อิทธิพลของความหนาแน่น และขนาดของกลุ่ม ต่อพฤติกรรม, ความเครียด และสวัสดิภาพ ของไก่ลูกผสมพันธุ์พื้นเมือง

นางสาวซิน ฮั่ว

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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EFFECTS OF STOCKING DENSITY AND GROUP SIZE ON BEHAVIOR, STRESS AND WELFARE OF THAI CROSSBRED CHICKENS

Xin Huo

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Animal Production Technology

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EFFECTS OF STOCKING DENSITY AND GROUP SIZE ON BEHAVIOR, STRESS AND WELFARE OF THAI CROSSBRED CHICKENS

Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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ซิน ฮั่ว : อิทธิพลของความหนาแน่น และขนาดของกลุ่ม ต่อพฤติกรรม, ความเครียด และสวัสดิภาพ ของไก่ลูกผสมพันธุ์พื้นเมือง (EFFECTS OF STOCKING DENSITY AND GROUP SIZE ON BEHAVIOR, STRESS AND WELFARE OF THAI CROSSBRED CHICKENS) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.พงษ์ชาญ ณ ลำปาง, 91 หน้า

งานวิจัยนี้มีวัตถุประสงค์ เพื่อศึกษาอิทธิพลของความหนาแน่น (8, 12 และ 16 ตัว/ตารางเมตร) และขนาดของกลุ่ม (50 และ 100 ตัว) ต่อผลผลิต พฤติกรรมทั่วไป พฤติกรรมการจิกขน พฤติกรรมก้าวร้าว ตัวชี้วัดสวัสดิภาพ และการกระจายเชิงพื้นที่ ของไก่ลูกผสมพันธุ์พื้นเมือง (จำนวนทั้งหมด 1,350 ตัว) ทำการเก็บบันทึกพารามิเตอร์การให้ผลผลิต พฤติกรรม และการกระจายเชิงพื้นที่สัปดาห์ละครั้ง ตัวชี้วัดสวัสดิภาพวัดเมื่ออายุ 12-13 สัปดาห์ ข้อมูลที่ได้ถูกนำมาวิเคราะห์ความแปรปรวนด้วยแผนการทดลองแบบ 2x3 factorial in CRD โดยทำ 3 ซ้ำต่อทรีตเมนต์

ผลการศึกษาพบแสดงว่า ความหนาแน่น และขนาดของกลุ่มไม่มีผลต่อ น้ำหนักตัว น้ำหนักตัวที่เพิ่มขึ้น และ อัตราการตายของไก่ ในช่วงอายุ 2-12 สัปดาห์ การกินได้ของไก่ในกลุ่ม 50 ตัวที่ความหนาแน่น 16 ตัว/ตารางเมตร สูงกว่ากลุ่ม 100 ตัวที่ความหนาแน่นอื่นๆ ($P<0.05$) ความหนาแน่นไม่มีผลต่ออัตราการเปลี่ยนอาหารเป็นน้ำหนักตัว (FCR) อย่างมีนัยสำคัญ แต่พบว่าขนาดของกลุ่มมีผลต่อ FCR อย่างมีนัยสำคัญ โดยที่ฝูงขนาด 100 ตัว มี FCR ต่ำกว่า ($P<0.05$) ฝูงขนาด 50 ตัว ความหนาแน่นไม่มีอิทธิพลต่อความถี่ทั้งหมดของพฤติกรรมทั่วไป ขนาดของฝูงแต่ไม่ใช่ความหนาแน่น มีอิทธิพลต่อพฤติกรรมทั่วไป ($P<0.01$) และพฤติกรรมการจิกขน ($P<0.01$) ขนาดของกลุ่มและความหนาแน่นไม่มีผลต่อความถี่ของพฤติกรรมก้าวร้าว เมื่อความหนาแน่นเพิ่มสูงขึ้น อัตราส่วนเฮทเทอโรฟิลต่อลิมโฟไซท์ของไก่ในกลุ่ม 50 ตัว มีแนวโน้มลดลง นอกจากนี้ ระยะเวลาที่ไก่นอนนิ่งไม่ไหวติง (Tonic immobility, TI) สูงขึ้นอย่างมีนัยสำคัญ ($P<0.05$) ความหนาแน่นและขนาดของกลุ่มไม่มีผลต่อค่าของ relative fluctuating asymmetry และคะแนนการเดิน ค่าคะแนนความเสียหายของขนพบต่ำที่สุดในกลุ่ม 50 ตัวที่ความหนาแน่นที่ 8 ตัว/ตารางเมตร ไก่ที่อายุ 12 สัปดาห์ชอบที่จะอยู่บริเวณริมผนังคอก พบว่าไก่ในกลุ่ม 100 ตัวที่ชอบอยู่ในบริเวณริมคอกนี้มีจำนวนสูงกว่าในกลุ่ม 50 ตัว ($P<0.01$) ความหนาแน่นไม่มีอิทธิพลต่อการกระจายเชิงพื้นที่ของไก่ในไก่กลุ่ม 50 ตัวเมื่อความหนาแน่นเพิ่มสูงขึ้นจำนวนของไก่ที่อยู่ในบริเวณคอนเกาะเพิ่มสูงขึ้น แต่ไม่พบความแตกต่างในไก่กลุ่ม 100 ตัว ทั้ง 3 ระดับความหนาแน่น

สรุปได้ว่า ไก่ลูกผสมพันธุ์พื้นเมืองสามารถเลี้ยงในความหนาแน่นได้ถึง 16 ตัว/ตารางเมตร โดยไม่เกิดผลเสียต่อการผลิต ขนาดของกลุ่มแต่ไม่ใช่ความหนาแน่นมีผลต่อ กิจกรรมทั่วไป พฤติกรรมการจิกขนและพฤติกรรมก้าวร้าวของไก่ ถึงแม้ว่าไก่ที่เลี้ยงในความหนาแน่นสูงจะมีความ

หวาดกลัวสูงกับสภาพของขนไม่เป็นที่พึงประสงค์ แต่สวัสดิภาพนั้นไม่ได้เลวร้ายไปกว่าไก่ที่เลี้ยงในความหนาแน่นที่ต่ำกว่า

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา ____________________
ปีการศึกษา 2555 ลายมือชื่ออาจารย์ที่ปรึกษา________________
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม ____________

XIN HUO : EFFECTS OF STOCKING DENSITY AND GROUP SIZE ON BEHAVIOR, STRESS AND WELFARE OF THAI CROSSBRED CHICKENS. THESIS ADVISOR : ASSOC. PROF. PONGCHAN NA-LAMPANG, Ph.D., 91 PP.

STOCKING DENSITY/GROUP SIZE/BEHAVIOR/WELFARE/THAI CROSSBRED CHICKENS

This research aimed to investigate the influence of stocking density (8, 12 and 16 birds/m^2) and group size (50 and 100 birds) on productivity; general behaviors, feather pecking, and aggressive behaviors; welfare indicators and spatial distribution of Thai crossbred chickens (n=1350 birds). The productivity, behavioral and spatial distribution parameters were recorded once a week. The welfare indicators were measured from 12 to 13 weeks of age. The data were subjected to analysis of variance with 2×3 factorial completely randomized design with 3 replicates per treatment.

The results showed that stocking density and group size had no effect on body weight, body weight gain and mortality of chickens from 2 to 12 weeks of age. Feed intake in the 50 bird group with 16 birds/m^2 density was higher than that in the 100 bird group with other densities ($P<0.05$). Feed conversion ratio (FCR) was not significantly affected by density but was significantly affected by group size. The lower FCR was in the 100 bird group ($P<0.05$). Stocking density had no effect on total frequency of general behaviors. Group size, rather than density, had a significant influence on general behaviors ($P<0.01$) and feather pecking ($P<0.01$). The frequency

of aggressive behaviors was not affected by group size and density. With an increase of density, there was a decreasing tendency in the heterophil to lymphocyte ratio of chickens in the 50 bird group, moreover, the tonic immobility duration of the chickens increased significantly ($P<0.05$). The relative fluctuating asymmetry values and gait score were not affected by stocking density and group size. The lowest feather damage score was found in the 50 bird group with 8 birds/m^2 density compared with other treatments. For spatial distribution, it was found that chickens preferred to stay in the wall area rather than in other areas of the pen. The highest number of chickens to stay in the walled area was in the 100 bird group compared with the 50 bird group ($P<0.01$). Density had no influence on the spatial distribution of the chickens. With an increase of density, the number of chickens in the perching area increased in the 50 bird group. There was no difference between the 100 bird groups at three levels of density.

In conclusion, Thai crossbred chickens could be stocked up to 16 birds/m^2 without adverse effects on productivity. Group size rather than density affected general activities, feather pecking and aggressive behaviors of chickens. Although the chickens in the high stocking density had a high fearfulness with adverse feather condition, their welfare was not any worse than that of the low stocking density chickens.

School of Animal Production Technology Student's Signature ____________________

Academic Year 2012 Advisor's Signature ____________________

Co-advisor's Signature ____________________

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Xin Huo

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

CHAPTER Ⅰ

INTRODUCTION

1.1 Rational of this study

Usually Thai native chickens are raised in the extensive backyard system. The meat of Thai native chicken has been very popular among Thai consumers because of its unique taste and texture which regarded as a greater delicacy over that of commercial broiler (Jaturasitha, Leangwunta, Leotaragul, Phongphaew, Apichartsrungkoon, Simasathitkul, Vearasilp, Worachai and ter Meulen, 2002; Wattanachant, Benjakul and Ledward, 2004; Wattanachant et al., 2005; Choprakarn and Wongpichet, 2007). The domestic market for Thai native chickens has increased significantly. Thai native chickens also have strong potential for overseas market. This leads to the change of practice in raising Thai native chickens.

Cross breeding of Thai native male with egg type female is use to get higher chick production than pure breeding of Thai native chickens. The crossbred chickens are raised in higher density and larger group than normally practice in the extensive backyard system. Since Thai native chickens derived from those extensively raised and have higher aggressiveness than commercial breeds (Jaturasitha et al., 2002), it is suspected that when Thai native or crossbred Thai native chickens are raised in intensive system their welfare would be compromised.

Stocking density is considered as one of the most important environmental factors because of the established effect on growth rate in broiler chickens (Škrbić, Pavlovski, Lukić, Perić and Milošević, 2009). Dozier, Thaxton, Branton, Morgan, Miles, Roush, Lott and Vizzier-Thaxton (2005) indicated that negative effects on live performance of heavy broilers happened with increasing density beyond 30 kg/m^2. In most of these studies the effects of group size are confounded with stocking density or with pen attributes due to the shape or size of the pen (Christman and Leone, 2007).

According to farm animal welfare council (FAWC) the five freedoms that are required to ensure that animals are in stress free environment are freedom to express most normal behavior by providing sufficient space, proper facilities and company of the animal's own kind (FAWC, 2009). Several authors have studied behavioral response of animal as a source of welfare information and assessment (Pettit-Riley, Estevez and Russek-Cohen, 2002). The behavior measurements and behavior test can also reveal whether the animals are adapted to production system (Veersamy, Lakritz, Ezeji and Lal, 2010).

With increasing group size more and more aggressive of animals has been shown (Al-Rawi and Craig, 1975; Hughes and Wood-Gush, 1977). However, studies conducted on larger group sizes have demonstrated that aggression has a tendency to decrease with increasing group size (Hughes, Carmichael, Walker and Grigor, 1997; Nicol, Gregory, Knowles, Parkman and Wilkins, 1999). Feather pecking was found to reduce welfare of the birds and increases economical losses due to increased feed consumption and mortality (Rodenburg, de Haas, Nielsen and Buitenhuis, 2010). It is a multi factorial problem affected by the genetic background of the birds, their early life history and environmental factors, such as availability of floor substrate, nutrition, adequate lighting and group size and stocking density (Rodenburg, Komen, Ellen, Uitdehaag and van Arendonk, 2008). Due to the fact that Thai crossbred chickens have aggressive temperament of Thai fighting cock, the aggressive and feather pecking behaviors that could cause adverse effects for chickens are considered in this study.

A combination of welfare indicators related to production system, husbandry routines and animal behavior and health is suggested to assess the welfare level of the individual farm (Veersamy et al., 2010). Determining the welfare of chicken can be done by using a number of indicators, such as tonic immobility (Campo, Teresa and Dávila, 2008), fluctuating asymmetry (Stige, Slagsvold and Vøllestad, 2005; Campo et al., 2008; Nuffel, Tuyttens, Dongen, Talloen, Poucke, Sonck, and Lens, 2007), heterophil to lymphocyte ratio (Gross and Siegel, 1983; Campo et al., 2008), gait score (Jones, Donnelly and Dawkins, 2005) and feather damage (Bilcík and Keeling, 1999; Rodenburg et al., 2010).

Distribution studies have the advantage of investigating the birds in the system in which they are normally kept, thus giving information that is relevant for raising system (Buijs, Keeling and Tuyttens, 2011). The spatial requirements of broiler chickens have most often been studied by looking at the adverse physical effects of high stocking densities (for instance decreased walking ability, increased contact dermatitis and mortality) or by studying changes in the behavioral repertoire (Buijs, Tuyttens, Baert, Vangeyte, Poucke and Keeling, 2008).

Although the Department of Livestock Development (DLD) of Thailand recommends the stocking density of Thai chicken: 0-6 week 22 birds/m^2; 7-16 week 8 birds/m^2, recently there is no research article about the effects of stocking density and group size on productivity, behavior or welfare of Thai crossbred chickens. So which level of stocking density and group size are suitable for raising Thai crossbred chickens and will offer a good welfare for chickens are needed to investigate meanwhile can bring the best benefits to the farmers.

1.2 Research objectives

1.2.1 To assess the effects of the stocking density and group size on productivity of Thai crossbred chicken.

1.2.2 To assess the effects of the stocking density and group size on general behaviors of Thai crossbred chicken.

1.2.3 To assess the effects of the stocking density and group size on aggressiveness of Thai crossbred chicken.

1.2.4 To assess the effects of the stocking density and group size on feather pecking of Thai crossbred chicken.

1.2.5 To assess the effects of the stocking density and group size on welfare of Thai crossbred chicken.

1.2.6 To assess the effects of the stocking density and group size on spatial distribution of Thai crossbred chicken in pen area.

1.3 Research hypothesis

1.3.1 With increasing of stocking density and group size, the productivity of Thai crossbred chicken will be reduced.

1.3.2 With increasing of stocking density and group size, the frequency of general behaviors of Thai crossbred chickens will be different.

1.3.3 With increasing of stocking density and group size, the frequency of aggressiveness of Thai crossbred chickens will be increased.

1.3.4 With increasing of stocking density and group size, the frequency of feather pecking of Thai crossbred chickens will be increased.

1.3.5 With increasing of stocking density and group size, the welfare of Thai crossbred chickens will be compromised.

1.3.6 With increasing of stocking density and group size, the spatial distribution of Thai crossbred chickens will be different.

1.4 Scope and limitation of the study

In order to investigate the effects of stocking density and group size on behavior, stress and welfare of Thai crossbred chickens, there were several parameters were measured in this study, such as productivity, occurrences of general behaviors, frequency of aggressive behaviors, frequency of feather pecking behaviors, welfare indicators and percentage of spatial distribution. Actually, group size used in this study was for small scale chicken farm, so results might not be applied for large commercial chicken farm.

1.5 Expected results

To indicate the optimal level of stocking density and group size for raising Thai crossbred chicken by using behaviors data of Thai crossbred chickens. The research results will contribute to assess a standard welfare of Thai crossbred chickens production in Thailand. This information will benefit the rural or small-scale farmer to ensure sustainable poultry production and food security.

CHAPTER II

LITERATUREREVIEW

2.1 Native chicken raised in Thailand

2.1.1 Thai chicken

Thai chickens (Gallus gallus domestics) are wild birds that have been domesticated in rural villages in Thailand. They have been Thai people's way of life at least since the time of the Ayutthaya Kingdom some 400 years ago (Choprakarn, 1976). They are a source of food protein and quick cash income. They are used in leisure pursuits and as offerings in various rituals and ceremonies as well (Choprakarn and Wongpichet, 2007). Compared with the commercial broiler and White Leghorn, the native chickens have better resistance against heat stress and many diseases, and their eggs and meat possess better eating qualities (Lee, 2006).

Meat of pure or crossbred Thai chicken has unique taste, tough and strong muscle characteristics. This regarded as of higher quality when compared with the over tenderness of broiler meat. Thailand is one of the world's leading countries in poultry egg and meat production. Poultry meat is produced mainly from broilers (86.4%). The lesser portions are from indigenous chickens (13.0%) and hybrid indigenous (0.6%) birds. Poultry meat is produced in the eastern, central, and northern regions with the ratios of 40%, 35%, and 12% respectively. Saraburi and Nakhon Ratchasima provinces are the main production areas in Thailand (Haitook, 2006).

Genetic variation of 4 breeds of Thai native chicken (based on the colors of the feather): "Loeng hang khao" (Yellow·White Tail), "Pradu hang dam" (Dark Black Tail), "Shee" and "Nokdang hang dang". The "Loeng hang khao" fighting cock is one of the most popular variety in Thailand because of its long history. The adult males of this variety have mainly black color on the ventral part with white dorsal plumage including the neck, hackle, saddle, back and wing bow region. Some feather at the middle of rectrix and primary wing are colored on the web. "Loeng hang khao" adult females have smaller body. There are evidences that "Loeng hang khao" variety has been manipulated since Ayoda era (Apichai, 1998). The number of "Loeng hang khao" is also very high in Thailand. Therefore, this variety of chicken would be easy for the breeding selection of the excellence characters and breeding development (Chatchawan, 2003).

The cross breeding of Thai native males with egg type females was aimed for higher chick production than pure breeding of Thai native chickens. The crossbred chickens: are similar in general physical appearance to the native chickens (i.e., with black feathers); are compatible with market prices; are easier to raise than pure exotic breeds; are able to utilize local feed resources; grow more rapidly than native chickens and have higher egg yields (Leotarakul and Pimkamlai, 1999; Haitook, 2006).

Thai crossbred chickens can reach a marketable live weight of 1.2 to 1.4 kg after 8 to 12 weeks while pure breed reach the same weight only at 16 weeks, with the same feed (Leotarakul and Pimkamlai, 1999). Crossbreeds of Thai chickens, only under good management conditions, have a higher production efficiency compared to pure breed chickens. However, under the rural area conditions, pure breed chickens perform better in all aspects (Choprakarn, Wattanakul, Wongpichet and Suriyachantratong, 1998).

2.1.2 Housing system

Thai chickens are generally raised under free range conditions. However, in the case of fighting cocks, production is much more intensive. Most small holders employ a "low input/low output" system, which is appropriate to their local conditions, while a few farmers (less than 10 percent) keep their chickens semi-intensively to supplement their incomes. Only a very small number of commercial farms exist, serving niche markets (Choprakarn and Wongpichet, 2007).

Most Thai farmers, generally, keep their Thai chickens in the backyard. Nevertheless, some farmers may take their birds to the fields when endemic diseases break out in the villages and/or during crop growing and harvesting seasons. Chickens are penned to protect them from predators and/or thieves at night. A pen is usually located under rice storage for ease of construction; if it stands alone it will still be close to the house. Thai chickens are fed twice daily, in the morning and evening, mostly by women. Chickens can move freely with their flock scavenging around for edible insects, seeds and fresh plant parts (Choprakarn and Wongpichet, 2007).

In most cases, there are no vaccination and de-worming for Thai chickens; but some farmers may have local herbs for prevention and/or curing. This practice tends to satisfy the farmers involved (Choprakarn and Wongpichet, 2007). The general picture of the Thai indigenous chicken production system is summarized in Figure 2.1.

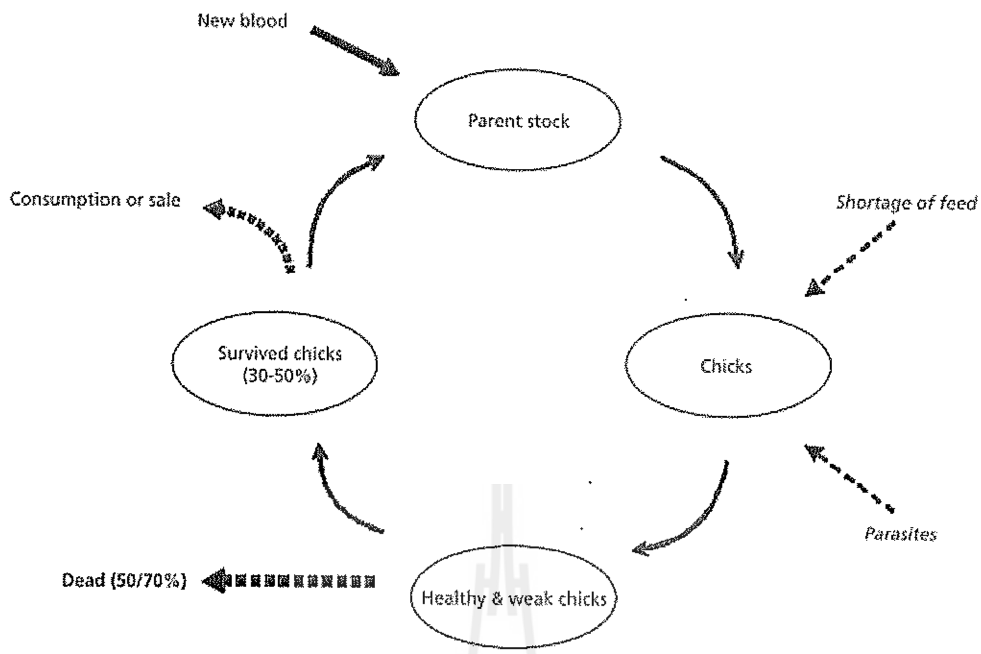

Figure 2.1 Raising indigenous chickens by Thai farmers under traditional system (Choprakarn et al., 1983 cited by Choprakarn and Wongpichet, 2007).

2.2 Stocking density

Stocking density is generally given as kg body weight (at slaughter age) or number of birds per m^2 (European Commision, 2000). There are many papers on the effect of stocking density on production of broilers as welfare indicator, and very wide scope of stocking densities was investigated (Škrbić et al., 2009).

The stocking densities used in different countries depend on climate and the costs of housing. In general, birds kept in hot climates are stocked less density to avoid overheating (Table 2.1). When stocking density is expressed as kg per m^2, it is a notional estimate of density at the end of the growing period. Stocking density reaches that level for a very short period, and if the sheds are cropped or depopulated at set times, sometimes the target weight is not achieved and the final stocking density is lower (Gregory, 2007).

Table 2.1 Examples of broiler stocking densities used in different countries

	Typical stocking densities (birds per m^2)
Thailand/ Libya	8-12
Brazil	10-12
USA	14
China	15-16
France	16-25
Netherlands	23

Source: Gregory (2007).

Arnould and Faure (2004) found that chickens at the higher density mainly stayed and lay in the free area. At the low density, they preferred to stay and lie near drinkers and feeders and had limited use of the free area. Chickens spontaneously limited their physical effort and only rarely went to some parts of the pen when reared at low density. These results emphasize the need to find situations that could stimulate activities to avoid local densities that could have deleterious effects on health and comfort (Alvino, Archer and Mench, 2009). A better understanding of the motivation of broiler chickens to go to and remain in a different part of the pen (or not) could help to adapt the organization of chicken rearing areas on farms in the future.

However, broilers do not seem to be averse to crowd. Febrer, Jones, Donnelly and Dawkins (2006) suggest that, even at high commercial stocking densities, broiler chickens may find the close proximity of other birds more attractive than aversive.

The birds' locomotory abilities would likely have been influenced by leg problems. So it is important to note that the higher stocking densities result in lower activity levels within the broiler house (Collins, 2008). Chickens grew more slowly at higher stocking densities and fewer birds had the best gaits. However, for the most obvious measures of welfare, i.e. the numbers of birds dying, being culled as unfit or showing leg defects, stocking density had no effect, although there were large differences between producer companies.

Some researchers said stocking density itself was less important to the physical health and mortality rates of the chickens than were the environmental consequences of increased stocking density, such as poorer air and litter quality that can result if the internal environment of a house is not adequately controlled (Dawkins, Donnelly and Jones, 2004; Jones et al., 2005).

A stocking density of 30 kg/m^2 is the RSPCA's (1995) recommended upper limit and 34 kg/m^2 is the upper limit recommended by the Farm Animal Welfare Council (1992), while current practice in the U.K. and the E.U. is up to 42.5 kg/m^2 (European Commission, 2000). Dozier et al. (2005) indicated that increasing the density beyond 30 kg/m^2 elicited some negative effects on live performance of heavy broilers. Thaxton, Dozier, Branton, Morgan, Miles, Roush, Lott and Vizzier-Thaxton (2006) support that stocking density did not cause physiological adaptive changes indicative of stress, and they agree with the conclusion of Dawkins et al. (2004) is that the environment had more impact on welfare than did stocking density.

2.3 Group size

Broiler chickens kept indoors and reared intensively in commercial conditions live in large groups and high stocking densities are used (Arnould and Faure, 2003). In large group, there are more individuals with which to interact and in all captive animal frequency of social interactions increases with group size. Birds in large group will have more difficulty learning to recognize their flock mates individually. Individual recognition in hens seems to be limited to groups of up to about 80 birds. In general, small group size is advantageous. For example, in cages for laying hens small groups show higher production levels compared to larger unit sizes. There is also evidence that in cages, stress increases linearly with group size (Michael, Barry, Hughes and Elson, 1992).

The effects of increasing group size on aggressive interactions in domestic fowl have been studied with conflicting results. Group size has been reported to influence levels of aggression in domestic fowl. As group size increases, aggression should decline as increasing numbers of animals switch from resource defense aggression to "scramble competition", defined as non-aggressive individual competition in local groups for available resources (Parker, 2000). For small experimental group sizes, aggression has been shown to increase with increasing group size (Al-Rawi and Craig, 1975; Hughes and Wood-Gush, 1977). Study conducted on larger group sizes have demonstrated that aggression has a tendency to decrease with increasing group size (Hughes et al., 1997; Nicol et al., 1999). Still others have observed reduced levels of aggression with increasing group size in young (Estevez, Newberry and Arias de Reyna, 1997) and adult domestic fowl (Carmichael, Walker and Hughes, 1999; Estevez, Newberry and Keeling, 2002; Hughes et al., 1997;

Nicol et al., 1999). Nearly all studies dealing with group size and vigilance in birds and mammals report a decrease in vigilance with increasing group size (Lee, 2006).

2.4 Behavior of chicken

2.4.1 General behavior

Actually, broiler chickens become increasingly inactive as they near market weight (Shields, Garner and Mench, 2005). The general activities of broiler chickens are affected by the legs weakness (Sørensen, Su and Kestin, 2000; Weeks, Danbury, Davies, Hunt and Kestin, 2000). The birds kept at the low stocking density in each case showed more walking, running, preening and calm behavior, spent less time concentrated in the areas around the feeders and drinkers and were more active in the last week before slaughter than birds kept at the high densities (Ferrante, Lolli, Marelli, Vezzoli, Sirri and Cavalchini, 2006).

The behavior of taking the dust bath is an important indicator of social welfare of the group (Olsson and Keeling, 2005; Dixon, Duncan and Mason, 2008), and this behavior is mainly affected by the bedding material (de Jong, Wolthuis-Fillerup and van Reenen, 2007). Fine materials such as sand or peat are preferred for dust bathing, probably because they are superior at penetrating the feathers to reach the downy portions (Mench, 2009). Pecking ground and scratching at a potential site usually precedes a dust-bathing bout (Shields, Garner and Mench 2004). Zimmermana, Lindberg, Pope, Glen, Bolhuis and Nicol (2006) observed that different sizes of groups provide significant differences in the expressions of aggressive behavior. Perching may also reduce the impact of leg problems in broilers, which can result in significant financial losses to broiler producers due to increased mortality, culling and carcass downgrading (Pettit-Riley and Estevez, 2001).

2.4.2 Social behavior

Social behavior is the clue that allows groups of animals to function and that allows us to interact with the domestic animals we care for. Some of the main types of social behaviors are competition, sexual behavior, parent-offspring interactions and play. All poultry species are highly social, although their wild ancestors show different forms of social organization (Weary and Fraser, 2009).

2.4.2.1 Affiliative behavior

Social facilitation, defined as "an increase in the frequency by an animal when in the presence of a con-specific engaged in the same activity" (Keeling and Hurnik, 1993). Affiliative behavior promotes group cohesion (friendly/positive gestures), e.g. grooming, touching, and hugging. It is characterized by maintaining proximity, providing food, protection or allogrooming between specific individuals. It may therefore play a major role in achieving a positive mood in animals. In farm animals, affiliative behavior has been much less well studied than social competition and it has only rarely been investigated with regard to affective states (Boissy, Manteuffel, Jensen, Moe, Spruijt, Keeling, Winckler, Forkman, Dimitrov, Langbein, Bakken, Veissier and Aubert, 2007).

An understanding of the different roles that animals may assume within a group can help us to design husbandry systems that will function adequately and will not excessively strain their capacity to cope. For example, permitting animals to establish 'friendships' with preferred associates or avoiding mixing of unfamiliar individuals whenever possible, is known to reduce aggression in pigs, poultry and horses (Keeling and Gonyou, 2001), so the affiliative behavior is one kind of indicators to assess the animal emotion, further, to value the animal welfare.

The main affiliative behavior shown by poultry is flocking. The tendency to form groups rather than move independently or avoid other members of the species evolved primarily for protection against predators. A behavior pattern of more immediate mutual advantage is the habit of pecking food, which has adhered to the face of another bird. The bird being pecked remains very still, often with its head back and its eyes closed, allowing the pecking to continue. This probably happens more often at high stocking density where birds are feeding in close proximity. In hens, it may also happen more often in cages than in other systems, because of the lack of suitable objects on which birds could clean their own faces, by beak wiping. There is a possibility that this could produce a disadvantage despite the obvious advantage. Such behavior may be a pre-disposing factor to feather pecking or cannibalism, because birds being pecked in those cases frequently also freeze, rather than trying to escape (Michael et al., 1992).

2.4.2.2 Aggression behavior

Agonistic interactions were classified into fight, attack-avoidance, chase-escape and threat-avoidance (Huang and Lee, 2005). Estevez et al. (2002) classified aggressive acts according to their apparent level of escalation as fights, leaps, chases, stand-offs, pecks, threats and avoidances, respectively. The aggression peaked early at 3-4 weeks of age in broiler (Pettit-Riley et al., 2002). The aggression was lower at the moderately crowded level of 0.067 m^2 per bird compared to least crowded level of 0.1 m^2 per bird, which supports to a certain extent the concept of increased social tolerance at higher densities proposed by Estevez et al. (1997).

In a wild group, the first aggression experienced by young birds is probably that received from other members of the flock, when they move too close, or when they are in the way of older birds. Later, particular chicks, ducklings or poultry themselves become aggressive to contemporaries. There are also usually certain individuals, perhaps smaller or weaker than others, which are attacked particularly frequently (Estevez et al., 2002). D'Eath and Keeling (2003) found the idea that in large groups hens become less aggressive and may change their social system to one where dominance is determined through direct assessment and 'status signaling' rather than the remembered individual assessment of a small group pecking order.

Many researchers want to find a good way to decrease the chicken aggression, however, Pettit-Riley et al. (2002) revealed that the provision of perches to the broilers did not necessarily result in lower levels of aggression, and that certain perch designs may in fact contribute to aggression.

2.4.2.3 Play behavior

Play behavior has been suggested to be an indicator of good welfare in wild as well as captive juveniles, and has been used to assess welfare in different farm environment. It is founded that playful behavior during the suckling period may help piglets to adapt to weaning and other stressful situations in domestic pigs (Donaldson, Newberry, Spinka and Cloutier, 2002). Play in calves is typically seen in a social context either as locomotor play, play fighting, ground play, where the calf rubs its neck and head against the ground while kneeling down (Jensen and Kyhn , 2000).

When Cloutier, Newberry and Honda (2004) observe the aggressive behavior in young domestic fowl they found that an interesting phenomenon. A chick runs carrying a worm-like object while flock mates follow and attempt to grab the object from its beak called worm-running. Worm-running ranks are not predictive of success in aggressive interactions. Instead, worm-running fits some criteria for play. Therefore, there are a few reports about the chicken play behavior until now.

2.4.2.4 Feather pecking and cannibalism

The most problematical social behaviors seen in commercial flock are feather pecking and cannibalism. These are abnormal behaviors, and are more common in large than small flocks (Mench, 2009). The experimental evidence showed that the social learning can contribute to the spread of cannibalistic behavior in domestic fowl (Cloutier, Newberry, Honda and Alldredge, 2002). Cannibalism sometimes follows on from feather pecking, for example, when exposed skin is injured, but it more often arises independently. In hens, the most common form is vent pecking. If the skin is broken, here or elsewhere on the body, other birds then join in pecking, because these species of birds are attracted to blood. Further pecking and consumption of flesh then frequently result in death. Vent pecking also sometimes occurs in cage systems (Michael et al., 1992).

Hen in battery cage pecking one another reduces economy for the manager and welfare for many hens, and it is likely the cause of the death of some. The feather pecking is considered as redirected ground-pecking (Blokhuis, 1986). It was shown that the motivation for non-aggressive pecking at con-specifics varies along with ground pecking motivation. Foraging behavior has a similar form in domesticated hens as in wild jungle fowl. Arnold (2005) use rubber bands to let hens show the nature behavior normally in order to reduce the hen-directed pecking (Chamove, 1989; Andersson, Nordin and Jensen, 2001; Arnold, 2005). The novel stimuli as appropriate foraging substrates is effective in reducing the incidence of cannibalism (Huber-Eicher and Wechsler, 1998).

2.5 Welfare indicators

2.5.1 Legs and skin conditions

Lameness is recognized as the single most important welfare issue in the broiler industry. Poor walking ability is common and in some bird it is painful. It is linked to high growth rates in modern broiler strains. The most effective way of avoiding lameness is by reducing the growth rate during the second week of life. Two views are emerging about whether or not there is suffering associated with leg disorders in broilers. One view is that birds with a gait score of three or more (on a 0 to 5 point scale) experience pain when walking. This is based on the findings that gait score 3 birds walk faster when given an analgesic, and they voluntarily select an analgesic when given the opportunity (Gregory, 2007).

The alternative view is that some birds may experience pain when walking, but others walk badly simply because they are physically unstable. The prevalence of pain and the path physiological conditions that cause pain have not been clearly defined. Once a bird is lame, it visits the feeder less frequently and it often eats whilst in the sitting position (Gregory, 2007). The welfare indicators relates to the condition of skin (dermatitis, lesions, injuries) and legs, moreover the physiological parameters relates to incidence of physiological adaptive changes which indicate the stress.

2.5.2 Stress in chicken

2.5.2.1 Stress

Stress is defined as a condition in an animal that results from the action of one or more stressors that may be of either external or internal origin. Whether a stressor can be considered as harmful depends on the way an organism is able to cope with a threatening situation as it regains a state of homeostasis (von Borell, 2001). Responses to stressors can include anatomical, physiological and/or behavioral changes (Mumma, Thaxton and Dodson, 2006).

Distress relates to the emotional content of noxious experiences and the resulting emotional state of an animal (Mellor, Cook and Stafford, 2000). In the context of animal well-being, this term has been used to differentiate between nonthreatening and threatening stress responses. Distress refers to a biological state in which the stress response to a threat (stressor) has a deleterious effect on the individual's well-being. Some decades ago, Selye (1979) already distinguished between good (eustress) and bad (distress) stressors. Others argue that such a distinction between stressors becomes unnecessary in that any stressor can have a detrimental effect on the organism if it occurs often enough, and it would be hard to determine when stress becomes distress (Selye cited by von Borell, 2001). The ability to adjust to some stressors (controllability), however, seems to be under the control of the amygdala through activation of the sympathetic nervous system and prepares the animal for fight and flight responses (see Figure 2.2).

The adjustment to stress induces a broad range of physiological and behavioral changes that allow for a rapid recovery or adaptation to the change. In the past, housing systems and handling procedures for farm animals were mainly assessed by descriptive behavioral studies using indicators presumed to be related to stress (von Borell, 2001).

Situations of uncertainty, social pressure and fear are potent stressors with relevance for the well-being of animals, leading to severe damage to specific target organs and tissues or even to death in some species. Whether a stressor can be considered as harmful depends on the way an organism is able to cope with a threatening situation as it maintains a state of homeostasis. In that way, stress can be measured and monitored in terms of behavioral and physiological alterations that might be indicative for the individual's state of well-being (von Borell, 2001).

2.5.2.2 Haematological response

The Heterophil/lymphocyte ratio appears to be a more reliable indicator of levels of corticosterone in the feed and to social stress than were the plasma corticosteroid levels (Gross and Siegel, 1983). So many researchers realized that the ratio of Heterophil/Leucocyte (H:L) is a reliable indicator of chicken well-being (Thaxton and Puvadolpirod 2000; Zulkufli, Al-Aqil, Omar, Sazili and Rajion, 2009; Dennis, Cheng and Cheng, 2008). The H:L ratio was increased by overcrowding stress (Karthiayini and Philomina (2008). However, Türkyilmaz (2006) found that H:L ratio is independent of stocking density at 15, 20 and 25 birds/m^2. During the heat stress the H:L ratio of the broilers was significantly higher than that of the Thai indigenous chicken crossbreds and Thai indigenous chickens (Aengwanich, 2007).

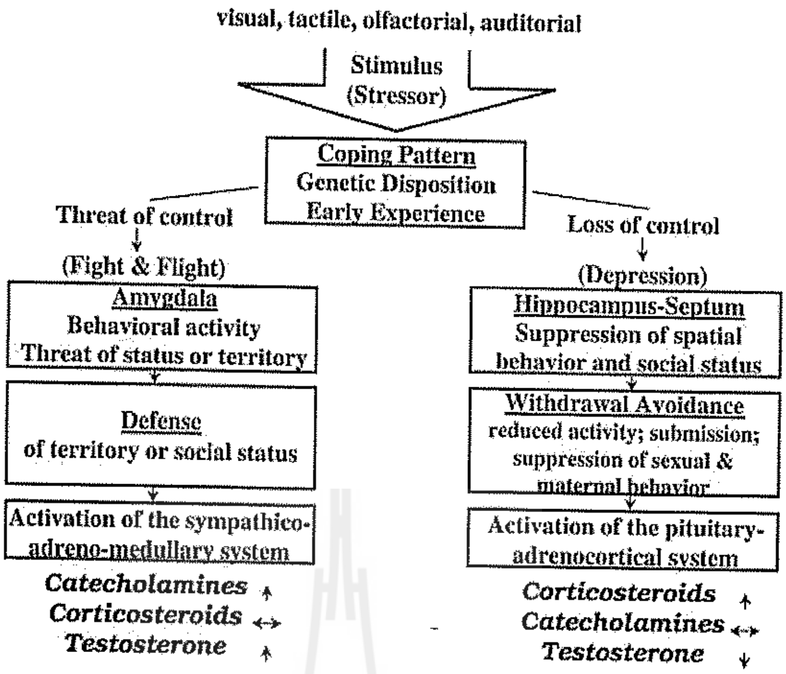

Figure 2.2 Coping/predictability concept (modified after Henry and Stephens, cited by von Borell, 2001)

2.5.3 Behavioral response

Behavior is a window of the body for the outside, enabling better estimate with precision the welfare of birds (Danilo, Sandra and Narima, 2011). The effects of behaviors observed in the welfare condition of the broiler breeders in the logistic models also corroborated with the observations of Spinu, Benveneste and Degen (2003), because when the behaviors of sitting and of drinking water occur more frequently, they will contribute positively to the welfare. Feed restricted birds show an increase in spot pecking, litter scratching and pecking and preening (Hocking, Maxwell and Mitchell, 1996). Increased drinking is also a behavioral response to feed restriction.Panting is an indication that the birds may be under heat stress (European Commission, 2000).

Andrews, Omed and Phillips (1997) concluded that a high stocking density reduces activity in broiler chickens. It has been suggested that social stress might exacerbate other behavioral problems such as cannibalism, feather pecking and hysteria, which are generally worse in large groups (D'Eath and Keeling, 2003).

2.5.4 Fearfulness

Dawkins (1980) and Duncan (1993) insisted on the idea that the welfare of animal results from its emotional state and mental states. What matters for an animal's welfare is not how this animal is but how it feels (Dawkins and Duncan cited by Veissier and Boissy, 2007). It is very important to use the emotion to be the indicators of assessing farm animal welfare.

Fearfulness is one of the primary emotions refer to a set of six to eight basic emotional reactions (e.g., fear, joy, disgust, sadness, surprise and anger) that are innate and found in human and non-human animals (Leventhal, 2000). Emotion is aroused state involving intense feeling, autonomic activation and related behavior (Blood and Studdert, 2007). It is considered as elementary modes of responding to affectively arousing events and are supposed to have appeared during evolution because they confer more fitness, e.g. negative emotional states protect animals from a negative environment because they trigger protective responses such as avoidance (Veissier and Boissy, 2007).

Determining whether the stocking density and group size will be the social stressors for chicken can use a number of methods. One of the ways to measure the stress is Tonic immobility (TI) test. It is common throughout the animal kingdom and it is considered a particularly useful behavioral method of assessing fearfulness

(Jones, 1989). Tonic immobility is an unlearned state of profound but reversible motor inhibition and reduced responsiveness to external stimulation which is induced by brief physical restraint. The TI reaction is prolonged upon exposure to frightening stimuli whereas fear-reducers shorten it. It is, therefore, thought to be positively related to fear and to represent the terminal reaction in a sequence of anti-predator responses. The immobility response is sensitive to a wide variety of genetic (breed, selection), environmental (pen-housing, cages, cage level), experiential (regular handling) and social (group size, isolation, hierarchy, alarm vocalizations and odors) factors. Therefore, TI is a measurement for evaluating fearful behavior in the chicken that exhibits a terminal defensive reaction and can be used as criterion for measuring the wellbeing and stress levels of the birds (Campo, Gil, Davila and Muñoz, 2006).

High stocking densities cause acute stress in broilers; the effects of low and intermediate stocking densities, however, are not so evident, particularly in relation to tonic immobility and response to acute stress (Villagrá, Ruiz de la Torre, Chacón, Lainez, Torres and Manteca, 2009). Increased density and group size were expected to increase conflicts between the birds, leading to an increase in stress, which would be expressed psychologically by increased fearfulness and physiologically by higher glucocorti-coid levels and decreased bursa weight (Ravindran, Thomas, Thomas and Morel, 2006).

2.5.5 Fluctuating asymmetry

Non-identical development of bilateral traits due to disturbing genetic or developmental factors is called fluctuating asymmetry (FA) if such deviations are continuously distributed. It is one frequently used indicators of welfare in chickens (Campo et al., 2006). Despite an increasing body of research, the link between FA and animal performance or welfare is reported to be inconsistent, possibly, among other reasons, due to inaccurate measuring protocols or incorrect statistical analyses (Nuffel et al., 2007). It refers to small, random departures from perfect morphological symmetry, and is typically measured as differences in sizes of structures between the right and left sides of bilaterally symmetric organisms.

It is used as a measure of developmental instability, which reflects the ability of an organism to consistently produce a targeted phenotype in a given environment (Stige et al., 2005). The stresses can be either genetic or environmental could affect the FA (Swaddle and Witter, 1994). It has been suggested that fluctuating asymmetry (FA) reflects an animal's ability to cope with the sum of all challenges during its growing period and, thus, is a potential welfare indicator (Knierim, Van Dongen, Forkman, Tuyttens, Spinka, Campo and Weissengruber, 2007). Ventura, Siewerdt and Estevez (2010) suggested that a negative effect of high density on broiler footpad health and FA in broiler chickens.

2.5.6 Feather pecking

Feather pecking (FP) remains a major welfare and economic problem in laying hens. FP has been found to be related to other behavioral characteristics, such as fearfulness. There are indications that fearful birds are more likely to engage in FP. Furthermore, FP can lead to increased fearfulness in victims. These results suggested that although relationships were found between feather damage and fear responses at cage level, lines divergently selected on feather pecking behavior do not differ in their fear responses. Divergent selection on feather pecking may have altered pecking motivation rather than fearfulness (Rodenburg et al., 2008).

Very poorly feathered hens showed shorter tonic immobility than did hens with perfect plumage. Hens with very poor plumage were less fearful and more stressed than hens with a perfect plumage. Studies suggest that very poor plumage is associated with indicators of fearfulness and stress (Campo, Gil, Torres and Davila, 2001).

2.6 Spatial distribution

Distribution studies have the advantage of investigating the birds in the system in which they are normally kept, thus giving information that is relevant for raising system (Buijs et al., 2011). The distribution of wild and feral chickens is influenced by the distribution of food and water, non-specifics and predators, availability of cover and roosting sites (for review, see Mench and Keeling, 2001).

Broiler chickens spatial distribution is affected when density is altered by changing either group size or pen size; but when group and pen size are altered simultaneously, thus keeping density equal, these effects are much smaller (Leone and Estevez, 2008; Leone, Christman, Douglass and Estevez, 2010). In all of the studies the effects of group size are confounded with stocking density or with pen attributes due to the shape or size of the pen (Christman and Leone, 2007). However, there is evidence supporting density, rather than pen size, as the main variable motivating chickens' spatial preferences when group size is kept constant.

Scientific Committee on Animal Health and Welfare (2000) reported the intensive domestic animal husbandry always compromises animal welfare by providing insufficient space per animal. Broiler chickens have a strong tendency to rest in particular areas of the house (Newberry and Hall, 1990) such as walls, corners, migration barriers, or around farm equipment that is left in the house. This typical spatial distribution takes place even when plenty of space may be still available in the central areas of the poultry houses (Estevez, 2003). Birds also spend less time resting at higher densities due to a resultant increase in disturbances (Hall, 2001).

Studies on spatial distribution are scarce although they offer opportunities to investigate the animal's spatial preference more directly. For example, if close proximity of pen mates is experienced as aversive by broiler chickens, they may position themselves further away from their con-specifics when given the opportunity to do so (Keeling, 1994) (thus, at lower stocking densities).

The spatial requirements of broiler chickens have most often been studied by looking at the adverse physical effects of high stocking densities (for instance decreased walking ability, increased contact dermatitis and mortality) or by studying changes in the behavioral repertoire. Exercise is particularly low at high densities (Newberry and Hall, 1990; Estevez et al., 1997), and this lack of activity may be one reason for the exacerbated leg weakness found in broilers at high densities (Sørensen et al., 2000).

Roosting is a natural behavior of jungle fowl (Collias and Collias, 1967). Increasing the complexity of the environment by adding enrichment can have a substantial impact on broiler welfare (Leone and Estevez, 2008; Bizeray, Estevez, Leterriera and Faurea, 2002), such as set perching serves as a good example in intensive broiler production. Therefore, providing chickens with perches could be a way to encourage exercise. However, modern lines of broilers are typically not provided with perches in commercial flocks, mainly because at moderate and relatively high densities perch use is relatively low (LeVan, Estevez and Stricklin, 2000; Pettit-Riley and Estevez, 2001).

Studies on the use of pen space by domestic chickens reared under commercial conditions mostly involve analysis of movements of a few identified birds (Arnould and Faure, 2004). Uneven distribution also seems to occur in broiler chickens reared under such commercial conditions. However, more information is needed for situations where facilities are arranged as near as possible to commercial conditions. Furthermore, only studies focusing on the behavior of whole group or large numbers of birds can reveal areas with high concentrations of chickens. However, none of these methods are suitable for use on commercial broiler farms, because they involve either complex equipment, removing birds from their peers to test them or working with small groups that do not represent real farm conditions (Febrer et al., 2006). There was short of the research about spatial distribution of Thai crossbred chicken in Thailand.

CHAPTER Ⅲ

MATERIALS AND METHODS

3.1 Animals

The Thai crossbred used in this experiment was a cross between Thai native males (‘Loeng hang khao’ that means yellow white tail) and ISA Brown commercial layer type females. One thousand three hundred and fifty mixed sex chicks, supplied by the project of “Establishment of ‘Korat Meat Chicken’ strain for small and micro community enterprise (SMCE) production”, were reared from one day old to 13 weeks of age without beak trimming. The experiment lasted from February to April, 2011.

3.2 Housing and rearing management

From the 2nd to 13th week, chicks were brooded for 2 weeks before being randomly assigned to the treatments. The gas heater and the 100W electric bulb, placed about 1 meter above the floor, were used to brood the chickens. At the end of 2nd week, the brooding gas heaters and bulbs were removed. The experimental pens were bedded with approximately 5 cm of rice husk. The pen sizes for the 100 birds treatment group had areas of 12.5, 8.33, and 6.25 m^2, and those for the 50 birds treatment group were 6.25, 4.17, and 3.12 m^2. This resulted in treatment densities of

8, 12 and 16 birds/m^2, respectively. Plastic curtains were used to prevent visual contact between the chickens in adjacent pens.

The birds were fed a standard commercial three phase broiler diet. Feed and water were fed *ad libitum* throughout the rearing period. During the first 3 weeks, feed was added 3 or 4 times a day. After that, the feed was added to 2 times a day (07:00 h and 16:30 h). The ratio of birds per feeder cup (diameter×high: 40 cm×30 cm) or water bottle (4L capacity) was 25 to 1. Before stocking the birds, the housing was sprayed with disinfectant. Natural lighting was used after brooding period until 13 weeks old. The chicken house was protected from the wind and rain with plastic sheeting, which also adjusted the ventilation.

3.3 Vaccination program

At the end of week 2 (14 days old), the chicks were vaccinated according to the recommendations of the Department of Livestock Development (DLD), Thailand (Table 3.1).

Table 3.1 Vaccination schedule

Vaccines	Age of chickens (day)	Method of Vaccination
ND + IB	5-7	Eye
IBD	12-14	Mouth
ND (Lasota) +IB	28	Eye
FP	42	Wing web
ND (Lasota) +IB	56	Eye

Source: Haitook (2006).

3.4 Experimental design

The experiments would be arranged in 2×3 factorial in completely randomized design (CRD) with repeated measurement. There were 3 replications for each treatment combination (Table 3.2).

Table 3.2 Design of experiment

Factor A (Group size)	Factor B (Stocking density)		
	B_1 (8 birds/m^2)	**B_2 (12 birds/m^2)**	**B_3 (16 birds/m^2)**
A_1 (50 birds)	A_1 B_1	A_1 B_2	A_1 B_3
A_2 (100 birds)	A_2 B_1	A_2 B_2	A_2 B_3

Model: Y_{ijk} =μ+ A_j + B_{jk}+ AB_{jk} + e_{ijk} (i=1,…r, a; j=1,…b; k=1,…,n)

Where: Y_{ijk} = observation k in level i of factor A and level j of factor B

μ = the overall mean

A_j = the effect of $level_j$ of factor A (j= 1,..a)

B_k = the effect of $level_k$ of factor B (k=1,..b)

AB_{jk}= Effect of interaction of factor A and factor B (jk)

e_{ijk} = error

3.5 Productivity assessment

The average body weight (BW) (random sampling 20% of each group), body weight gain (BWG), feed intake (FI) and the feed conversion ratio (FCR) were recorded weekly with restriction of feed 12 hours before weighing. Additionally mortality rate was determined daily in each pen.

3.6 Behavior Observation

3.6.1 General Activities

Each pen was observed once from 09:00 to12:00 at the end of 12 weeks of age. The observer stood in front of the pen about 5 min before observation. The Canon digital camera A3100 IS was used when scanned all birds. The general ethogram (Bokkers and Koene, 2003) was given in Table 3.3.

Table 3.3 Ethogram of recorded behaviors

Behavior	Description
Feeding	With head above or in the feeder
Drinking	Pecking to a drinking nipple or drinking out of the cup beneath the drinking nipple
Preening	Grooming of own feathers with the beak
Head grooming	Grooming of own head with the foot
Stretching	Stretching of wing and/or leg
Aggression	Pecks directed to the head of a pen mate or sparring
Standing idle	Standing without any other activity
Sitting idle	Sitting with hocks resting on ground without any other activity
Walking	Locomotion with a normal speed or with quick steps
Wing flapping	Bilateral up-and-down wing flapping
Dust bathing	Performed with fluffed feathers while lying, head rubbed on floor, wings opened, scratching at ground
Lying	With head flat on the bedding or with the head under a wing either with eyes open or closed
Flying	to move through the air using wings
Exploring	Searching for and active investigation of novel situations in the absence of a pressing physiological need
Pecking	Pecking the plastic material
Perching	All behaviors showed in the perching area
Other	All other behavior not mentioned above

3.6.2 Aggressive behavior

Each pen was observed once from 09:00 to 12:00, 10 min interval for each pen observation at the end of 12 weeks of age. The observer stood in front of the pen about 5 min before observation. All birds in the pen were observed in each pen (Martin and Bateson, 1986). The frequency of different types of aggression were recorded (Table 3.4).

3.6.3 Feather pecking

Each pen was observed once from 09:00 to 12:00, 10 min interval for each pen observation at the end of 12 weeks of age. The observer stood in front of the adjacent pen about 5 min before observation. All birds in the pen were observed in each pen (Martin and Bateson, 1986).

According to the methods of Wechsler and Huber-Eicher (1998), feather pecks that were successively directed at the same receiver were recorded as one interaction. An interaction ended when there were no more pecks during a period of 4 s. It was differentiated whether the interaction was composed of 1-4, 5-9 or more than 10 single feather pecks. This categorization allowed us to limit the amount of time we would pay attention to interactions that were composed of more than 10 single pecks in favor of recording all occurrences of feather pecking interactions. For each feather pecking interaction, the number of pecks was counted in relation to the area of the body that was pecked, namely the head, neck, breast, wings, back, rump and tail.

Table 3.4 Aggressiveness ethogram

Type of aggression	Definition
Chase	When one bird at the patch ran after another bird for more than three steps in an aggressive manner (which was very different from food running)
Fight	When two birds standing in front of each other were threatening and delivering pecks to each other in rapid succession, sometimes accompanied by leaps
Fight with peck	All criteria for a fight with the bird delivering at least one peck to the opponent
Leap	When a bird jumped and kicked its feet forwards at her opponents
Peck	When one bird raised its head and vigorously stabbed its beak at the other bird (usually directed towards the comb)
Stand-off	When two birds stood staring at each other for >2 s
Threat	When one bird stood with its head clearly raised (sometimes accompanied by rising of the neck feathers) in front of a second bird who held its head at a lower level
Avoidance [a]	When a bird suddenly lowered its head and walked away from another bird

[a] Avoidance: only when observer had not observed an aggressive act being delivered by the other bird, possibly because it was too subtle to be unambiguously apparent to observer (Estevez et al., 2002).

Only pecks at feathered parts were classified as feather pecking. Pecks at legs, beaks, combs or wattles were neglected. Every feather pecking interaction was attributed with increasing intensity to one of the following 4 types of behavior: ‘Pecking’ at a feather without pinching; ‘Pinching’ a feather and pulling slightly; ‘Pulling’ at a feather with a vigorous backward movement of the head; ‘Plucking’ a feather. Interactions that were composed of repeated pecks were classified according to the most intense type of behavior observed.

3.7 Spatial distribution

The spatial distribution was determined when all birds were observed in each pen at the end of 12 weeks of age. To test the validity of the distribution obtained by direct observations, the distribution was also assessed by photographs taken per pen when observed the general behaviors. Photographs were taken without flash. The special areas can separate as the feeding, drinking, perching and wall area (Arnould and Faure, 2004) (see Figure 3.1).

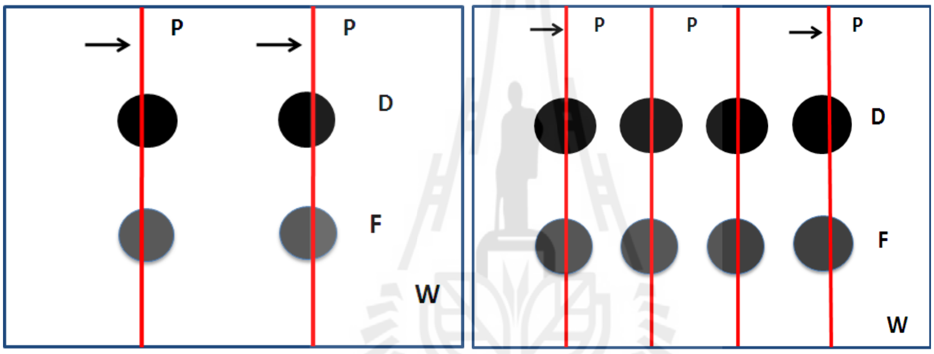

Figure 3.1 Pen design
(F: Feeding area; D: Drinking area; P: Perching area; W: Wall area). Fig.(left side): group size of 50 birds, Fig.(right size): group size of 100 birds

3.8 Hematological parameters

At the end of the 12th weeks (84 days old), a total of 15 randomly selected chickens from each group were gently removed from each pen. One ml blood sample from each bird was obtained, using a needle, from the alar vein in the wing within 2 min of removal from its pen. Blood sample was kept in a tube containing the anticoagulant EDTA K2 (Türkyilmaz, 2008). Blood smeared was used to determine heterophil to lymphocyte (H:L) ratio (Campo et al., 2008). Total Red Blood Cell (RBC), total White Cell (WBC), the packed cell volume (PCV) and Hemoglobin concentration (Hb) were checked by RIA Lab Company in Korat.

3.9 Tonic immobility (TI) test

During the 13th week (from 85 to 88 days old), 7 birds, randomly chosen from each pen, were tested for the duration of tonic immobility in a separate place of the chicken house. Tonic immobility was induced as soon as the bird was caught, by placing the animal on its back with the head hanging in a V-shaped plastic cradle (length×width×height: 30×24×20 cm). The method was similar to that described by Campo et al. (2008). The bird was restrained for 10 s. The researcher sat in full view of the bird, about 1 m away, and fixed her eyes on the bird to cause the fear-inducing properties of eye contact. If the bird remained immobile for 10 s after the researcher removed her hands, a stopwatch was started to record latencies until the bird righted itself. If the bird righted itself in less than 10 s, and the restraint procedure was repeated (3 times maximum), then it was considered that tonic immobility had not been induced, so a 0 s score was given. If the bird did not show a righting response over the 10 min test period, a maximum score of 600 s was given for righting time.

3.10 Fluctuating asymmetry (FA)

Fluctuating Asymmetry was measured with the same sample birds (n=7) after TI test finished. Digital vernier caliper and ruler were used to measure leg length (tarsal bone) and width (tarsometarsus and tibia joints) and wing length on both left (L) and right (R) sides (de Beer Lockwood, Raijmakers, Raijmakers, Scott, Oschadleus and Underhill, 2001; Kemper et al., 2007) and then calculated the relative FA for each trait using formula [2|R-L|/(R+L)] (Campo et al., 2008).

3.11 Gait score

Ten randomly selected chickens per pen were assessed for leg health such as gait score, hockburn and pad dermatitis when they were 89-91 days old. The method of assessment of gait score followed that of Jones et al. (2005).

3.12 Feather damage score

After gait score assessment, 15 birds per pen were randomly chosen to be scored for feather damage in breast, legs, vent, back, rump, wings, tail and primaries areas. The method followed the scoring system of Wechsler and Huber-Eicher, (1998). Using a scoring system of 1 (perfect plumage), 2 (feathers damaged, no skin area denuded), 3 (denuded area up to 3×3 cm) or 4 (denuded area greater than 3×3 cm) points for six individual parts of the body: breast, legs, vent, back, rump, wings. In addition, the tail and the primaries were given a score of 1 (perfect) and 2 (damaged). In the analysis, a total 'feather loss' score range (6 to 24) was calculated for each bird by adding the scores of breast, legs, vent, back, rump and wings.

3.13 Data analysis

The experimental unit considered was the pen. The data were subjected to analysis of variance (ANOVA) with 2×3 factorial arrangement of treatments in a CRD with 3 replicates per treatment.

The factorial was made on the effects of stocking density and group size and they were considered fixed effects. The General Linear Model (GLM) procedure of SPSS 16.0 was used. If the data appeared to have non-normally distribution, transforming should be used. The frequency behavior data were transformed to square root prior to analysis (Estevez et al., 2002). TI duration and feather damage score data were logarithmically transformed, and the relative FA and H:L ratio data were transformed to square root prior to analysis. Group size and stocking density were considered fixed effects. When there was an interaction between stocking density and group size, the all means of 6 treatments were compared. When multiple comparisons were involved and significance was indicated, differences among treatment means were tested by Duncan's multiple range tests. The level of significance was determined at $P<0.05$.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Productivity

It was found that different levels of stocking density and group size did not affect BW, BWG and mortality (Table 4.1). Interaction between stocking density and group size was not found for BW, BWG and mortality from 2 to 12 weeks of age. The final BW of Thai crossbred chickens were sufficient to reach the marketable live weight of 1.2 kg after 12 weeks (Haitook, Tawfik and Zöbisch, 2003).

Many factors affect growth performance of poultry including genotype, production system, diet, age, sex, stocking density, photoperiod, temperature, and activity (Fanatico, Pillai, Hester, Falcone, Mench, Owens and Emmert, 2008). Our findings were in agreement with those of Feddes, Emmanuel and Zuidhof, (2002) and Ravindran et al., (2006), who reported the similar BW and BWG of birds reared at three levels low, middle, and high of densities. In another evaluations involving stocking density ranges of 10 to 24 birds per m^2, increasing population density had no influence on feed per gain. Dawkins et al. (2004) and Jones et al. (2005) supported that stocking density itself was less important to the physical health and mortality rates of the chickens than the environmental consequences. Besides, Hall (2001) established a significant increase of mortality in high stocking density in commercial farms. However, our results agreed with Thomas, Ravindran, Thomas , Camden, Cottam, Morel and Cook (2004) found stocking density had no effect on broiler mortality.

Table 4.1 Effects of stocking density (D) and group size (GS) on productivity of Thai crossbred chickens from 2 to 12 weeks of age[1]

GS (birds)	D (birds/m^2)	BW (g)	BWG (g)	FI (g)	FCR	Mortality (%)
50	8	1253.30	1141.70	3384.80	2.99	2.67
50	12	1270.00	1157.30	3544.90	3.07	3.33
50	16	1190.00	1082.00	3632.00	3.36	2.67
100	8	1293.30	1187.70	3367.60	2.85	1.67
100	12	1242.20	1137.60	3348.20	2.96	1.00
100	16	1275.00	1164.50	3423.90	2.95	0.33
ANOVA	GS	NS	NS	**	*	NS
	D	NS	NS	*	NS	NS
	GS×D	NS	NS	NS	NS	NS
	SE	56.64	56.92	61.12	0.15	0.02

[1]Data are expressed as means;
GS×D: interaction between group size and stocking density;
*P<0.05; **P<0.01; NS=P>0.05.

It was found that the mean FI (Table 4.1) was higher in the group of 50 birds than that of chickens in the 100 bird group (P<0.01). Stocking density significantly increased FI of Thai crossbred chickens (P<0.05). The mean FI in 16 birds/m^2 density was significantly higher than that of chickens in 8 birds/m^2 density. There was no difference between 8 and 12 birds/m^2 densities, or 12 and 16 birds/m^2 densities for FI. No interaction between stoking density and group size was found for FI of chickens. Group size significantly influenced FCR (P<0.05) (Table 4.1), i.e. 100 bird group had lower FCR than 50 bird group. The effect of stocking density was not found for FCR. There was no interaction between stocking density and group size in FCR.

Although the feeding competition exists in each group, when birds get a share of resources in their local group, the competition that they showed is called scramble competition (Parker, 2000). In the behavioral ecology sense, there are no clear winners or losers in scrambles, individuals compete by increasing their effort in harvesting the resource. The results of FI in the 50 bird group were higher than birds in 100 group, possibly because of increased disturbances from other Thai crossbred chickens in larger group size.

A novel discovery is that FI of Thai crossbred chickens increased as stocking density increased. Some researchers found that stocking density did not affect FI of broiler (Ravindran et al., 2006). Shanawany (1988) found that reducing stocking density, food consumption and body weight gain increased. Although the reasons for observed discrepancy in FI are unclear, our findings indicate that Thai crossbred chicken has characteristics of fighting cocks, when they are raised at intensive commercial system it is different from the researches in breeders and broilers (Škrbić, et al., 2009).

4.2 Behavior observation

4.2.1 General activities

Group size had significantly affected the total frequency of feeding ($P<0.01$), drinking ($P<0.05$), preening ($P<0.01$), standing idle ($P<0.01$), sitting idle ($P<0.01$), walking ($P<0.01$), exploring ($P<0.01$) and perching ($P<0.01$) from week 4 to 12, but not on pecking. In the 100 bird group, the total frequency of general behaviors was significantly higher than that in the 50 bird group (Table 4.2). Stocking density did not affect the total frequency of general behaviors. Since an interaction between stocking density and group size was found in total frequency of standing idle ($P<0.05$), sitting idle ($P<0.05$) and perching ($P<0.05$), then the effects of stocking density were analyzed within each group size.

In the 50 bird group, the total frequency of standing idle in 16 birds/m^2 density was significant lower than that in 12 birds/m^2 density ($P<0.05$) and in group of 100 birds with 3 levels of stocking densities. The total frequency of standing idle in the 50 bird group with 8 and 12 birds/m^2 stocking densities were similar. There was no difference among 100 birds group with 3 levels of stocking densities.

The high total frequency of sitting idle of chickens was found in the 100 bird group with 16 birds/m^2 density. The low total frequency of sitting idle of chickens was found in the 50 bird group with 16 birds/m^2 density. It was similar as that in the 50 bird group with 12 birds/m^2 density. The total frequency of sitting idle of chickens in the 50 bird group with 8 birds/m^2 was not significant different with that in the 100 bird group with 3 levels of stocking densities. There was no difference of total frequency of sitting idle of chickens among the 100 bird group with 3 levels of densities.

The total frequency of perching in the 50 bird group with 8 birds/m^2

density was lower ($P<0.05$) than that in the 50 bird group with 16 birds/m^2 density, and the 100 bird group with 3 levels of densities. There was not different from the 50 bird group with 8 and 12 birds/m^2 densities. No difference was found between the 50 bird group with 12 and 16 birds/m^2 densities. In the 100 bird group, no difference was found among 3 levels of densities.

However, no interaction between stocking density and group size was found for other general activities. At the week 12, the percentage of sitting idle ($P<0.01$) and walking ($P<0.05$) were affected by group size (Table 4.3). Group size had no influence on other general activities. Stocking density did not affect the all general activities of chickens except perching behavior. No interaction was found between group size and stocking density. The high percentage of sitting idle and walking was happened in the 50 bird group and the 100 bird group respectively. However, there was no significant interaction between group size and stocking density on each treatment. The frequency of drinking behavior was rare event found in each treatment at the week 12.

Stocking density had significantly influenced on percentage of perching behavior for chickens ($P<0.05$) at the week 12. The percentage of perching behavior for the chickens in 8 birds/m^2 density was significant lower than that in 12 and 16 birds/m^2 densities ($P<0.05$). No different percentage of perching behavior was found between 12 and 16 birds/m^2 densities. There was a significant interaction between group size and stocking density for this trait (Table 4.3). With increased stocking density from 8 to 16 birds/m^2 densities, the percentage of perching behavior was increased specially in the 50 bird group not in 100 bird group.

Table 4.2 Effects of stocking density (D) and group size (GS) on total frequency of general behaviors from week 4 to 12 of Thai crossbred chickens[1]

GS (birds)	D ($birds/m^2$)	Eating	Drinking	Preening	Standing idle	Sitting idle	Walking	Exploring	Pecking	Perching
50	8	49.67	3.33	10.67	42.67^{ab}	70.67^{bc}	15.67	163.00	15.67	40.67^{a}
50	12	46.33	7.33	13.00	52.00^{bc}	53.33^{ab}	21.67	152.33	8.33	66.00^{ab}
50	16	51.00	5.00	12.33	32.33^{a}	30.67^{a}	16.33	155.67	19.33	91.67^{bc}
100	8	99.00	9.67	22.33	67.00^{cd}	78.67^{bc}	34.00	385.00	13.00	135.67^{d}
100	12	101.00	13.33	20.00	70.67^{d}	68.00^{bc}	25.67	424.33	19.67	121.67^{cd}
100	16	98.67	13.33	21.33	82.67^{d}	86.00^{c}	28.67	369.67	34.00	112.67^{cd}
ANOVA	GS	**	*	**	**	**	**	**	NS	**
	D	NS	NS	NS	NS	NS	NS	NS	NS	NS
	GS×D	NS	NS	NS	*	*	NS	NS	NS	*
	SE	5.04	3.17	2.86	5.10	8.77	3.72	13.31	5.84	12.70

[1]Data are expressed as means;
GS×D: interaction between group size and stocking density:
[a, b, c, d] means within the same column with different superscripts were significant difference at P<0.05;
*P<0.05 **P<0.01 NS: P>0.05.

Table 4.3 Effects of stocking density (D) and group size (GS) on percentage of general activities in Thai crossbred chickens at the 12 weeks of age[1]

GS (birds)	D (birds/m^2)	Total frequency	Eating	Preening	Standing idle	Sitting idle	Walking	Exploring	Pecking	Perching
50	8	49.67	8.88	4.71	8.66	10.85	2.67	45.57	3.40	11.22^{a}
50	12	47.67	10.51	2.76	9.51	12.11	0.72	32.41	2.61	25.89^{ab}
50	16	48.00	5.56	2.08	11.11	6.94	0.69	31.25	2.08	36.81^{b}
100	8	98.33	5.78	3.06	4.46	4.07	4.05	47.76	2.69	26.06^{ab}
100	12	96.67	4.82	2.07	7.59	3.10	3.11	42.73	1.72	33.13^{b}
100	16	101.67	4.63	0.99	7.26	5.90	3.25	46.44	3.97	25.58^{ab}
ANOVA	GS	**	NS	NS	NS	**	*	NS	NS	NS
	D	NS	NS	NS	NS	NS	NS	NS	NS	*
	GS×D	NS	NS	NS	NS	NS	NS	NS	NS	*
	SE	1.24	0.03	0.01	0.02	0.02	0.01	0.07	0.02	0.05

[1]Data are expressed as means;
GS×D: interaction between Group size and stocking density;
**P<0.01, NS: P>0.05;

Table 4.4 Effects of stocking density (D) and group size (GS) on total frequency of general behaviors from the week 4 to 12 of Thai crossbred chickens

GS (birds)	D (birds/m^2)	Wing flapping	Dust bathing	Lying	Flying	Head grooming	Stretching	Aggressive
50	8	1.67	2.67	3.33	1.00^a	4.00	0.33	0.00
	12	1.67	3.33	4.67	3.00^a	1.33	1.67	1.00
	16	2.00	1.00	2.00	2.00^a	3.00	1.33	1.00
100	8	2.67	2.33	5.67	3.33^b	5.33	1.00	0.67
	12	1.33	5.33	4.67	0.00^a	3.67	1.67	1.67
	16	1.33	3.67	5.67	1.33^a	4.33	2.33	0.67
ANOVA	GS	NS	NS	NS	NS	NS	NS	NS
	D	NS	NS	NS	NS	NS	NS	NS
	GS×D	NS	NS	NS	**	NS	NS	NS
	SE	0.84	1.71	1.47	0.65	1.14	0.81	0.71

[1]Data are expressed as means;
*P<0.05 **P<0.01 NS: P>0.05;
GS×D: interaction between Group size and stocking density
[a, b] means within the same column with different superscripts were significant difference at P<0.05.

In this study, some behaviors such as wing flapping, dust bathing, lying, flying, head grooming, stretching and aggressive were rare events. The occurrences of those behaviors were close to zero, which happened to Thai crossbred chickens in each pen when scanned once per week from the 4 to 12 weeks of age (Table 4.4).

Broiler chickens exhibit social facilitation (Keeling and Hurnik, 1993) in feeding, which has been defined as "an increase in the frequency by an animal when in the presence of a con-specific engaged in the same activity". Single birds staying for short intervals at a particular place along the feeder do not induce other birds to arrive (Collins and Sumpter, 2007). However, with increasing the stocking density the eating and drinking behaviors of chickens were not different in each treatment after feeding time. In another words maybe the ad libitum-fed decreased the motivation of feed intake of chickens or drinking when few birds come to eat.

No different preening behavior showed in each treatment. It was probably because the slow growing broilers did not show such an increase in preening behavior with increased age (Bokkers and Koene, 2003). In this study, the standing idle of chickens might be limited in large group. However, the frequency of walking could not be limited in large group size. The frequency of exploring behavior was the highest behavior among all general activities of Thai crossbred chickens during the observation. It indicated that the birds were motivated to explore even in the absence of a need to do so (Mench, 2009).

The most problematical social behaviors seen in commercial flock are feather pecking and cannibalism. These are abnormal behaviors, and are more common in large than small flocks (Mench, 2009). In order to reduce the frequency of those kinds of abnormal behaviors the researchers added the plastic materials in each treatment for chickens pecking available. So general activities observations we ignored the aggressive pecking and feather pecking, just only focus on birds pecked the materials. Although group size and stocking density did not affect this behavior, putting the materials for chickens pecking is a good way to reduce the frequency of feather pecking and aggressive behavior for Thai crossbred chickens in all pens.

When the chickens reached the final market BW at the 12 weeks of age, most of chickens motivated to show perching with increasing density in the small group size. It might be a way to avoid disturbances from con-specifics in the same pen (Pettit-Riley and Estevez, 2001). However, there was no different frequency of perching was found for birds in the large group size no matter which level of stocking density. It was realized that the large group size with high stocking density might not cause any crowding situation for Thai crossbred chickens. Moreover, Thai crossbred chickens did not have leg problems in each pen, it is possible that perching may also reduce the impact of leg problems in broilers, which can result in significant financial losses to broiler producers due to increased mortality, culling and carcass downgrading (Pettit-Riley and Estevez, 2001).

Although preening and dust bathing are two primary behaviors to maintain birds plumage condition (Mench, 2009), Thai crossbred chickens showed rare frequency of dust bathing during the observation. The behavior of taking the dust bath is an important indicator of social welfare of the group (Dixon et al., 2008; Olsson and Keeling, 2005), and this behavior is mainly affected by the bedding material (de Jong et al., 2007).

Fine materials such as sand or peat are preferred for dust bathing, probably because they are superior at penetrating the feathers to reach the downy portions (Mench, 2009). Shields et al., (2004) reported that sand is attractive to broiler chickens and is a potent stimulus for dust bathing, rather than the paper or the wood shavings. There was no dust bath in rice hulls in broiler chickens. Therefore, that may be one reason why the low frequency of dust bathing in Thai crossbred chickens. In practice, the rice hulls are used widely to be bedding material in raising chickens in Thailand. Although we did not change the bedding material until the end of raising period, we picked up the litter everyday in order to keep the good floor condition and to avoid leg problem for chickens.

Zimmermana et al. (2006) observed that different sizes of groups provide significant differences in the expressions of aggressive behavior. However, the aggressive behavior of Thai crossbred chickens was rare event in each treatment, and it was not affected by group size and stocking density. When the chickens become older and older, the social hierarchy is nearly stable, so the frequency of aggressive behavior might be low.

4.2.2 The aggressive behavior

Analysis of the total different type frequency occurrences of aggressive behaviors from week 4 to 12 indicated that the main aggressive behaviors were stand off and leap for Thai crossbred chickens. Stocking density had no significant effects on the frequency occurrences of stand off, fight with peck, threat, leap, chase, avoidance, fight and peck behaviors. Group size significantly affected peck ($P<0.01$) in Thai crossbred chickens. In the 100 bird group, the mean occurrences of peck behavior was higher than that in the 50 bird group. However, group size had no significant effect on other kinds of aggressive behaviors. There was no interaction between stocking density and group size (Table 4.5).

At the week 12, the aggressive behaviors such as stand off, fight with peck, threat, leap, chase, avoidance, fight and peck were rare events. The occurrences of those behaviors were close to zero, which happened to Thai crossbred chickens in each treatment.

Group size has been reported to influence the levels of aggression in domestic fowl. The authors did not observe the aggressive behaviors during feeding time, in order to avoid the non-aggressive individual competition (Estevez et al., 2002). Normally broiler chickens exhibit little agonistic behavior relative to other domestic fowls (Estevez et al., 1997).

Estevez et al., (1997) found broiler crowding increased from density of 5 to 20 birds/m^2 (group size 50 to 200) the frequency of threats was significantly lower. In our study, we found the aggressive behaviors such as threat, fight, avoidance, threat, fighting with peck, peck and chase were rare events during 10 min observation in Thai crossbred chicken at the 12 weeks of age. It meant that Thai crossbred chickens were not aggressive when they reach the final market weight. Sometimes Thai crossbred chicken showed leap before stand off or other aggressive behaviors. When a dominance hierarchy is established, the aggressive pecks were replaced by stand-off rather than threats (Nicol et al., 1999).

Although stocking density had no significant effects on total frequency occurrences of aggressive behaviors in Thai crossbred chickens, the tendency of those results indicated that the total frequency occurrences of aggressive behaviors was lower in 12 birds/m^2 compared with other density groups (8 and 16 birds/m^2). It was consistent with the frequency of threats and other types of aggression were lower in the moderately crowded groups (15 birds/m^2) compared to other crowding levels (10 and

20 birds/m^2) (Pettit-Riley et al., 2002).

Aggressive interactions both divert energy from growth and may reduce bird welfare (Guaryahu, Ararat, Asaf, Lev,- Weller, Robinzon and Snapir, 1994). Stocking density and group size did not affect the frequency of aggressive behaviors of Thai crossbred chicken, which was different from Keeling and Savenije (1995) who found a positive relationship between frequency of aggression and group size, and results of Estevez et al., (1997) reduced levels of aggression at larger group sizes, respectively. The frequency of aggressive behaviors even in large group size was not different from that in small group size. There was a possible reason that was lack of social structure in large flocks might be a factor in minimizing agonistic interactions between individuals (Hughes et al., 1997).

A bird suffers from frequently attacked by pen mates. However, high frequency of agonistic interactions in a flock does not imply any bird is under high social stress if aggressions are not concentrated to a few birds (Huang and Lee, 2005). Even at high commercial stocking densities, broiler chickens may find the close proximity of other birds more attractive than aversive (Febrer et al., 2006). In fact, from another angle, aggressive interactions are communication between con-species in the same group (Magnus, 1985).

Table 4.5 Effects of stocking density (D) and group size (GS) on frequency of aggressive behaviors in Thai crossbred chicken from the week 4 to 12[1]

GS (birds)	D (birds/m^2)	Stand off	Fight with peck	Threat	Leap	Chase	Avoidance	Fight	Peck
50	8	24.00	6.00	4.33	11.67	4.00	1.67	0.00	8.33
	12	13.00	6.67	1.67	8.33	2.33	1.67	0.00	2.67
	16	18.33	7.33	2.67	11.33	2.33	0.67	0.33	3.00
100	8	28.00	8.00	4.33	15.67	5.67	2.00	0.67	6.67
	12	16.67	5.67	4.33	15.00	6.00	1.33	0.67	11.00
	16	30.67	10.67	3.67	13.00	4.67	2.33	0.00	8.33
ANOVA	GS	NS	NS	NS	NS	NS	NS	NS	*
	D	NS	NS	NS	NS	NS	NS	NS	NS
	GS×D	NS	NS	NS	NS	NS	NS	NS	NS
	SE	0.67	0.46	0.27	0.64	0.49	0.47	0.27	0.51

[1]Data are expressed as means;
GS×D: interaction between group size and stocking density;
*P<0.05; NS=P>0.05.

4.2.3 Feather pecking

4.2.3.1 Body area

It was found that group size had a significant effect on the frequency of feather pecking in head ($P<0.05$), neck ($P<0.01$) and wing ($P<0.01$) areas, but did not have significant effect in head, breast, rump and tail areas (Table 4.6). The feather pecking frequency in the group of 100 birds was higher than that in the 50 bird group.

Stocking density had no effect on frequency of feather pecking on different body areas. There was no interaction between group size and stocking density on the frequency of feather pecking on different body areas except in the tail area ($P<0.05$). In the 50 bird group with 12 birds/m^2 density and in the 100 bird group with 8 birds/m^2 density the frequency of feather pecking in the tail area was significantly higher than that in the 50 bird group with 8 and 16 birds/m^2 densities. However, there was no difference between 8 and 16 birds/m^2 densities in the 50 bird group, and those in the 50 bird group with 12 birds/m^2 density, and in the 100 bird group with 12 and 16 birds/m^2 densities.

Under commercial conditions, an increased stocking density is often accompanied by large group size. An increase in group size is associated with higher levels of feather pecking (Nicol et al., 1999; Bilčík and Keeling, 2000). The results from our study support previous studies, which found that the most feather pecking activity occurred in the largest group size (Allen and Perry, 1975; Bilčík and Keeling, 2000). To a large extent, these results are in line with our hypothesis.

Table 4.6 Effects of stocking density (D) and group size (GS) on mean frequency of feather pecking in Thai crossbred chicken from the week 4 to 12[1]

GS (birds)	D (birds/m^2)	Back	Breast	Head	Neck	Rump	Tail	Wings
50	8	4.00	0.00	1.17	0.83	0.00	1.83^a	2.17
50	12	8.50	1.00	0.67	0.67	1.33	9.67^b	2.00
50	16	9.17	1.00	1.17	1.33	1.00	3.17^a	3.17
100	8	10.33	0.17	1.17	1.83	0.67	9.33^b	6.00
100	12	11.83	0.17	3.00	2.00	2.00	7.67^{ab}	6.83
100	16	7.83	0.00	2.50	1.83	0.50	6.83^{ab}	2.83
ANOVA	GS	NS	NS	*	**	NS	NS	**
	D	NS	NS	NS	NS	NS	NS	NS
	GS×D	NS	NS	NS	NS	NS	*	NS
	SE	2.80	0.36	0.66	0.53	0.62	2.14	1.04

[1]Data are expressed as means;
GS×D=interaction between group size and stocking density;
$^{a, b}$ means within the same column with different superscripts were significant difference (P<0.05);
*P<0.05; **P<0.01; NS=P>0.05.

Table 4.7 Effects of stocking density (D) and group size (GS) on mean frequency of intensity pecking in Thai crossbred chicken from the week 4 to 12[1]

GS (birds)	D (birds/m^2)	Per time		Intensity			
		1-4	5-9	Pecking	Pinching	Pulling	Plucking
50	8	9.83	0.33	6.83	2.00	1.00	0.50
50	12	20.33	1.17	13.17	4.50	2.67	1.83
50	16	17.50	2.00	14.00	2.33	2.00	0.67
100	8	28.33	0.83	19.17	4.67	4.83	0.83
100	12	29.50	1.33	23.83	4.50	2.50	1.17
100	16	20.67	1.00	12.17	4.33	3.33	1.5
ANOVA	GS	*	NS	*	NS	*	NS
	D	NS	NS	NS	NS	NS	NS
	GS×D	NS	NS	NS	NS	NS	NS
	SE	5.24	0.59	4.17	1.49	0.56	0.90

[1]Data are expressed as means;
GS×D: interaction between group size and stocking density;
*P<0.05; NS=P>0.05.

Table 4.8 Effects of stocking density (D) and group size (GS) on frequency of intensity pecking in Thai crossbred chickens at the 12 weeks of age[1]

GS	D	Per time		Intensity			
(birds)	(birds/m^2)	1-4	5-9	Pecking	Pinching	Pulling	Plucking
50	8	4.33	0.17	2.67	1.67	0.00	0.33
50	12	5.67	0.00	3.67	0.33	2.00	1.33
50	16	2.33	0.00	1.33	0.33	1.33	0.00
100	8	7.33	0.00	4.67	1.33	1.67	0.33
100	12	11.33	0.67	9.67	2.33	0.00	0.33
100	16	4.00	0.00	3.00	1.00	0.33	0.20
ANOVA	GS	NS	NS	NS	NS	NS	NS
	D	NS	NS	NS	NS	NS	NS
	GS×D	NS	NS	NS	NS	NS	NS
	SE	3.23	0.39	2.04	1.02	0.78	0.86

[1]Data are expressed as means;
GS×D: interaction between group size and stocking density;
NS=P>0.05.

Some researchers suggest that, in order to reduce feather pecking, chicks should be reared at low densities (Hansen and Braastad, 1994; Huber-Eicher and Audigé, 1999). However, in our study stocking density had no effect on the occurrence of feather pecking in Thai crossbred chickens. These findings are in support of the results presented by Carmichael et al. (1999) on stocking density which varied from 9.9 to 19 birds/m^2 densities and had no effect on feather pecking. The lack of stocking density effect found in our study might be that the densities used were not high enough to show adverse effects. This study is also in line with the claim of Stanislaus (2000) that group size rather than stocking density is the important controlling factor in relation to feather pecking.

Wood-Gush and Rowland (1973) reported that most feather pecks were delivered to the rump, followed by the tail and back. Nørgaard-Nielson et al., (1993) found feather pecking on the breast and back of White Leghorns was the most pronounced. Savory and Mann (1997) reported that most feather pecks were delivered on the back in Hisex and White Leghorn hens. A high occurrence of feather pecking in the back, the tail and the wings area was found in our study. Nicol et al. (1999) reported that the plumage condition of laying hens was worsened with increased group size and stocking density. Perhaps, the damage of feather pecking to the welfare of chickens was not fully realized in our study because of the low counts of pecks per bout and the low intensity levels.

4.2.3.2 Single feather pecks and intensity

Group size significantly affected the occurrences of 1 to 4 pecks per bout ($P<0.05$) (Table 4.7). For the group of 50 birds, the occurrence of 1 to 4 pecks per bout was lower than that in the group of 100 birds. Group size did not affect the occurrence of 5 to 9 pecks per bout. The occurrence of more than 10 pecks per bout was close to zero. Stocking density had no effects on either the occurrence of 1 to 4 pecks per bout or 5 to 9 pecks per bout. Interaction between group size and stocking density on the occurrences of pecks per interaction was not found either.

At the week 12, the frequency of single pecking was not significantly affected by group size and stocking density (Table 4.8). The frequency of 1 to 4 pecks per bout was higher than in 5 to 9 pecks per bout. The results of feather pecking in the 12 weeks of age were the same as the situation in the total frequency of feather pecking from week 4 to 12. However, there was no significant interaction between group size and stocking density on the trait.

Group size significantly affected the frequencies of "pecking" and "pulling" ($P<0.05$), but it did not affect that of "pinching" or "plucking" (Table 4.7). The frequencies of "pecking" and "pulling" in the 50 bird group were higher than that in the 100 bird group. Stocking density had no effect on the intensity of feather pecking. No interaction was found between group size and stocking density.

At the week 12, the intensity of feather pecking was not significantly affected by group size and stocking density (Table 4.8). Although the intensity of "pecking" was higher than other pecking intensity, there was no significant interaction between group size and stocking density on each treatment.

Thai crossbred chickens have the traits of fighting cocks when they are raised in high intensity groups. Theoretically, one would expect a high frequency of feather pecking in high stocking density and large group size. However, the results were not what we expected. There may be several reasons for this. Firstly, the pecking material such as rubber bands or foraging substrates were found to reduce feather pecking (Andersson et al., 2001; Arnold, 2005; Chamove, 1989) and perches was found to provide refuges for birds who are being pecked (Savory, 1995).

Secondly, the temperament of Thai crossbred chickens may be more 'gentle' than Thai fighting cocks. According to the pecking intensities classified, the high frequency of the lowest pecking intensity and higher pecking bouts occurred 1-4 times more than the other intensity levels and bouts in each pen. As reported by Kjare and Vestergaard (1998) the bout size (number of feather pecks per bout) might say more about the severity and risk of damage than the total number of feather pecks.

4.3 Welfare indicators

4.3.1 Hematological values

The group size and stocking density had no effect on concentration of Hb, PCV and number of WBC. However there is an interaction between group size and stocking density affected on the number of WBC ($P<0.01$). The number of WBC in chickens in the 50 bird group with was significantly lower ($P<0.05$) than that in 50 bird group with 16 birds/m^2 density, and the 100 bird group with 8 and 12 birds/m^2 densities. There was no difference found among the 50 bird group with 8 and 12 birds/m^2 density, and the 100 bird group with 16 birds/m^2 density. No different number of WBC was found in the 50 bird group with 12 birds/m2 density, and 100 bird group with 8 and 12 birds/m2 densities (Table 4.9).

Table 4.9 Effects of stocking density (D) and group size (GS) on Hematological values in Thai crossbred chickens[1]

GS (birds)	D (birds/m^2)	Hb (g/dl)	PCV (%)	WBC ($\times 10^4/\mu l$)	RBC ($\times 10^6/\mu l$)	H:L ratio
50	8	11.88	35.30	2.33^a	1.68	0.74^c
	12	11.29	34.70	3.12^{ab}	1.93	0.59^{bc}
	16	11.32	35.07	3.96^b	2.37	0.46^a
100	8	11.44	35.07	3.69^b	2.30	0.62^{abc}
	12	11.34	34.43	3.73^b	2.29	0.55^{ab}
	16	12.19	36.83	2.39^a	2.39	0.65^{bc}
ANOVA	GS	NS	NS	NS	**	NS
	D	NS	NS	NS	*	NS
	GS×D	NS	NS	**	NS	*
	SE	0.26	0.74	0.28	0.14	0.06

[1]Data are expressed as means;

GS×D: interaction between Group size and stocking density;

[a, b, c] means within the same column with different superscripts were significant difference (P<0.05);

*P<0.05; NS=P>0.05.

The group size had significant effects on the number of RBC. The number of RBC in 50 birds group was significantly higher than in 100 birds groups (P<0.01). The stocking density also affected the number of RBC significantly (P<0.05). The number of RBC in 8 birds/m^2 density was significantly lower than 16 birds/m^2 density (P<0.05). There was no different compared with 8 and 12 birds/m^2 densities; also in 12 and 16 birds/m^2 densities. There was no interaction between group size and stocking density effects on number of RBC (P>0.05).

It was found that stocking density and group size did not affect H:L ratio, while interaction between stocking density and group size was found for H:L ratio

($P<0.05$) (Table 4.9). When the means of the H:L ratio were compared for the 6 treatments, it was found that the H:L ratio of chickens in the 50 bird group with 16 birds/m^2 was lower than that of chickens in the 50 bird group with 8 birds/m^2 and the 100 bird group with 16 birds/m^2 ($P<0.05$). The H:L ratio of chickens in the 100 bird group with 12 birds/m^2 was lower than that of chickens in the 50 bird group with 8 birds/m^2. With an increase of stocking density, there was a decreasing tendency of the H:L ratio of chickens found in the 50 bird group but not in the 100 bird group.

Stocking density in intensive broiler production does not have significant effect on broiler performance and physiological parameters (Tayeb, Siamand, Merkhan, Shawkat, Gulizar and Asia, 2011; Yakubu, Gwaska and Salako, 2009). Thaxton et al. (2006) reported that stocking density did not cause physiological adaptive changes indicative of stress. Our study found that density did not exert any influence on the hematological characteristics (such as Hb, PCV and WBC), as very similar values were recorded for the three stocking densities investigated. This is an indication of the body homeostasis of the birds and hence health of the birds was not adversely disturbed as a result of housing the birds up to 16 birds/m^2.

It should be noted that the PCV and RBC values of all the experimental birds were within the normal range for chickens (28-37 %) and ($2\text{-}3\times10^6$ cell/μl) as reported by Simaraks, Chinrasri and Aengwanich (2004). The Hb of Thai crossbred chicken was a little bit higher than the report that did by Simaraks et al., (2004). However, the reference of hematological values was for 6-8 months age of Thai pure breed chicken. The total WBC of Thai crossbred chickens in the 50 bird group with 12 and 16 birds/m^2 densities, and the 100 birds group with 8 and 12 birds/m^2 densities were a little bit higher than the normal range for chickens ($1.6\text{-}2.5\times10^4$ cell/μl). It might indicate that the high stocking density might not cause the adverse effects on Thai crossbred chickens.

Gross and Siegel (1993) suggested that values of H:L ratios of about 0.2, 0.5, and 0.8 were characteristic of low, optimal, and high degrees of stress, respectively (Gross and Siegel cited by Campo et al., 2006). The H:L ratio range between 0.46 and 0.74 found in our study indicated that stocking density from 8, 12 and 16 birds/m^2 might cause Thai crossbred chickens stress. Although an interaction was found between stocking density and group size, we found no statistically significant effects of stocking density or group size either on the H:L ratios of Thai crossbred chickens. This result was similar to that found by Spinu et al., (2003) and Türkyilmaz, (2008). Our study showed a trend of decreasing H:L ratio with increasing stocking density from 8 to 16 birds/m^2 in the 50 bird group, which was in line with Cravener, Roush and Mashaly, (1992) in broiler breeder hens, but not with Türkyilmaz (2008) for broilers.

4.3.2 Tonic immobility (TI) duration

It was found that stocking density but not group size affected TI duration of the chickens (Table 4.10). When stocking density was increased from 8 birds/m^2 to 16 birds/m^2, the TI duration of the chickens increased significantly ($P<0.05$). The mean TI duration of chickens in 12 birds/m^2 density was not significantly different from those of both the lower and the higher densities. No interaction was found between group size and stocking density.

The duration of tonic immobility response to manual restraint is widely considered to be positively related to the antecedent fear state and to thereby represent a useful behavioral index of fear (Marin, Freytes, Guzman and Jones, 2001). The increase in stocking density caused longer TI duration, which made the chickens more fearful, which is the same as the findings of Andrews et al. (1997) and Onbaşılar, Poyraz, Erdem and Őztürk (2008). The finding that group size had no effect on the fearfulness of Thai crossbred chickens found in this study agreed with Lee (1989). We suspect that the group size of 100 birds is not high enough to cause any adverse effect on the fearfulness of Thai crossbred chickens.

Table 4.10 Effects of stocking density (D) and group size (GS) on fluctuating asymmetry (FA) and tonic immobility (TI) duration in Thai crossbred chickens[1]

GS (birds)	D (birds/m^2)	TI duration (s)×10^2	Leg length	Leg width	Wing length
50	8	2.67	2.06	2.22	2.76
	12	3.17	2.38	1.77	2.35
	16	3.41	2.48	1.28	2.07
100	8	2.84	3.20	2.37	2.72
	12	3.27	2.48	2.43	2.58
	16	4.32	1.96	2.30	2.01
ANOVA	GS	NS	NS	NS	NS
	D	*	NS	NS	NS
	GS×D	NS	NS	NS	NS
	SE	0.09	0.13	0.15	0.15

[1]Data are expressed as means;
GS×D: interaction between Group size and stocking density;
NS: P>0.05.

4.3.3 Fluctuating Asymmetry (FA)

The relative FA values for leg length, leg width and wing length were not affected by stocking density and group size (Table 4.10). No interaction between stocking density and group size was found either.

The relative FA was proved to be a potential welfare indicator (Knierim et al., 2007). That stocking density and group size had no effect on the relative FA of Thai crossbred chickens as found in our study shows the levels of density and group sizes used in our study were not high enough to cause any adverse effects. On the other hand, the FA is not a sensitive indicator of welfare in ad libitum fed animals because of the absence of energy allocation constraints (Poucke, Nuffel, Van Dongen, Sonck, Lens and Tuyttens, 2007).

4.3.4 Gait Score

All the sample birds had a gait score of 0, i.e. there were no health problems were found for the legs, such as discoloration, hock burn, or pad dermatitis in any of the pens.

Even though it was found in broilers that higher stocking densities caused more leg problems (Dawkins et al., 2004), Thai crossbred chickens in different stocking densities and group sizes in our study showed no leg problems. One reason for this is that the body weight of these birds was not heavy enough to cause any leg problems. Another reason may have been the exemplary raising management used during the growth period, for example, the litter was shoveled daily in order to decrease pad dermatitis in these chickens.

4.3.5 Feather damage score

The effect of group size was significant only for back and tail feather damage scores ($p<0.01$) (Table 4.11). Stocking density had a significant effect on back ($P<0.01$), leg ($P<0.05$), rump ($P<0.01$), total body ($P<0.01$) and primaries ($P<0.05$) feather damage scores. Interaction between stocking density and group size was found for back ($P<0.01$), wing ($P<0.05$), total body ($P<0.01$) and tail ($P<0.01$) feather damage scores. No effect of group size and stocking density was found for breast and vent feather damage score.

The back feather damage score in the 100 bird group was significantly higher than that in the 50 bird group. Stocking density had a significant effect on back feather damage ($P<0.01$). The mean of the back feather damage score in for 8 birds/m^2 density was significantly lower than that for 12 and 16 birds/m^2 densities. There was no difference between 12 and 16 birds/m^2 densities. It was found that the back feather damage score in the 50 bird group with 8 birds/m^2 density was the lowest of all 6 treatments. There was no difference found among the other treatments.

Even though the interaction between stocking density and group size was found for the wing feather damage score ($P<0.05$), the effect of stocking density was not significant either in the 50 or the 100 bird group. When the means of the 6 treatments were compared, it was found that the wing feather damage score in the group of 50 birds with 16 birds/m^2 density was significantly lower ($P<0.05$) than that for the 50 bird group with 12 birds/m^2 density. There was no difference between 8 and 16 birds/m^2 densities in the 50 bird group. Moreover, the wing feather damage score in the 50 bird group with 16 birds/m^2 density was similar to that of the 100 bird group at 3 levels of stocking densities. The wing feather damage score in the 50 bird group with 12 birds/m^2 density was significantly higher than that for the 100 bird group with 8 bird/m^2 density ($P<0.05$).

The mean of the leg feather damage score of chickens in 8 birds/m^2 density was significantly lower than that for the other densities ($P<0.05$). There was no difference between 12 birds/m^2 and 16 birds/m^2 densities. There was no interaction between stocking density and group size for this trait. The mean of the rump feather damage score of chickens in 8 birds/m^2 density was significantly lower than that of the chickens in 12 birds/m^2 density ($P<0.01$). But there was no difference in rump feather damage scores between the chickens in 8 and 16 birds/m^2 densities, and 12 and 16 birds/m^2 densities. There was no interaction between stocking density and group size for this trait.

The means of the total feather damage score of chickens was not affected by group size. The means of the total feather damage score of chickens in 12 birds/m^2 density was significantly higher than that for those in 8 birds/m^2 density ($P<0.01$). There was no difference in the total feather damage score of chickens between 8 and 16 birds/m^2 densities, and 12 and 16 birds/m^2 densities. Interaction between group size and stocking density was found ($P<0.01$). The means of the total feather damage score of chickens in the 50 bird group with 8 birds/m^2 density was the lowest among all the treatments. The highest mean was found in the 50 bird group with 12 birds/m^2 density. However, it was not significantly higher than that for the 50 bird group with 16 birds/m^2 density, and for the 100 bird group with 12 and 16 birds/m^2 densities.

The mean of primaries feather damage score of the chickens in 12 birds/m^2 density was significantly higher than that of the chickens in 8 birds/m^2 density ($P<0.05$), but similar to that of the birds in 16 birds/m^2 density. However, the difference of primaries feather damage scores between the chickens in 8 and 16 birds/m^2 densities was not significant. There was no significant interaction between stocking density and group size for this trait.

The mean of the tail feather damage score of chickens in the 100 bird group was higher ($P<0.01$) than that for the chickens in the 50 bird group. The effect of stocking density was not significant. It was found that the tail feather damage score in the group of 50 birds with 8 birds/m^2 density was the lowest of all the treatments. The tail feather damage score in the 100 bird group with 8 birds/m^2 density was similar to that in the 50 bird group with 12 and 16 birds/m^2 densities, and in the 100 bird group with 12 birds/m^2 density as well. The tail feather damage score for the 100 bird group with 16 birds/m^2 was lower than ($P<0.01$) that for the 100 bird group with 8 birds/m^2 density.

Actually, the feather damage scores in breast, back, wing, leg, vent and rump of Thai crossbred chickens were not high in each treatment. There was no perfect tail or primaries in either group. With the increasing of group size, the feather condition would be severe (Bilcík and Keeling, 1999). Most feather pecking activities occurred in the large group size and there was some evidence of an increasing frequency of aggressive pecks with increasing group size (Bilcík and Keeling, 2000). However, group size did not have statistically significant effects on feather damage scores, except on back feather damage, in our study. It is possible that group size was not large enough to cause severe feather damage to our experimental chickens.

Our findings lend support to those of Hansen and Braastad (1994) who found that the birds in low density groups have better plumage condition. Increasing stocking densities result in increasing feather damage and cannibalism (Mench and Keeling, 2001). However, the total feather damage scores indicated that the Thai crossbred chickens raised in 12 birds/m^2 density had the worst feather condition rather than those in the high density group.

Table 4.11 Effects of stocking density (D) and group size (GS) on body feather damage score in Thai crossbred chickens at the 12 weeks of age[1]

GS (birds)	D (birds/m^2)	Breast	Back	Wings	Legs	Vent	Rump	Total Body	Primaries	Tail
50	8	1.44	1.71^a	2.02^{ab}	1.42	1.04	1.16	8.80^a	1.93	1.24^a
	12	1.51	3.09^b	2.18^b	1.67	1.09	1.78	11.31^c	2.00	1.64^{bc}
	16	1.51	2.89^b	1.93^a	1.71	1.04	1.56	10.64^{bc}	1.87	1.62^{bc}
100	8	1.29	3.11^b	1.96^a	1.24	1.02	1.44	10.07^b	1.80	1.80^c
	12	1.40	3.22^b	1.96^{ab}	1.53	1.07	1.76	10.93^{bc}	1.95	1.64^{bc}
	16	1.38	2.76^b	2.09^{ab}	1.67	0.00	1.58	10.47^{bc}	1.90	1.58^b
ANOVA	GS	NS	**	NS	NS	NS	NS	NS	NS	**
	D	NS	**	NS	*	NS	**	**	*	NS
	GS×D	NS	**	*	NS	NS	NS	**	NS	**
	SE	0.03	0.03	0.02	0.03	0.01	0.03	0.02	0.01	0.07

[1]Data are expressed as means;
GS×D=interaction between group size and stocking density;
[a,b,c] means within the same column with different superscripts showed a significantly difference ($P<0.05$);
*$P<0.05$; **$P<0.01$; NS=$P>0.05$.

4.4 Spatial distribution

The most of chickens preferred to stay in wall area at the 12 weeks of age. Group size had significantly affected the mean number of chickens to distribute in feeding area ($P<0.05$), perching area ($P<0.01$) and wall area ($P<0.01$) (Table 4.12) at the 12 weeks of age. The distribution in drinking area of chickens was not affected by group size. The high number of chickens distributed in feeding, perching and wall area was in the 100 bird group. Stocking density did not affect the number of chickens distributed in feeding, drinking, perching and wall area. There was an interaction between group size and stocking density in spatial distribution in feeding area ($P<0.05$) not in other areas. The high number of chickens in feeding area was in the 100 bird group with 16 birds/m^2 density. It was significantly higher than other treatments except in the 100 bird group with 12 birds/m^2 density ($P<0.05$). No difference among the 50 bird group with 3 levels of stocking density, and the 100 bird group with 8 and 12 birds/m^2 densities was found for this trait.

The observation scan started after feeding about 2 hours, Thai crossbred chickens stayed at feeding area it was possible they was motivated to eat or explore. We still found that the chickens in large group size with high density preferred to stay in feeding area more than in other densities. This result was contrary to Arnould and Faure (2004) who found that the broiler chickens at low density (2 birds/m^2) preferred to stay and lie near drinker and feeders.

Different from the experimental design of Buijs et al., (2010), we proved perching area for Thai crossbred chickens in order to decrease the crowding effects caused by group size and stocking density, in addition, to improve the health and welfare of domestic fowl. It is possible that exercise in the form of perching may also reduce the impact of leg problems in broilers and avoid disturbances from con-specifics (Pettit-Riley and Estevez, 2001).

Environmental enrichment is constrained by financial costs and time demands on caretakers, and providing live prey to enrich the environment of predators raises ethical concerns (Newberry, 1995). We set the plastic materials to block the visual of chickens between neighbor pens. At the same time, chickens pecked the plastic materials in order to decrease feather pecking damage by pen mates. Therefore, that was the reason why the high number of Thai crossbred chickens stayed at wall area was the highest compared with other areas.

Buijs et al., (2010) found that the broilers stocked at medium to high density showed a preference for the area along the walls, but this effect was not observed at low densities. The increased use of the wall area may be an indicator that birds are experiencing crowding. The preference for the wall area at higher densities is more likely to result from avoiding disturbance by con-specifics than from seeking cover from predators.

The number of chickens increased in high stocking density especially in small group size rather than in large group in perching area. These results agree with the findings of Martrenchar, Huonnic, Cotte, Boilletot and Morisse (2000) who reported the percentage of perching birds increased with age and density. The chickens preferred to stay in perching area indicated that they might be relative to prefer the best ventilation in the pen (Pettit-Riley and Estevez, 2001).

A better understanding of the motivation of broiler chickens to go and remain in a different part of the pen could help to adapt the organization of chicken rearing areas on farms in the future. Uneven distribution also seems to occur in broiler chickens reared under such commercial conditions. However, more information is needed for situations where facilities are arranged as near as possible to commercial conditions (Arnould and Faure, 2004).

Table 4.12 Effects of stocking density (D) and group size (GS) on spatial distribution (number of chickens) in Thai crossbred chickens at the 12 weeks of age[1]

GS (birds)	D (birds/m²)	Feeding area	Drinking area	Perching area	Wall area
50	8	8.33^{a}	3.67	6.67	30.00
50	12	8.33^{a}	1.67	14.00	28.33
50	16	6.67^{a}	2.67	19.00	20.67
100	8	8.00^{a}	3.33	25.67	61.00
100	12	10.00^{ab}	3.33	32.00	54.00
100	16	13.30^{b}	7.00	26.00	53.67
ANOVA	GS	*	NS	**	**
	D	NS	NS	NS	NS
	GS×D	*	NS	NS	NS
	SE	1.28	1.35	3.60	4.06

[1]Data are expressed as mean;
GS×D: interaction between group size and stocking density;
[a, b] means within the same column with different superscripts were significant difference (P<0.05);
*P<0.05; **P<0.01; NS=P>0.05.

CHAPTER V

CONCLUSION

In the present study, both stocking density and group size affected FI only. Group size alone affected FCR. Out of these, there were no effect of group size and density on BW, BWG and mortality of Thai crossbred chicken. Thus, it is safe to say that Thai crossbred chickens could be stocked up to 16 birds/m^2 without any adverse effects on productivity. The frequencies of the most general behaviors of Thai crossbred chickens were affected by neither stocking density nor group size. Group size rather than stocking density affects feather pecking and aggressive behaviors of Thai crossbred chickens. Although Thai crossbred chickens in high stocking density had a longer tonic immobility duration, a little bit higher feather damage score, they showed a lower H:L ratio level than that of chickens in low stocking density. Moreover, no leg problem and fluctuating asymmetry were found in each treatment. According to the statements of welfare of chickens, the welfare of Thai crossbred chickens in high density was not worse than in low density. In addition, with increased group size, the spatial distribution of Thai crossbred chickens was different.

The author expected the experiment results could be applied for small scale chicken farms. Actually, group size of 50 and 100 birds are easy to manage by small scale chicken farmers.

According to the behavioral observation, Thai crossbred chickens used in this study were not as aggressive as general Thai fighting cocks. Moreover, the frequency of feather pecking in Thai crossbred chicken was not quite high. It might be relative to use environmental enrichment such as perching area and pecking materials for chickens. So it was recommended to use environmental enrichment for raising Thai crossbred chickens in order to avoid the feather pecking or crowding situation caused by increasing stocking density in the future chicken raising.

The high level of stocking density used in this study (16 birds/m^2) was not high enough to cause any adverse effects on Thai crossbred chickens. Moreover, the results of spatial distribution that the higher proportion of chickens stayed in the wall area rather than evenly spread all over the pen area indicated that the pen area was more than enough for the chickens. Therefore further research on higher stocking density of Thai crossbred chickens is needed.

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