-Gush, 1989; Ellegaard et al., 2010) until a new hierarchy is established. Although pigs in our experiment all came from the same social group, division into a smaller group could have provoked fights about leadership as the originally dominant individuals might have been lost. As changes in judgement bias were especially prone at 3, 7 and 10 days after splitting the groups, i.e. hierarchy should have already been established at this point, the observed changes were probably not a direct result of stress caused by fighting but more an indirect effect of the underlying social tension in the group and the stress caused by disruption of formerly established social bonds with animals remaining in the larger group. This is in line with the fact that regrouping has been shown to cause reductions in performance of pigs, even after the animals were re-united with their familiar counterparts (Stookey and Gonyou, 1994). In 8-week-old pigs regrouping did not have any long-term effect on the pigs' performance, indicating that regrouping is a short-term stressor which the pigs can overcome if given adequate time (Heetkamp et al., 1995), however, the minimum required time for disappearance of these effects has not been determined. Thus, stress caused by social instability could be one possible explanation for the negative judgement bias shown by pigs separated into the smaller group.

Although it is possible that there were pessimistic and optimistic animals both in large and small groups, our results show that initially the pigs of the two groups did not differ in cognitive bias. With division of the pigs into the smaller and the large group, not only the social environment of the pigs changed, but also their housing environment. Pigs were moved from the larger pen of 71.5m² into a smaller pen of only 8.8 m². Thus, the available floor space per pig remained equal, but the pigs in the smaller group had a reduced total space available to them for movement and exploration. Pigs are highly intelligent and inquisitive animals, spending a big percentage of their time budget in the wild with rooting and exploratory behaviour (Graves, 1984). Limiting the animals to a smaller area, and reduced social partners, can cause and/or increase boredom and frustration in the pigs, leading to the more negative judgement bias observed.

Interestingly, changes in judgement bias of pigs kept in the small group were found at 3, 7 and 10 days, but no significant difference between the two groups existed anymore at 13 days after splitting the groups. This could indicate that the pigs of the smaller group habituated to their new environment and that the stressors causing the negative judgement bias of pigs were only of short-term nature. On the other hand, this could also reflect an anomaly in results, which cannot be satisfactorily determined without having data of judgment bias for the period after 13 days after splitting of groups.

In comparison to results from absolute latency time to approach ambiguous stimuli in different groups, almost similar results were observed using relative latency time for all tests, except before splitting. For ambiguous stimuli in the test before splitting, higher and significant relative latency value was observed in the small group. In contrast, higher but insignificant absolute latency time was obtained in the large group. This result is associated with the relatively higher absolute latency time value for black in the large group where the difference between absolute time for black and grey was less than corresponding difference in the small group. However, relative values for latency in test 1, 2, 3 and 5 showed the similar pattern like absolute latency time.

In current experiment due to technical problems and time constraints in the experimental farm, it was not possible to work for more than 8 hours per day; therefore we used an equal number of pigs in small and large group (7 pigs in small group; 7 pigs in large group) for training and cognitive bias tests in order to manage training session and cognitive bias tests in one day. However in current experiment the numbers of cognitive bias tests were more than similar experiments in pigs (this experiment=6 tests; Düpjan et al., 2013=5 tests; Douglas et al., 2012= 5 tests).

Since the untrained ambiguous visual cue was presented in the same location as the positive and negative cues and inserted within a sequence of reinforced positive and negative cues, it was hypothesized that it would be ambiguous to the pigs in this context, rather than merely novel, and that it could, therefore, be used to assess their judgment biases. In contrast to a conventional novel object test, the animals had a learned expectation that the ambiguous object, presented in the specific context, was associated with either reward or punishment (Rowe and Healy, 2014).

4-5-2- Preference test

Pigs tested in this study showed no significant preferences for returning to the small or the larger group after testing, but rather tended to choose the group that they were reared in, irrespective of pigs coming from the small or the large group. However, pigs were generally very reluctant to re-enter the group pens, but instead seemed to strongly prefer to return to the test arena, which they associated with a possible food reward. This indicates that results of the preference test in this study does not reflect an active choice of group size made by the pigs and can thus not be used to draw any conclusions about pigs' preferences. Also, there were some problems during group preference test, for example other pigs of the group blocked the sheep doors from inside of housing pen and it was needed to be driven away by helper, these problems also might have influenced choices by pigs during group preference tests.

Hughes, 1997, in similar study with two-choice tests in laying hens, indicated that in practice, it was obvious that an empty cage or cage with less members were the most attractive and became progressively less attractive as the number of birds in cages increased. Also, Hughes, 1997 indicated that the differences in group preference over the time (comparing results for day 1 with those of a later day) were not great, but were consistent between groups. Dawkins, 1982 evaluated the preferred group size in domestic hens and concluded that hens have a preference for being on their own and are decreasingly attracted to larger groups of their cage-mates. To the best of our knowledge there is no study with group preference test in fattening pigs and results of studies from other farm animal, even in same experimental design, cannot be expanded to behavior of fattening pigs. Pigs exhibit novel-induced anxiety when submitted to a novel area (Janczak et al., 2003). When pigs introduced into new groups, they have to cope with social challenges, which may cause physiological reactions and injuries (Meese and Ewbank, 1972). Repeated relocation with mixing unfamiliar pigs results in agonistic behavior and hormonal changes and causes a decrease in daily weight gain (Coutellier et al., 2007). These agonistic behaviors can be attributed to the absence of a social hierarchy among unfamiliar pigs and to the uncertainty about fighting ability of the unfamiliar pen mate (Puppe, 1998). In our experiment we did not determine that preferences were stable or varied over time. Also in this experiment, after pigs made a choice, regardless of which group they originated from, they remained for two hours in the chosen group to ensure they would associate the consequence of staying in the chosen group with the choice they made.Therefore, maybe because of consequences of staying in the chosen group, either pigs from small groups chose large groups or pigs from large group chose small groups, caused some agonistic behaviors and forced pigs to choose the group that they were reared in, regardless of pigs coming from the small or the large group.

4-6- **Conclusion**

Our study revealed that pigs kept in a small group of 11 animals per pen showed a more negative judgement bias in comparison to pigs housed in a group of 80 pigs per pen, suggesting that the welfare of pigs kept in the small group was negatively affected for at least 10 days after separation of the large group. This might either have been a result of more short-term stressors linked to the change in social and housing environment, but could also reflect a generally impaired welfare of pigs kept in small groups due to reduced total space available to the pigs. Future research assessing the long-term changes in cognitive bias of pigs housed in different group sizes is necessary to allow a more complete understanding how pigs' welfare is influenced by group size.

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5th Chapter

General Discussion

5-1- Preface and Overview

This thesis studies the relevance of and preference for different group sizes, and associated husbandry management to performance, health and welfare in monogastric animals with focus in chicken and pigs. These factors were reviewed and studied in **chapters 2**, **3** and **4**. In this chapter first an overview of the results from **chapters 2**, **3** and **4** is provided, afterwards the challenges in welfare assessment methods and possible solution are discussed. We studied a variety of factors and parameters that affect the performance, welfare, behavior and cognition in poultry and pigs.

In chapter 2 we reviewed the scientific literature pertaining to the effects of group sizing on behavior, welfare, and productivity of poultry including chicken, quail and turkey.

In chapter 3 we assessed Performance parameters [average daily feed intake (FI), average daily weight gain (ADG), body weight (BW) gain and feed conversion ratio (FCR)] as well as mortality and health and welfare parameters [gait score, hock burn, tibial dyschondroplasia (TD), foot pad dermatitis, plumage cleanliness] of broiler chickens housed in different group sizes (100, 300, 1000 and 5000 birds per group).

In chapter 4 we investigated the emotional state of fattening pigs when housed in a small vs very large group by cognitive bias and preference tests.

5-2- Effects of group sizing on behavior, welfare, and productivity of poultry

We explained that because of large economic and husbandry effect of reducing group size in poultry farms, it is very important to determine the relationship between group size and welfare as precisely as possible so that decisions can be taken on what group size is acceptable from an animal welfare point of view.

Studies which examine the effects of group size involve some degree of confounding with density, and enclosure size (Christman and Leone, 2007). Also, different reported results might be because of differences in study design (under controlled circumstances vs. on-farm, varying group size by changing density or pen size, and so on) and others by the use of different indicators of welfare. Some researchers have shown that decreasing group size can increase distress in chickens. For example Marx et al. (2001) showed that the number of distress call (characterized by declining frequency with high energy) increased, as group size decreased. Marx et al. (2001) concluded that vocalization, as an indicator for mental states in animals, is strongly dependent on social contacts in the chick and that different degrees of social deficits find their expression in discrete changes in the pattern and elements of vocalization.

Some other researchers tried to find the relationship of behavioral parameters with different husbandry practice or strategies. Newberry and Hall (1990) conducted an experiment to investigate the effects of age and pen size on the use of pen space by male broiler chickens. Results of their experiment indicted that distance moved per hour declined with age in both pen sizes. Although movements were non-random, the chickens did not restrict their movements to small areas in which they could become acquainted with their neighbors over the period from 4 to 9 weeks of age. These researchers concluded that mixing among flock members possibly increase the uniformity among birds at different locations within pens and may affect the incidence of certain diseases and abnormalities, such as cannibalism or behavioral disorders, within a pen. Beauchamp (2001) compared some models to examine how changes in searching and exploring behaviors with group size could alter the shape of the function relating vigilance to group size. Results of this study indicate that increased behavioral effects that are dependent on group size, such as scrounging, may lead to changes in the shape of the vigilance function and may mitigate against any gain garnered from a reduction in individual levels of vigilance in larger groups. This researcher concluded that as displacement and kleptoparasitic attacks may be more frequent in larger groups, the contribution of these activities could, like scrounging behavior, alter the shape of the function relating vigilance to group size.

5-3- **Aggressive behaviors in pigs and poultry**

The direction in which the environment affects aggression, however, appears to differ both between and within species (Van Loo et al., 2002; van de Weerd and Day, 2009). The same holds for pigs, where enriched pens may lead to less aggression(Lyons et al., 1995), no difference in aggression (Scott et al., 2006), or more aggression (Spoolder et al., 2000) as compared to barren pens of equal size. In the current study, pigs in straw-enriched pens had more skin lesions under stable social conditions, but showed less nonreciprocal biting than pigs in barren pens. Though the number of skin lesions may be underestimated due to skin dirtiness(Guy et al., 2002), as pigs in the barren pens had a more dirty skin, lesions were clearly visible and were scored when the observer was in close proximity to the animal.

On the other hand, it is believed that environmental enrichment can satisfy the behavioral needs of pigs to explore and forage and to help the animals to adapt to their environment (Ferguson, 2014). It has been shown that behaviors of pigs can be different in barren or enriched environments, reflected in different time budgets. Some studies have revealed that pigs housed in barren environments were less active, less explorative and showed less play behavior (Beattie et al., 2000; Bolhuis et al., 2005). Grimberg-Henrici et al. (2016) conducted a study to investigate the cognitive performance of pigs that were housed in a barren or an enriched environment from weaning. Their study provided some evidence that pigs reared in an enriched environment after weaning show a better cognitive performance in a spatial holeboard task compared to pigs reared in a barren environment. Enriched-housed pigs showed a better reference memory performance during the acquisition phase, and a marginally better general working memory performance during the reversal phase.

While group size is increasing, establishing dominance relationships with all group members takes too much time and effort and the social organization of domesticated poultry changes from a dominance hierarchy established through aggression to a tolerant social system characterized by low aggression (Estevez et al., 1997).

Some researcher hypothesized that a dominance hierarchy will only be established when the chances of reencountering the same bird are relatively high, which will be more likely in small than large social groups ,(Pagel and Dawkins, 1997; Nicol et al., 1999). These hypotheses were supported by several studies which reported exhibition of relatively little aggression in large groups of domestic fowl (Hughes et al., 1997; Carmichael et al., 1999; Estevez et al., 2002).

However, formation and patterns of aggressive behaviors during ontogeny have not been studied systematically over a different range of group sizes. Estevez et al. (2003) conducted an experiment to answer these questions: Do domestic fowl attempt to establish dominance and then give up when reared in large groups? Or do they form a low-aggression tolerant social system directly? These researchers investigated the effects of group size on the ontogeny of aggressive behavior in flocks of 15, 30, 60 and 120 female chickens while controlling for stocking density. They hypothesized that, because the establishment of dominance in large groups is too costly, birds in large groups adopt a low-aggression (tolerant) social strategy. Results of their experiment showed that frequency of pecks and threats received per focal bird showed the opposite pattern, being higher in larger than smaller groups. They concluded that it is possible that, whereas the majority of birds in the larger groups adopted a low-cost, tolerant (low-aggression) social strategy, birds with good fighting ability were still able to obtain a net benefit by being aggressive and, hence, their threshold to give up an aggressive strategy was higher.

5-4- **Group size and leg disorders in Broilers**

Broiler chickens are the most frequent farmed species. During the last four decades they have been subjected to increasingly intensive genetic selection for performance and production parameters including growth rate and food conversion efficiency. The consequences of this genetic alteration was chickens with adult body size but still very immature in many aspects of physiological development which can be reflected in altered behavior (Weeks et al., 2000). Leg disorders are among the most severe welfare problems in commercial broiler production (Farm Animal Welfare council, 1992; Kestin et al., 1992). There are several studies which reported effects of broiler behaviors on welfare indicators and lameness (Preston et al., 1983; Newberry et al., 1988; Weeks et al., 1994) but to our knowledge there is no study where group size has been varied in the present range and stocking density was kept constant. Stocking density and group size are often confounded in broiler welfare experiments in a way that higher stocking density is achieved through increasing group size.

5-5- **Cognitive bias and group preference in pigs**

Results of our experiment showed that after splitting pigs into small and big groups, pigs in large group had more optimistic statement rather than pigs in small group when making decisions under ambiguity and risk, particularly 2 and 3 days after splitting. Also According to the result of our experiment for group preference test, there was no significant difference for selecting small and big group by pigs reared in both groups.

Since first cognitive bias test in rats (Harding et al.2004), a large number of studies have been conducted in different species. Several studies in pigs have been published using designs based of Harding et al. (2004) (Douglas et al., 2012; Scollo et al., 2014; Brajon et al., 2015; Murphy et al., 2015) but most of these studied have used modified methods with several differences in order to improve the assessment of the judgment bias in pigs. The type of stimuli, type of punishment and reward, numbers of the training and test session have noticeable impacts on cognition assessment which differ between published studies in pigs. The main aim of most cognitive bias studies is to find and assess differences of affective state using different treatments, such as environmental enrichment or housing conditions (Bateson and Matheson, 2007; Löckener et al., 2016; Douglas et al., 2012).

A judgment of ambiguous stimuli is outcome of several interdependent processes including attention and recognition of stimulus, evaluation of stimulus, decision-making and selection of e response. These processes are implemented by multiple inter-connected and parallel-processing circuits distributed throughout the brain (Mendl et al., 2009). Affective state like anxiety or depression can lead to a biased evaluation of stimulus (Dunlop and Nemeroff, 2007), thus providing a potential link between reward and affective state. Most studies on cognitive bias have used the judgment bias task to compare two or more groups of animal subjected to different treatments, such as different housing systems or husbandry conditions (Douglas et al., 2012; Düpjan et al., 2013; Papciak et al., 2013). For instance, Düpjan et al.(2013) conducted a cognitive bias test with spatial judgment paradigm, and revealed that there were no difference in judgment bias between pigs kept isolated and pigs kept in group.

Recently common neurophysiological mechanisms underlying cognitive processes were investigated by several researchers. For example Stracke et al. (2017) investigated the effects of central serotonin depletion on affective states and cognitive processing in pigs. In short, these researchers conducted a spatial judgment task, providing information about the effects of the affective state on cognitive processing, and the open field/novel object test, which measures behavioral reactions to novelty that are assumed to reflect affective state. These researchers concluded that serotonergic system is a key player in cognitive-emotional processing and the serotonin depletion model and the spatial judgment task can increase understanding of the basic mechanisms underlying both human neuropsychiatric disorders and animal welfare. Cortisol is used to assess if the treatment has an effect on physiological parameters and if this effect is similar on results of the cognitive bias test. Scollo et al. (2014) also failed to find differences in the latency to reach the bucket between groups of pigs raised at different densities.

It is now generally accepted that different animals may have different types of cognitive processes according to their different physiological and ecological conditions. The rearing conditions of industrial animals are often has a distinctive difference from the way of life of their wild progenitors. In order to understand the behavior of a domesticated species, it can therefore be necessary to understand the behavior of the wild ancestor. The holeboard task is a valid measurement instrument for spatial discrimination learning in pigs. However, it is unclear to what extent the holeboard testing procedure itself could have provided enrichment that could (partially) have counteracted the effects of living in a barren environment and may lead to underestimation of the effects of a barren environment. Therefore, it may be difficult to test effects of different environments on cognitive performance in pigs using longer-lasting, appetitively motivated complex testing procedures (Grimberg-Henrici et al., 2016).

Furthermore, some researchers have shown that there are intraspecific correlations between group size and memorial and problem-solving abilities in animals; and they explained this phenomenon by more adept at solving new or ambiguous problems for individuals in larger groups than those living in smaller groups (Croney and Newberry, 2007; Mirville et al., 2016). These can be explained as follows: united awareness among individuals make able members of group to spent more time in problem-solving and social challenges activities, the ‘pool of competence’ hypothesis which suggests that groups with more members have more individuals and consequently have a more chance of having individuals competent at solving novel problems (Aplin et al., 2013), and finally, animals living in groups with larger sizes may show better individual problem-solving abilities because of their inherent cognitive abilities obtained from monitoring and/or maintaining more social interactions and relationships (Cronin et al., 2014). These explanations define the potential correlation between sociality and individual problem-solving ability and shows how solving more multipart social challenges may rise the cognitive domain of an individual to solve non-social challenges as well.

5-6- **Effective factors in behavior assessment of animals**

It has been suggested that many factors including territory quality, unit size, intruder pressure, risk of predation, and sensitivity of territories to resource depletion and cost of parental care can affect the fitness of an individual in a group (Brown, 1982). All of these parameters, as well as sex and social status, must be viewed in the perspective of time-energy budgets. Each individual in a population of specified density and distribution of territory qualities is perceived as having a threshold of sociality. Optimal group size is expressed as a compromise between advantages gained by sharing costs and disadvantages arising from resource depletion. Brown (1982) suggested that the use of time-energy budgets to study group territoriality provides new insights and approaches to the study of helping behavior. Enkel et al. (2010) showed that both a pharmacological challenge mimicking acute stress-like conditions and genetic risk factors effect on ambiguous cue interpretation in rats, inducing distinct forms of response biases. These researchers described that described model in their study is suitable to analyze the neuronal mechanisms of decision making under ambiguity and offers the opportunity to analyze the basis of a cognitive bias with respect to changes in positive and/or negative responsiveness.

Furthermore, indirect genetic effect (IGE), known as social genetic effect, is another effective or competitive variable which is a heritable effect of an individual on the trait values of its social partners or group mates (Wolf et al., 1998; Muir, 2005). Camerlink et al. (2013) assessed the potential of a new breeding method using information on indirect genetic effects, to reduce aggression in pigs. They showed that the two distinct indirect genetic effect growth groups did not differ in number of skin lesions, or in amount of reciprocal fighting, both under stable social conditions and in confrontation with unfamiliar pigs in a 24 h regrouping test. These researchers also demonstrated that pigs selected for a positive effect on the growth of their group members, performed less non-reciprocal biting and showed considerably less aggression at reunion with familiar group members after they had been separated during a 24 h regrouping test. The enriched environment was associated with more skin lesions but less non-reciprocal biting under stable social conditions. Changes in aggression between pigs selected for indirect genetic effects on growth were not influenced by G×E interactions with regard to the level of environmental enrichment. It is likely that selection on indirect genetic effects on growth targets a behavioral strategy, rather than a single behavioral trait such as aggressiveness. Aggression may be one facet of the possible ways in which group housed animals may influence each other's growth. If indirect genetic effects on growth are included in the breeding criteria it would be important to consider the possible changes in behavior over generations.

Many researchers measured animals' behaviors in social isolation either in order to avoid confounding between social factors and the resource in question, or because of more convenient data collection (Sherwin and Nicol, 1998). However, Matthews and Ladewig (1994), using operant conditioning techniques, showed that social contact may in itself be a behavioral need in pigs. Several studies have shown that individual housing compared to group housing can change the eating behaviour and feed consumption in pigs (Nielsen et al., 1996; Bornett et al., 2000). Furthermore, Nielsen et al. (1996) described that pigs housed in isolation spend more time rooting than pigs housed in groups and their pattern of eating is also affected. Pedersen et al. (2002) investigated if demand curves generated using operant conditioning techniques were affected by testing the pigs in isolation. The results showed that the effect of testing in isolation may vary depending on the resource in question. Thus, the priority of different behaviors may be affected by the social context in which the animals are tested. Since, pigs are social animals and since they are also housed in groups under commercial production conditions, it would not be meaningful to assess their behavioral priorities in isolation. It would not reflect their behavioral priorities under the conditions in which the animals are housed. Even though being tested with a companion pig in an adjoin pen is not the same as being tested in a group situation, it may, however, resemble the natural situation more closely than being tested in isolation.

Conclusion

Based on the result of this thesis, it can be concluded that the group size, beside the density and space availability, is a parameter that affects welfare of animals in intensive farming. Also future research in welfare and behaviors in poultry and pig should focus on the effect of group size on more specific responses and separate the effect of group size from other correlated factors. We also suggest that the assumption of increasing group sizes leading to decreasing harmful social interaction needs to be reassessed, especially for new genetic commercial lines.

5-7- **References**

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