ABSTRACT

Title Thesis: **EFFECTS OF MARKS ON AGGRESSION AND STRESS IN THE DOMESTIC FOWL (*GALLUS GALLUS DOMESTICUS*)**

Rachel Lynn Dennis, Master of Science, 2004

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Animals are marked for identification in a range of experiments from behavior and wildlife management, to reproduction and pharmacological studies. However, little is known of the impact marks have on animals. The objective of our experiment was to determine the impact of marking on aggression and stress in the domestic fowl. Broilers, in groups of 10 and 50 were housed with 20%, 50% or 100% of the birds marked. Aggressive interactions, given and received, were observed from 3 to 10 wks. Our results revealed that marked birds received significantly more aggression than unmarked birds, and subsequently deliver fewer aggressions to pen mates. Marked birds appear more stressed than their unmarked pen mates, especially in 20% pens. Marked birds in 20% pens also had a lower epinephrine response to manual restraint. Our findings show that marks can impact both the aggressive behavior and stress of the birds bearing them.

**EFFECTS OF MARKS ON AGGRESSION AND STRESS IN THE
DOMESTIC FOWL (*GALLUS GALLUS DOMESTICUS*)**

By

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Chapter 1: Literature Review

1.1 Aggressive behavior in the domestic fowl

Aggression in the domestic fowl (*Gallus gallus domesticus*) reared in commercial conditions is of importance from both welfare and production stand point. Alleviating aggression would increase the quality of life of birds normally subjected to aggression. Aggressive interactions may also cause a loss of productivity, and birds may be bruised or otherwise injured from agonistic encounters. Increased aggression is also of great concern for the well being of the birds.

Aggressive behavior in domestic fowl is most often analyzed in close relationship with social dominance (Candland et al. 1969). The majority of aggressive interactions within a group, which has an established dominance hierarchy, have been shown to be directed toward the subordinate individuals (Guhl, 1969). Aggressiveness has been linked to several traits including previous experience, body mass and comb size (Guhl and Ortman, 1953; Cloutier and Newberry, 2002) as well as familiarity or lack there of, of the participants (Beaugrand and Zayan, 1985; Blanchard et al., 1988). Familiarity was determined to be the leading factor influencing dominance by Beaugrand and Zayan (1985). A winning experience in a recent confrontation with another individual was found to be the second most important factor to influence dominance and the outcome of future

aggressive encounters (Hsu et al., 1999; Hsu et al, 2001; Beacham, J. L., 2003). It has been shown that prior loosing experience is correlated with the likelihood of loosing in subsequent encounters (Hsu et al., 1999; Cloutier and Newberry, 2000; Hsu et al., 2001). It is possible that a losing cycle could be maintained by increased stress caused by defeat.

1.2 Effect of group size on aggression

Estevez et al. (1997) and the model proposed by Pagel and Dawkins (1997) both state that when group size is large, social hierarchies are not established because it is no longer an efficient strategy. In large groups the cost of establishing the relationship with another individual severely outweighs the benefit when the chance of encountering that individual again becomes low (Pagel and Dawkins, 1997). In addition the number of pair wise relationships that would need to be established becomes increasingly great with increasing group size (Chase, 1985). The model of social dominance hierarchy proposed by Pagel and Dawkins (1997) suggests that the establishment of such hierarchies is only beneficial when there is a high likelihood of re-encountering the familiar birds. Therefore, dominance hierarchies will not be established in social situations in which the chances of encountering familiar birds are low. The tolerant hypothesis proposed by Estevez et al. (1997) suggests that broiler chickens show plasticity of social behavior dependent on their environment, increased tolerance is apparent at larger groups. They proposed that a tolerant strategy is more economical at large group sizes. Similarly, Hughes et al. (1997) found that the

apparent lack of individual recognition in large flocks of 700 birds minimized the overall number of aggressive interactions within the group when compared to smaller group sizes of 300 birds. This phenomenon causes significant reduction in the aggressive interactions required to establish dominance relationships with increased GS.

In a study by Estevez et al. (2002) levels of aggressive interactions at a food patch, were determined at four different group sizes (15, 30, 60, and 120), while maintaining the same density in all groups. They found that aggressive behavior around the food patches declined as group size increased from 15 to 120. Utilizing the same group sizes (Keeling et al., 2003) found that intermediate group sizes might be problematic in terms of incidence of behavioral problems. Hens in group sizes of 30 were found to be smaller in body mass and produce significantly smaller eggs, possibly indicating an increase in social disruption. They proposed that group sizes around 30 birds could represent an intermediate group size between coping tactics for small (simulating natural group size) and large group sizes. Nicol et al. (1999) examined birds in four group sizes, ranging from 72 to 368 birds, at varying rearing densities. They found that aggressive interactions, again, were most frequent in the small group sizes, and in larger flocks a non-aggressive tolerant tactic was adopted. These studies show that regardless of whether density is constant or varying among group sizes, birds maintained at larger group sizes are characteristically less aggressive than when maintained at smaller group sizes. The only exception to this is

in intermediate group sizes of about 30, between the natural state and large group sizes.

Studies on use of space in the domestic fowl in large flocks have shown that birds do not establish territories which are characteristic of smaller flocks (Keeling and Savenije, 1995). It has also been found to be much more difficult to control detrimental social interactions, such as aggressive behaviors, in large flocks (Appleby et. al. 1992). Group size has been shown to affect behavioral and physiological traits in group housed fowl. As group size increases birds show increased mortality and damage to feathers and skin, reduced egg production and smaller eggs, reduced body mass, increased fear response, but fewer aggressive interactions (Hughes and Duncan 1972; Adams and Craig, 1984; Tauson, 1998; Bilcik and Keeling, 1999; Nicol et al., 1999; Keeling et al., 2003; Estevez et al., 2003).

1.3 Differential aggression based on recognition and physical "badges"

McBride (1964) defined a social group as a "unit of lowered intraspecific aggression, which requires recognition of others." However, little is understood about the way in which birds perceive one another in large groups. It has been shown that, in large flocks, birds do not appear to recognize conspecifics as familiar or unfamiliar when compared to birds in small flocks (Hughes et. al. 1997). The number of conspecifics, which a bird can recognize has not been definitively established. Guhl (1953) determined that hierarchies did exist in flocks up to 96 birds; however

individual recognition was not determined. Douglis (1948) showed that hens had the ability to recognize up to 27 individuals.

Aggressive displays toward both familiar and unfamiliar conspecifics were extremely rare in large groups compared with hens in small group sizes (D'Earth and Keeling, 2003). Hens in large groups almost never initiated aggressive behaviors toward familiar birds and were 10 times less likely to initiate aggressive behaviors toward unfamiliar birds than were hens from small group sizes. D'Eath and Keeling (2003) determined that birds were far more likely to fight with an unfamiliar bird than a familiar one when housed in small groups of 10. In large groups with 120 birds, however, there was no difference in aggressive encounters between familiar and unfamiliar birds. Lindberg and Nicol (1996) showed that less familiar birds are more likely fight with greater frequency than will more familiar birds. Bradshaw (1991) proposed that the formation of dominance hierarchies within groups of fowl, and their propensity to show aggression toward unfamiliar birds, are probably related to individual recognition. Chickens housed in large commercial groups may be unable to establish large numbers of pair wise relationships and therefore they are unable to form dominance relationships with them all (McBride and Foenander, 1962). Pagel and Dawkins (1997) suggested that in large groups status signals take the place of active dominance relationships, suggesting that identifiable marks, or "status badges", may increase the likelihood of recognition of the social status of the bird, but not the individual itself.

In a study examining the effects of group size (Estevez et al., 2003) flocks of chickens of varying group sizes but containing the same number of marked focal birds were found to have decreasing frequencies of aggression given by the focal birds as group size increased as expected. However, the rate of aggression received by marked focal birds increased as group size increased. Since the number of marked birds remained unchanged as group size increased, the percentage marked within the group decreased with increasing group size. Hughes et al. (1997) reported a similar discrepancy between aggression received and given by marked focal birds. Estevez et al. (2003) suggest the possibility of a methodological bias causing marked birds to receive greater amounts of aggression from their flock mates. When marked individuals compose a small portion of the social group, the markings could distinguish them from the rest of the social group, causing them to be attacked with greater frequency.

Guhl and Ortman (1953) made alterations to the physical appearance of individual chickens by dying feathers, adding feather extensions and comb alterations. These chickens were then returned to their original flock and the reactions of the flock mates to these birds were noted. Altered birds, especially those with alterations to the comb and feather color, were met with significantly increased aggression from their prior subordinates as well as the dominants. A prior study by Guhl (1953) determined that prior subordinates attacked hens that had been removed and dubbed upon their return to the original flock. In an study by Marks et al. (1960) in which dubbed and non-dubbed pullets were intermingled, he found that dubbed

birds were relegated to the lower social status positions within the flock. Siegel and Hurst (1962) determined that flocks of dubbed chickens exhibited greater frequency of aggressive behavior than did flocks of non-dubbed chickens, suggesting that alterations to the physical appearance of birds increases the frequency of aggressive behaviors that they will receive. According to the social peck order in the domestic fowl, the majority of pecks during aggressive encounters are directed toward birds at the bottom of the social order (Guhl 1969, McBride 1960). These results suggest that the social order of birds can be altered by an increase in physical differences among individuals within the group.

1.4 Kin Selection and Filial Imprinting

As precocious young, newly hatched chicks are immediately exposed to conspicuous stimuli. The young chicks will quickly establish social preferences toward these stimuli. This early learning process (known as filial imprinting) (Cook, 1993; Bolhuis, 1999) is divided into three main processes: analysis of the features of the stimulus, recognition of the features or feature combination as a familiar stimulus, and association and implementation of the appropriate filial response (Bateson and Horn, 1994). Ultimately, kin recognition and altruism depend on the indicators present in the population. Visual and olfactory cues are often shown to be utilized by individuals for this purpose (Hamilton 1964, Keller 1997).

Male alliances have been studied in many species and shown to be a result of kin selection or degree of relatedness. Populations of bottlenose dolphins have been shown to form male alliances almost solely characterized by maternal relatedness (Parsons et al., 2003). In studies of tadpoles (Waldman, 1985) individuals were found to orient toward siblings in preference to non-siblings. However, in populations in which there may be a low probability of correctly identifying kin, there may be a decrease in the frequency of otherwise normally kin-biased behavior (Keller, 1997). In other words, in populations whose members are all closely related and have little phenotypic variation, there could be little evidence of selection for certain individuals over others within the population. Commercial flocks of domestic fowl are likely closely related and show small of phenotypic difference within the flock. However, the alteration of appearance of certain members such as by artificially marking the birds would incorporate the phenotypic variability required for kin selection to be a dominant force in motivating altruistic behaviors.

Kin recognition has been documented in domestic chicks. Chicks have been shown to have the ability to learn to differentiate between two different calls made by broody hens (Halpin, 1991). They also exhibited preferences for their own mother's call and for those calls which were paired with moving stimuli. Discrimination and preference for the mother's call has been documented in several other species of birds (Stonehouse, 1960; Rowley, 1980; Halpin, 1991). Early imprinting in the domestic chick has been shown to be an irreversible process, which leaves the bird with a

preference for associations with conspecifics most similar in appearance to those with which the individual was imprinted (Johnson, 1991).

In a study of adopted gull chicks (Bukacinski et. al. 2000), chicks were rejected less frequently by adopting neighbors when a higher degree of band-sharing, a measure of genetic similarity, existed between the chick and the adopted parents. This indicates that neighboring gulls were more likely to reject a chick that was more distantly related. The means by which an individual can detect familiality in an unknown individual are not fully understood, but it has been shown that more closely related individuals are generally closer in physical appearance, among other things, than more distantly related individuals (Hamilton 1964, Hancock and DeBruine, 2003). Jaisson (1991) suggested that increased aggression can be instigated by the perception of differences between individuals.

1.5 Stress and Animal Welfare

Stress, from a physiological perspective, is not inherently bad. It is the type of stressor and the degree of stress, which can cause problems for the organism (Haller et al., 1997; Mostl and Palme, 2002). Unfortunately, there is no single means of measuring stress. The physical and social environment of the animal can cause stress. The animals' environment is dynamic, and the birds are constantly adjusting to the stress of an ever-changing environment through behavioral and physiological adaptation (Mostl and Palme, 2002). Numerous studies have shown aggression and

dominance order to be affected by the stress of an individual (Matt et al., 1996; Hennessy et al., 2002; Wood et al., 2003). Male rats have been shown to have significantly fewer aggressive encounters with conspecifics following exposure to acute stress when compared with males that were not exposed to the stressor (Wood et al., 2003). However, in a study of aggressiveness in males using rats, they found that males exposed to a chronic stressor exhibited significantly more aggressive behaviors compared with control rats, as seen previously in other species (Mostl and Palme, 2002).

Animal welfare has become an area of increasing interest and importance to the public and industry. The drive to raise animals in a less stressful environment has prompted researchers to delve more deeply into the causes of stress on animals in production systems and the means by which it can be alleviated.

Environmental manipulation and breeding programs for increasing production traits have come into public concern. Selection for production traits in commercial flocks, such as increased body mass, egg size and egg number may be negatively impacting animal welfare. Nicol et al. (1999) found that birds reared in smaller flock sizes and lower stocking densities had the highest egg production. Several studies have shown that broilers reared in smaller flock sizes have increased body mass (BM) (Estevez et al., 1997). However, in order to increase the overall production and lower production costs, there is a push to maintain birds at larger flock sizes and increased stocking densities. Through genetic manipulation, broilers have increased in BM,

growth rate and yield greatly over the past several decades. However, birds now

suffer increased leg problems due to the extra weight they must carry which is an

important welfare concern (Kestin et al., 1992).

1.6 Indicators of Stress

Fluctuating Asymmetry (FA) portrays the subtle, random deviations from

symmetry in characters that ideally possess bilateral symmetry (Ludwig, 1932;

Palmer and Strobeck, 1986; Allenbach et al. 1999). Stress, both genetic and

environmental, is believed to be the major contributing factor to increased FA

(Palmer and Strobeck, 1986; Parsons, 1990; Møller et al. 1995).

Fluctuating asymmetry has been positively correlated with an individual's low

capacity to safeguard against stress, both environmental and genetic (Yngvesson and

Keeling, 2001). In cannibalistic attacks in laying hens Yngvesson and Keeling (2001)

found that cannibalism victims were found to exhibit greater amounts of FA.

Similarly, Cloutier and Newberry (2002) analyzed the ornamental traits of laying hen

victims of cannibalistic attacks. They divided the victims into attacks on the head and

neck area, and attacks to other body parts. They found that victims of cannibalistic

attacks to the head and neck displayed larger combs relative to their flock mates.

However, they also found that victims of attacks to the head and neck area had a

higher degree of FA of the metatarsus and higher composite asymmetry. FA

measurements were also assessed in two genetically selected lines of Japanese quail

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(Satterlee et al., 2000). The two lines were selected by their low stress and high stress plasma corticosterone response to restraint. Both diameter and length of the shank was measured for FA assessment. A significant increase in bilateral asymmetry of the shank length was found in the quail bred for exaggerated stress response.

Tonic Immobility (TI) is a response to a brief period of physical restraint which is characterized by a reduced responsiveness "catatonic-like" state (Jones, 1986). Typically the bird is laid upside down in a U- or V-shaped cradle and restrained by light pressure on the sternum for a brief period. After this initial restraint the bird voluntarily remains in the characteristic catatonic-like state for a varied period of time afterward. This reaction is thought to be an evolutionary adaptation to predation, in which the bird would enter the immobile state after it was caught until the predator lightened or let go of its grip, giving the bird a chance to escape (Jones, 1996). However, TI is also used to show the level of stress related to the fear response of an individual (Jones, 1986). A long duration of immobility is indicative of a high level of fear.

Body mass (BM) is a common measure of the level of stress of an individual used in many experiments with domestic fowl (Bilcik and Keeling, 1999; Nicol et al., 1999; Keeling et al., 2003). BM has been shown to vary with the stress of an individual. Estevez et al. (1997) showed that BM decreased with increasing group size from 50 to 200 birds. They also showed that this phenomenon was not due to monopolization of resources by dominant individuals. They suggested a number of

stressors that could be responsible for this trend such as increased levels of disturbance while resting, reduction of thermoregulatory capacity and deterioration of the environmental quality.

1.7 Hormones and mediation of stress and aggression

Numerous researchers have evaluated the behavior and endocrinology of aggressive and docile strains of a domestic fowl (Keer-Keer, et. al. 1996, Korte et. al. 1997, Hocking, et. al. 2001). There have been far fewer studies of the hormone levels of docile and highly aggressive individuals within the same strains. Do the behavior and hormonal patterns of the aggressors in a common strain match those of an aggressive strain?

Behavioral and physiological responses to stress are mediated by the hypothalamic-pituitary-adrenal (HPA) axis and the sympatho-adreno-medullary system (Mostl and Palme, 2002; Wood et. al., 2003). The adrenal gland and central and peripheral nervous systems increase secretion of glucocorticoids and/or catecholamines in response to unfavorable stimulations. Epinephrine (EP) is synthesized and stored in the adrenal medulla and released to the body via the systemic circulation. Norepinephrine (NE) is synthesized and stored in the peripheral nerve endings. Both EP and NE also act as neurotransmitters of the central nervous system (CNS). Dopamine (DA) is synthesized and stored in the periventricular nucleus (PVN) of the hypothalamus and acts as a neurotransmitter of the CNS.

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Catecholamines are fast acting hormones used by the body to cope with stress and are indicative of acute stress. Hormonal catecholamines, EPI and NE, are connected to the body's metabolic and cardiovascular preparation for an impending fight (Haller et al., 1997). Plasma DA has been found to be involved in the control of behavior and an organism's ability to cope with stress (Haller et al., 1997; Driscoll et al., 1998; Kuikka et al., 1998; Mostl and Palme, 2002).

Aggression and dominance order are strongly associated with the stress of the individual (Harding and Follet 1979, Matt et. al., 1996, Hiebert et. al., 2000, Hennessy et. al., 2002). Hormonal parameters are often used to determine the effects of the physical and social environment on the animal's stress levels, glucocorticoids being most commonly used stress indicators (Mostl and Palme, 2002). However the key hormones used in overcoming stressful situations are both the glucocorticoids and the catecholamines (Mostl and Palme, 2002). Since corticosterone (CORT), the predominant glucocorticoid in birds, has a feedback system involved in aggressive behavior (Kruk *et al.,* 1998), catecholamines should be used as an additional indicator of stress. Mabry et al., (1995) showed that aged rats have exaggerated sympathetic-adrenal-medullary responses to acute swim stress at low temperatures. Mabry measured both EP and NE plasma concentrations. Korte, *et al.*(1997) described two lines of layers with distinct reactions to stress; active and passive behavioral responses. They found that the passive response strain had a significantly higher basal plasma concentration of CORT, while there was no significant difference in basal NE or EP. Contrarily, in response to an additional manual restraint stressor, the

active response strain showed higher NE levels. Kruk, et al., (1998) suggested that the increase in stress hormone levels, such as that of CORT, was due to the stimulation itself and not caused by the stress of fighting. Japanese quail that had been selected for short TI, or low fear response, had relatively low concentrations of CORT in response to a stressor than did those birds selected for long TI, or high fear response (Launay, 1993 as cited in Korte et al., 1997). These results show that hormonal indicators of stress are better looked at in combination, as suggested by Mostl and Palme (2002).

Intermingling laying hens of different strains was also shown to increase the social stress of the birds (Cheng et al., 2002). Hens of different strains selected for high and low group productivity and survivability (HGPS and LGPS, respectively), when intermingled with those of the Dekalb XL strain (a commercial strain), were found to have altered DA and CORT concentrations. LGPS hens had an increased DA and CORT responses, when housed with hens from a different strain, compared with HGPS hens. Layers from the Dekalb XL strain were also found to have an increased CORT response to intermingled housing, when compared to the high survivability strain.

Sgoifo et al., (1996) analyzed EPI, NE and CORT responses in rats of various levels of aggressiveness, to both social stress (a defeat experience in an aggressive encounter with an alien male) and nonsocial stress (presentation of a shock-prod). Social stress was found to induce a much greater catecholaminergic and

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glucocorticoid effect than did the nonsocial stress. Aggressiveness was defined as the latency to attack in an inevitable agonistic encounter. Comparison of high and low aggressive animals determined that the more aggressive the animal, the greater the chatecholaminergic reactivity to both social and nonsocial stressors.

Catecholaminergic reactivity to stress was measured in male tree lizards by Matt et al. (1996). They discovered a significant and rapid increase in EP, NE and DA in free living males as a response to a manual restraint stressor. From the results of this experiment they suggested that catecholamines measured following restraint requires that time to bleed be used as a covariate. Concentrations of EPI and NE increased 10 to 30 times their basal levels during aggressive encounters and remained high for a period following the encounter. DA was found to be slightly higher during and following aggressive encounters, but not significantly different from baseline levels. Variability of DA was considerably high and animals were not separated into winner and loser categories for analysis.

An experiment on the behavior and hormone levels in cats (Kojima et al., 1995) showed that NE increased only with threats but not with restlessness, while EP and cortisol (the predominant glucocorticoid measured in most mammalian species) levels increased with both threats and restlessness. This suggests that different catecholaminergic responses between strictly active behaviors and aggressive behaviors. Porta et al. (1995) showed that rats previously exposed to high levels of EP had higher dopaminergic reactions to later stress than did those without previous

exposure. The pretreated rats were also found to have significantly decreased free NE

concentrations and significantly increased free EP concentration. Their results

indicate that in addition to having different mediating abilities on the body's ability to

cope with stressful situations, there may be a feedback loop between catecholamines.

Epinephrine concentrations are of high economic importance in the poultry

industry as it has been linked to productivity and survivability in laying hens (Cheng,

et al., 2001). Cheng showed that although there was no significant difference in NE

levels alone between high and low group productivity and survivability (HGPS and

LGPS, respectively); the ratio of EP to NE was greater in the low group productivity

and survivability. These results indicate that the sympathetic-adreno-medullary

system is linked to the welfare and productivity of birds.   HGPS birds have been

suggested to have a unique coping capability to their domestic environment as well as

their survivability.

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Chapter 2: The Effects of Artificial Marking on Aggression

2.1 Abstract

Animals are often marked in a wide range of experiments from behavior and wildlife management, to reproduction and pharmacological studies for identification. However little is know regarding the consequences that marks may have for the animals, particularly when only a small proportion of group members received them. The objective of our investigation was to determine the impact of marking on the level of aggressive interactions in the domestic fowl (*Gallus gallus domesticus*). Specifically we wished to determine whether the effects may be different at different experimental group sizes and when different proportions of individuals are marked. We hypothesized that aggression to marked bird will increase as proportion of marked birds in the group decreases and these effects will be evident in both large and small GS We used 1260 one day old broilers that were divided into groups of 10 and 50. Within each of these group sizes we marked 20%, 50% or 100% (positive control) of the individuals. Each group size by percent mark treatment combination was replicated six times. Aggressive interactions of all individuals in the pen were observed from 3 to 10 wks of age and we recorded whether the bird giving or receiving the aggression was mark or unmarked. Our results revealed that, as hypothesized, marked individuals received significantly more aggression per bird than did their unmarked pen mates. Marked birds in both 20% and 50% groups received significantly more pecks (0.083 and 0.065 pecks/bird/10 min, respectively)

than the unmarked birds in 20% and 50% and marked birds in 100% groups (0.024, 0.021, and 0.033 pecks/bird/10 min, respectively, P<0.0001). Marked individuals in 20% groups also received significantly more threats (0.095 threats/bird/10 min) than their unmarked counterparts and the birds in 100% groups (0.045 and 0.052 threats/bird/10 min, respectively, P=0.0018). Moreover, marked individuals were also found to deliver significantly fewer aggressive acts when housed in groups of 50 birds than their unmarked pen mates. Pecks delivered by unmarked birds in groups of 50 during period 4 in both 20% and 50% pens (0.086 and 0.074 pecks/bird/10 min, respectively) were significantly higher than for marked birds (0.046 and 0.037 pecks/bird/10 min, respectively; P=0.0063). Our results provide strong evidence that marking affects the level of aggression received by the domestic fowl. However the differential results obtained when the totality of the group members are marked suggest that marked birds become a "target" only when some are marked.

Key Words: aggression, aggressive behavior, marking, domestic fowl, methodological bias

2.2 Introduction

In a study examining the effects of varying group size (Estevez et al., 2003) in flocks of the domestic fowl, but containing the same number of focal birds across group size (GS), focal birds were found to deliver fewer aggressive acts but to receive more as GS increased. In that study, since the number of marked individuals remained unchanged as GS increased, the percentage marked within the group

progressively decreased. Hughes et al. (1997) reported a similar discrepancy between aggression received and given by focal birds. Estevez et al. (2003) suggested that when marked individuals comprise a small portion of the group, the markings could distinguish them from the rest of the group members, causing them to be attacked at a greater frequency.

Early studies on the social behavior of the domestic fowl by different authors revealed that morphological changes impair individual recognition of group members. Guhl and Ortman (1953) changed the physical appearance of individual chickens by dying their feathers, adding feather extensions and comb alterations. These chickens were then returned to their original flock. Guhl and Ortman's results indicated that altered birds, and especially those with changes to the comb and feather color, were met with increased aggression by dominants but also by birds that previously were subordinate to the altered birds. Guhl (1953) demonstrated that subordinates attacked hens that had been removed and dubbed upon their return to the original flock. In a related study by Marks et al. (1960) in which dubbed and non-dubbed pullets were intermingled, dubbed birds had lower social status than intact birds. Similarly, Siegel and Hurst (1962) determined that flocks of dubbed chickens exhibited a greater frequency of aggressive interactions than did flocks of non-dubbed chickens, possibly due to increased difficulties with individual recognition of group members that were dubbed.

In a social hierarchy of domestic fowl, the majority of aggressive pecks are directed towards birds at the bottom of the social order (McBride, 1960; Guhl, 1969). It is also well established that less familiar birds fight with greater frequency than will more familiar birds (Lindberg and Nicol, 1996). The number of conspecifics that a bird can recognize has not been definitively established. Douglis (1948) showed that hens had the ability to recognize up to 27 individuals. Guhl (1953) determined that hierarchies existed in flocks of up to 96 birds. However, individual recognition was not assessed.

D'Eath and Keeling (2003) demonstrated that birds were far more likely to fight with an unfamiliar than a familiar bird when housed in small groups of 10. In large groups with 120 birds there was no difference in the frequency of aggressive encounters between familiar and unfamiliar birds. Bradshaw (1991) proposed that the formation of dominance hierarchies within groups of fowl and their propensity to show aggression toward unfamiliar birds are probably associated with individual recognition. Chickens housed in large commercial groups may be unable to recognize large numbers of individuals and therefore be unable to form stable dominance relationships (McBride and Foenander, 1962).

Estevez et al., (1997) and Pagel and Dawkins (1997) suggest that the establishment of a hierarchy is only beneficial in small groups when there is a high likelihood of encountering the same bird. Therefore, a dominance hierarchy will not be established in situations in which the chances of encountering the same bird

multiple times is low. Furthermore, Pagel and Dawkin's model (1997) suggests that, in large groups where individual recognition may be difficult, status signals take the place of active dominance relationships, and identifiable marks, or "status badges", may increase the likelihood of recognition of the birds' dominance status (Pagel and Dawkins 1997). It is possible, therefore, that artificial marks used for the purpose of individual recognition in behavioral studies may inadvertently act as negative "status badges" and affect the social relationships among birds within a group, particularly if the number of these marked birds is low in relation to the other, unmarked, group members.

The purpose of this study was to test the hypothesis that artificial marks were indeed a disruptive modification of the bird's appearance. We predicted that these marks would attract increased aggression from conspecifics. Subsequently, aggression given by these birds would be depressed. It is our contention that this effect would be more severe as percent marked decreased and would be consistent for all ages and GS.

2.3 Materials and methods

2.3.1 Animals and Management

See Appendix 6.1

2.3.2 Experimental Design

This experiment was set up as an incomplete 2 x 3 x 2 factorial design. Pens were set in a randomized complete block design, with seven complete blocks, to control for location within the house. All birds were divided on day one into 42 groups of 10 or 50 chicks (21 pens per GS). Each of these groups were then assigned randomly to one of three percentages of marking in which the number of total birds marked was 20%, 50%, or 100% of the group. The group containing 100% marked birds was our positive control group in which all individuals were marked, but none was different from the other group members. The third factor was the marking or lack of marking applied as a within pen treatment. Pen size was constant for all experimental group sizes; therefore bird density varied simultaneously with group size. Rearing densities maintained from day one through week 11, were 2.22 birds/m^2 and 11.11 birds/m^2 for groups of 10 and 50, respectively.

Marked birds were tagged using a dual marking system. Using the Swiftack[1] system we attached two laminated paper disks approximately 3.5 cm in diameter to either side of the neck of the bird. The disks were attached with a plastic filament. This is a fairly painless procedure and has been used in the past for individual bird identification with good results (Cornetto and Estevez, 2001). Laminated paper disks were used due to the light weight as well as the versatility to individual experimental needs and ease of replacement. The disks were numbered to distinguish between individual birds. All tag numbers used were double digit so that all birds carried

[1] Swiftack for poultry identification system. Heartland Animal Health, Inc. Fair Play, Missouri

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approximately the same amount of marking. Marked birds also carried an additional

mark on the back of the head area. The head mark was a large black dot

(approximately 5-6 cm in diameter) made with a non-toxic marker. Retagging

occurred only in sporadic occasions as tags fell out, but remarking of the head mark

was necessary every two weeks from five to ten weeks of age.

2.3.3 Observational Techniques

Behavioral observations were taken from the alley between the pens. The

observer stood at the front of the neighboring pen to interfere as little as possible with

the interactions of the birds. Pens within a block were assigned to 10 minute periods

for observation within the same hour. Observations across blocks were balanced for

time of day and day within each week. Each pen was observed for 10 minutes, 5

times every 2 wk period from 3 to 10 wks of age. Observations included all

aggressive behaviors given, aggressive behaviors received, and identification of any

tagged individuals involved in the interaction. Aggressive behaviors recorded are

described below. Observational data were recorded with the aid of a laptop computer

using the Observer software package from Noldus[2].

2.3.4 Behavioral Definitions

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[2] Noldus Information Technology. 1995. Version 3.0 Wageningen, The Netherlands

Behaviors observed (modified from Estevez et. al., 2002) were the following. Avoidance, one bird actively evades another bird. Avoidances recorded the two birds involved. Avoidance was counted toward the bird actively evading a conspecific. Times avoided was counted toward the bird which was being avoided, when the object of avoidance was apparent. Avoidance were considered received aggressions because they imply a lack of aggressive propensity. Times avoided were considered with given aggressions because they imply a propensity for aggression as a possible reason for conspecifics to avoid it. Chase was recorded when one bird took more than 2 steps following another bird in an aggressive manner. Fight was noted when at least one kick was delivered accompanied by at least one peck to the head. The fight was noted as given by the bird that delivered the peck in the fight, if both birds delivered pecks, both birds were noted separately as givers in the fight. Fight with peck to the body, was considered when at least one kick was delivered accompanied by at least one peck to the body or legs. Peck, at least one peck to the head was delivered. Peck to the body, at least one peck to the body was given. Peck to the tag, was recorded when at least one non-aggressive peck was delivered to the identification tag of a marked bird. Threat was considered when one bird stood with head held higher than the other bird and at times with the neck feathers raised.

2.3.5 Statistical Analysis

Aggressive behavior data were analyzed as a blocked factorial using a Mix Model repeated measure analysis of variance (ANOVA). Age of the birds was

considered the repeated measure and was fit to an appropriate covariance matrix for each behavior. Age of the birds was fit into 4 periods of 2 wks each from 3 to 10 wks of age. Variance partitioning was used to correct for heterogeneity of variance and log transformation was used when needed to correct for normality. Aggressive behaviors, both received and given by marked and unmarked individuals were compared across blocks, marked treatment, GS, and period. Treatment groups for the purposes of statistical analysis were a combination of the group size treatment, percentage of birds marked, and within pen treatment, whether the birds were marked or unmarked, making a total of 5 treatment groups (marked birds from 100% marked pens, marked birds from 50% marked pens, unmarked birds from 50% marked pens, and marked birds from 20% marked pens, and unmarked birds from 20% marked pens). Least square means were reported for all treatment groups, GS and periods. Contrasts were used to determine significance using the Sidak adjustment to maintain an experimental alpha of 0.05. Main effects and interactions were tested using contrasts for marked compared with unmarked (in 20 and 50% marked groups only), and for percentage marked between 20, 50, and 100% marked pens (using marked birds only). Comparisons were made within treatment group, GS, and period. Comparisons across treatment groups included comparisons within percent marked pens, each treatment to control, and marked across all percent mixtures as well as unmarked across all percent treatments. Data were analyzed using SAS 8.2 software[3].

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[3] SAS Institute Inc., 1999 Version 8.2 ed. Cary, NC

2.4 Results

2.4.1 Aggression received

Treatment by period interactions revealed that marked birds received significantly more fights (Table 2-1, Fig. 2-1) than unmarked birds in 50% pens and significantly more than the positive control (100% marked) during period 2. Unmarked birds in 20% pens received significantly less fights than the control in period 1. No significant differences were found in fights in periods 3 and 4, nor due to group size or on its interaction with other factors (Table 2-1).

Marked birds in both 50 and 20% pens were found to receive significantly more pecks than their unmarked pen mates (Table 2-1, Fig. 2-2) and the marked birds in the positive control group. No significant interactions were found between treatment and group size for pecks received. A significant GS by period interaction was found in the frequency of pecks received. Birds in GS 50 were found to receive significantly more pecks in period 4 than in period 2, while no differences were found in the other periods or in the group size of 10 (Table 2-2). Similarly, a significant treatment effect was found for threats received. Marked birds in 20% pens received significantly more threats than their unmarked pen mates (Table 2-1, Fig. 2-3) or the birds in the 100% pens, but not significantly more than marked birds in 50% pens. Frequency of threats was also higher for marked birds in 50% pens as compared with unmarked, however these frequencies were not significantly different from each other. A significant GS by period interaction was also found in threats received.

Birds in GS of 50 received significantly fewer threats per bird in groups of 10 in both periods 1 and 2. No significant difference was found between GS in periods 3 and 4, or within each GS across age (Table 2-1 and 2-2).

In GS 10, marked birds in 20% pens were found to avoid other birds significantly more than their unmarked pen mates (Table 2-1, Fig. 2-4), the marked birds of 50% pens, and the control birds. Marked birds in 20% pens were found to avoid conspecifics more frequently in GS 10 compared to GS 50 (Fig. 2-4). Analysis of avoidances revealed a significant GS by period interaction. Birds in GS 10 were found to avoid conspecifics significantly more frequently than birds in GS 50 during period 1. No significant differences were found among treatment groups in the other 3 periods. Birds in GS 10 avoided conspecifics significantly more often in period 1 than time periods 3 and 4 (Table 2-1 and 2-2).

Marked birds received significantly more pecks to the tag in 20% and 50% pens compared to the birds of 100% pens during the first period (Table 2-1, Fig. 2-5). Pecks to the tag in 20% and 50% pens were significantly more frequent during period one compared to the later periods; however, this difference across periods was not seen in 100% pens. No significant differences in pecks to tag were found during the other time periods (Fig. 2-5).

Other behaviors such as chase, peck to the body and fights with peck to the body were extremely rare. Means and standard errors are represented in Table 3.

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Significant differences were found only in the pecks to the body in 20% pens, in which marked birds received significantly more pecks to the body than their unmarked counterparts (Table 2-3).

Table 2-1 Model and ANOVA results for aggressive behaviors received.

Source	df	F	P
Fight			
Group Size (GS)	1,71	0.11	0.763
Treatment	4,71	4.89	0.0015
Treatment* GS	4,71	1.43	0.2343
Period	3,90	14.04	0.0001
Treatment*Period	12,126	2.50	0.0057
GS*Period	3,90	0.11	0.9561
Treatment*GS*Period	12,126	1.29	0.2303
Pecks	df	F	P
Group Size (GS)	1,44	0.65	0.4254
Treatment	4,111	16.83	0.0001
Treatment*GS	4,111	1.32	0.2687
Period	3,113	4.77	0.0036
Treatment*Period	12,153	1.24	0.2632
GS*Period	3,113	4.28	0.0066
Treatment*GS*Period	12,153	1.63	0.0899
Threats	df	F	P
Group Size (GS)	1,37	17.15	0.0002
Treatment	4,39	5.25	0.0018
Treatment*GS	4,39	0.46	0.7665
Period	3,64	2.20	0.0964
Treatment*Period	12,64	1.58	0.1134
GS*Period	3,64	4.09	0.0101
Treatment*GS*Period	12,81	1.70	0.0823
Avoidances	df	F	P
Group Size (GS)	1,44	15.62	0.0003
Treatment	4,44	6.76	0.0003
Treatment*GS	4,44	4.36	0.0047
Period	3,69	5.66	0.0016
Treatment*Period	12,89	1.42	0.1733
GS*Period	3,69	4.80	0.0043
Treatment*GS*Period	12,89	1.11	0.3610
Peck at Tag	Df	F	P
Group Size (GS)	1,30	8.27	0.0095
Treatment	2,20	5.85	0.0102
Treatment*GS	2,20	1.87	0.1806
Period	3,87	37.28	0.0001
Treatment*Period	6,87	4.94	0.0002
GS*Period	3,87	9.66	0.0001
Treatment*GS*Period	6,87	2.00	0.0740

Table 2-2 Mean ± SEM number of aggressive behaviors for period x GS interaction represented in number per bird in 10 min. AB letters represent significant differences (P<0.05) between GS of the same period. ab letters represent significant difference (P<0.05) between periods in the same GS.

Aggressions Received	GS	Period 1	Period 2	Period 3	Period 4
Peck	10	0.058 ± 0.0098^{Aa}	0.041 ± 0.0061^{Aa}	0.028 ± 0.0080^{Aa}	0.042 ± 0.0100^{Aa}
	50	0.048 ± 0.0098^{Aab}	0.025 ± 0.0061^{Aa}	0.045 ± 0.0078^{Aab}	0.074 ± 0.0096^{Ab}
Threat	10	0.099 ± 0.0157^{Aa}	0.122 ± 0.0236^{Aa}	0.067 ± 0.0138^{Aa}	0.058 ± 0.0102^{Aa}
	50	0.047 ± 0.0053^{Ba}	0.044 ± 0.0040^{Ba}	0.051 ± 0.0066^{Aa}	0.053 ± 0.0047^{Aa}
Avoidance	10	0.015 ± 0.0036^{Aa}	0.013 ± 0.0027^{Aab}	0.002 ± 0.0015^{Ab}	0.006 ± 0.0028^{Ab}
	50	0.004 ± 0.0011^{Ba}	0.004 ± 0.0014^{Aa}	0.004 ± 0.0010^{Aa}	0.001 ± 0.0005^{Aa}
Peck to	10	0.072 ± 0.0054^{Aa}	0.008 ± 0.0054^{Ab}	0.002 ± 0.0055^{Ab}	0.000 ± 0.0055^{Ab}
Tag	50	0.026 ± 0.0054^{Ba}	0.006 ± 0.0054^{Bb}	0.001 ± 0.0054^{Ab}	0.003 ± 0.0054^{Ab}
Aggressions Given					
Threat	10	0.089 ± 0.0142^{Aa}	0.098 ± 0.0120^{Aa}	0.062 ± 0.0144^{Aa}	0.063 ± 0.0105^{Aa}
	50	0.042 ± 0.0045^{Ba}	0.038 ± 0.0036^{Ba}	0.046 ± 0.0057^{Aa}	0.043 ± 0.0036^{Aa}
Times	10	0.029 ± 0.0063^{Aa}	0.018 ± 0.0048^{Aa}	0.004 ± 0.0032^{Ab}	0.008 ± 0.0038^{Aab}
Avoided	50	0.005 ± 0.0012^{Ba}	0.005 ± 0.0014^{Ba}	0.005 ± 0.0014^{Aa}	0.002 ± 0.0008^{Aa}

Table 2-3 Mean ± SE number of behaviors received per bird in 10 min. ab letters represent significant differences (P<0.05)

Behavior Received	100%	50%		20%	
	Marked	Marked	Unmarked	Marked	Unmarked
Chase	0.003 ± 0.002^a	0.005 ± 0.002^a	0.003 ± 0.002^a	0.006 ± 0.002^a	0.003 ± 0.002^a
Fight w Body Peck	0.0005 ± 0.0002^a	0.0005 ± 0.0002^a	0.0 ± 0.0002^a	0.0 ± 0.0003^a	0.0 ± 0.0003^a
Peck to Body	0.002 ± 0.002^{ab}	0.003 ± 0.002^{ab}	0.002 ± 0.002^{ab}	0.008 ± 0.002^a	0.0005 ± 0.002^b

Figure 2-1 Mean fights received (± SE) for each treatment and period. AB letters represent significant differences (P<0.05) between periods of the same treatment; ab letters represent significant differences (P<0.05) within the same period across treatments.
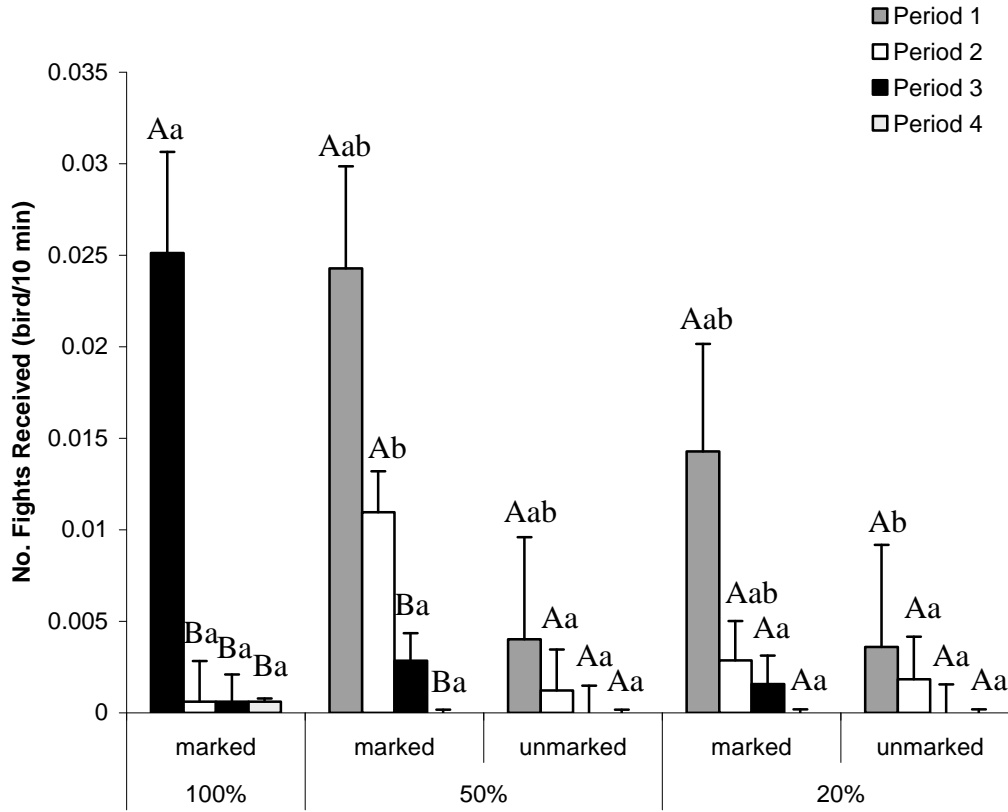
Figure 2-2 Mean pecks received (± SE) for each treatment. ab letters represent significant differences (P<0.05) across treatments.
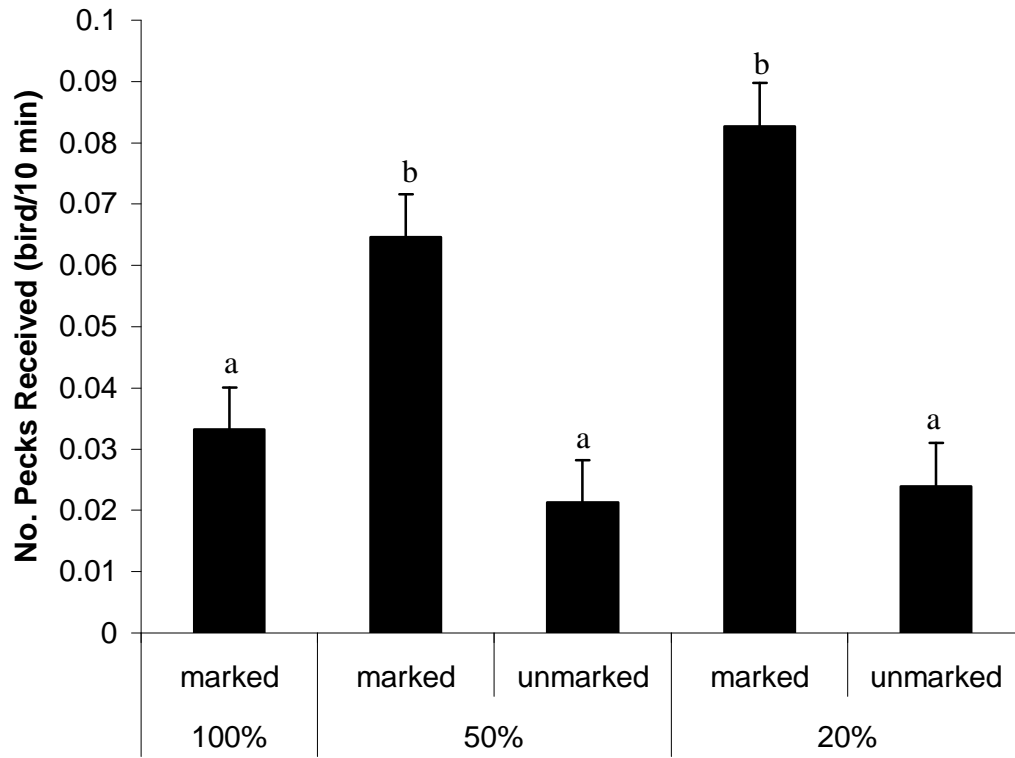
Figure 2-3 Mean threats received (± SE) for each treatment. ab letters represent significant differences (P<0.05) across treatments.
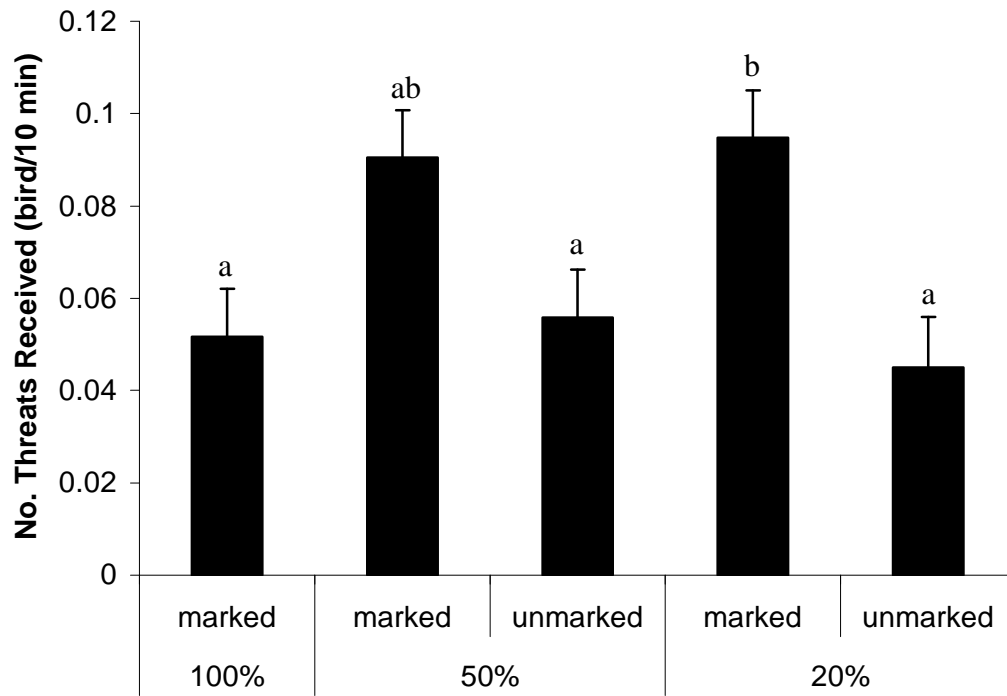
Figure 2-4 Mean avoidances (± SE) for each treatment and GS. AB letters represent significant differences (P<0.05) between GS of the same treatment; ab letters represent significant differences (P<0.05) within the same GS across treatments.
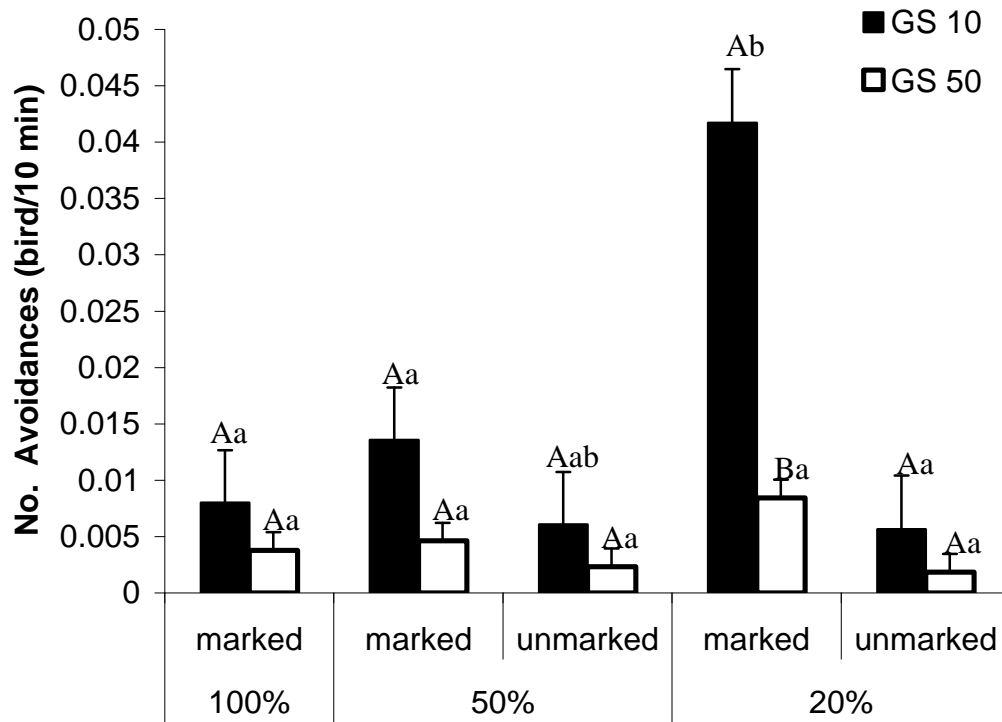
Figure 2-5 Mean pecks to tag received (± SE) for each treatment and period. AB letters represent significant differences (P<0.05) between periods of the same treatment; ab letters represent significant differences (P<0.05) within the same period across treatments.
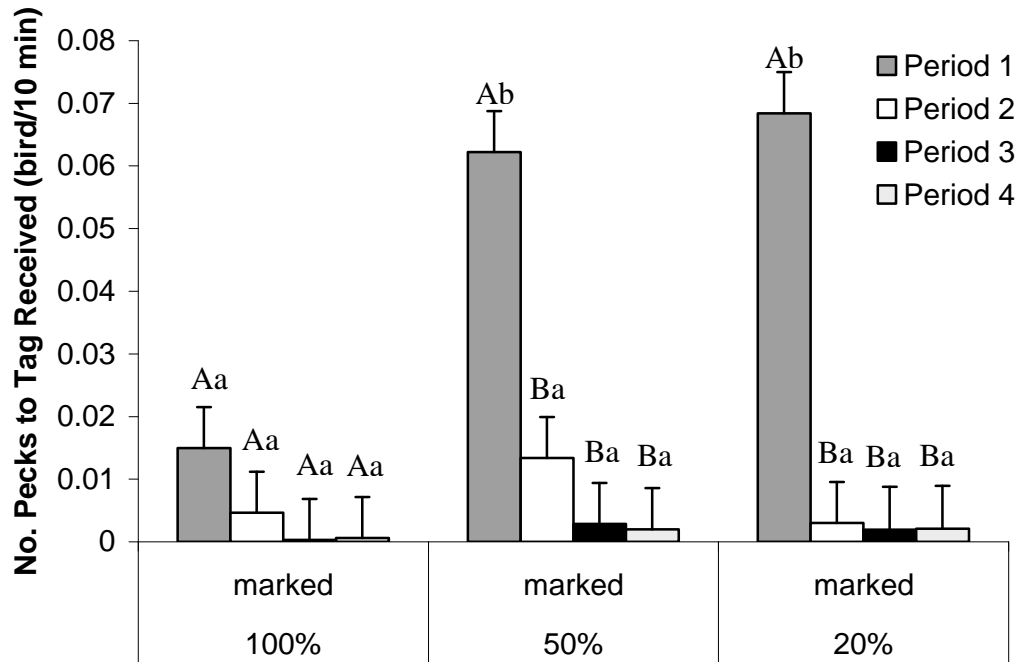
2.4.2 Aggression given

Marked birds from 20% and 50% pens, as well as unmarked birds form 20% pens, gave significantly less fights than did the control birds in period 1 (Table 2-4, Fig. 2-6). However, there were no significant differences in the frequency of fights given by unmarked birds in the 50% pens compared to the control and other treatments groups in period 1. During the second period unmarked birds from 50% pens gave significantly more fights with pecks than did their marked pen mates, the marked birds of 20% pens or the control birds (100% pens). No significant differences in fights given were found between treatments in the third or fourth periods. Fights were given significantly more frequently in period 1 in 100% pens, and in period 1 and 2 by unmarked birds in 50% pens.

There was a treatment by GS by period interaction for pecks given. There was no significant difference in pecks given between marked and unmarked groups in GS 10 (Table 2-4, Fig. 2-7a). However, unmarked birds in GS 50 for 20 and 50% pens both exhibited a higher frequency of pecks during period 4 when compared with periods 1 and 2, and when compared with period 3 in 20% pens. There was a significant difference in the unmarked birds of 50% pens between time period 1 and 3. Unmarked birds in GS of 50, of both 20% and 50% pens, were found to give more pecks than their marked pen mates or the control birds during period 4 (Fig. 2-7b).

Regarding the frequency of threats given, unmarked birds in 50% pens delivered significantly more threats than any of the birds in other treatments during period 1 (Table 2-4, Fig. 2-8). In period 2 unmarked birds of 50% pens delivered significantly more threats than control birds, but there was no significant difference between them and their marked pen mates or the unmarked birds in 20% pen. There was no significant difference in threats delivered during periods 3 and 4. Unmarked birds in 50% pens delivered significantly more threats during period 1 and 2 than during period 4 (Fig. 2-8). Frequency of threats given was also found to be affected by a significant GS by period interaction. There was a significant increase in threats given in GS 10 compared with GS 50 for both periods 1 and 2 (Table 2-2) but not for periods 3 and 4. During periods 1 and 2, birds in GS 10 were avoided more often then birds in GS 50 (Table 2-2).

Unmarked birds in 20% pens were avoided significantly more often than their marked pen mates (Table 2-4, Fig. 2-9), but not when compared with the control birds. Unmarked birds in GS 10 were avoided significantly more frequently than were birds in GS 50. A significant GS by period interaction was also found in the number of times a bird was avoided. Birds in GS 10 exhibited a significant decrease in the number of instances of being avoided during period 3 compared to periods 1 and 2.

Regarding pecks to the tag, there was a significant interaction between treatment, period and GS (Table 2-4). During period one in GS 10, marked birds in

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50% pens delivered more pecks to the tag than any other treatment group (Fig. 2-10). Unmarked birds of GS 10 in 50% pens delivered less pecks to the tag than their marked penmates, but more than the control or the unmarked birds of 20% pens during period one (Fig. 2-10). Marked birds of GS 10 in 20% pens delivered significantly less pecks to the tag than birds of any other treatment during this period (Fig 2-10). All birds of GS 10, except marked birds of 20% pens, delivered significantly more pecks to the tag during period one than all other periods. No significant differences in pecks to the tags were found between treatments during any other period. During period one, unmarked birds of 50% pens as well as marked birds of both 50% and 20% pens delivered significantly more pecks to the tag in GS 10 compared to birds of GS 50 of the same treatment (Fig. 2-10). No significant differences were found between treatment or period in birds of GS 50.

Other behaviors such as chase, peck to the body and fights with peck to the body were rare and no significant differences were found. Means and standard errors are represented in Table 2-5.

Table 2-4 Model and ANOVA results for aggressive behaviors given.

Source	df	F	P
Fights			
Group Size (GS)	1,73	0.37	0.5452
Treatment	4,73	7.58	0.0001
Treatment*GS	4,73	1.40	0.2439
Period	3,89	17.61	0.0001
Treatment*Period	12,125	3.71	0.0001
GS*Period	3,89	0.34	0.7960
Treatment*GS*Period	12,125	1.28	0.2393
Pecks	df	F	P
Group Size (GS)	1,35	0.02	0.8828
Treatment	4,35	2.96	0.0332
Treatment*GS	4,35	1.11	0.3659
Period	3,125	2.58	0.0566
Treatment*Period	12,125	1.47	0.1424
GS*Period	3,125	4.32	0.0062
Treatment*GS*Period	12,125	2.47	0.0063
Threats	df	F	P
Group Size (GS)	1,30	26.00	0.0001
Treatment	4,30	11.29	0.0001
Treatment*GS	4,30	2.74	0.0467
Period	3,61	1.50	0.2224
Treatment*Period	12,77	2.07	0.0293
GS*Period	3,61	2.76	0.0497
Treatment*GS*Period	12,77	1.53	0.1316
Times Avoided	df	F	P
Group Size (GS)	1,40	23.33	0.0001
Treatment	4,39	12.63	0.0001
Treatment*GS	4,39	6.48	0.0004
Period	3,61	5.01	0.0036
Treatment*Period	12,76	1.72	0.0780
GS*Period	3,61	4.17	0.0094
Treatment*GS*Period	12,76	1.33	0.2171
Peck to Tag	df	F	P
Group Size (GS)	1,31	9.02	0.0053
Treatment	4,90	5.83	0.0003
Treatment*GS	4,90	3.31	0.0140
Period	3,208	41.21	0.0001
Treatment*Period	12,207	2.89	0.0010
GS*Period	3,208	8.08	0.0001
Treatment*GS*Period	12,207	2.29	0.0094

Table 2-5 Mean ± SE number of behaviors given per bird in 10 min. ab letters represent significant differences across treatments for each behavior (P<0.05).

Behavior Given	100%	50%		20%	
	Marked	Marked	Unmarked	Marked	Unmarked
Chase	0.003 ± 0.002^a	0.002 ± 0.002^a	0.007 ± 0.002^a	0.003 ± 0.002^a	0.004 ± 0.002^a
Fight w Body Peck	0.0005 ± 0.0002^a	0.0003 ± 0.0002^a	0.0001 ± 0.0002^a	0.0000 ± 0.0002^a	0.0000 ± 0.0002^a
Peck to Body	0.002 ± 0.001^a	0.002 ± 0.001^a	0.004 ± 0.001^a	0.001 ± 0.001^a	0.002 ± 0.001^a

Figure 2-6 Mean fights given (± SE) for each treatment and period. AB letters represent significant differences (P<0.05) between periods of the same treatment; ab letters represent significant differences (P<0.05) within the same period across treatments.

Figure 2-7 Mean pecks given (± SE) for each treatment and period in (a) GS 10 and (b) GS 50. AB letters represent significant differences (P<0.05) between periods of the same treatment; ab different letters represent significant differences (P<0.05) within the same period across treatments; (*) represents significant differences (P<0.05) with in the same period and treatment across GS.
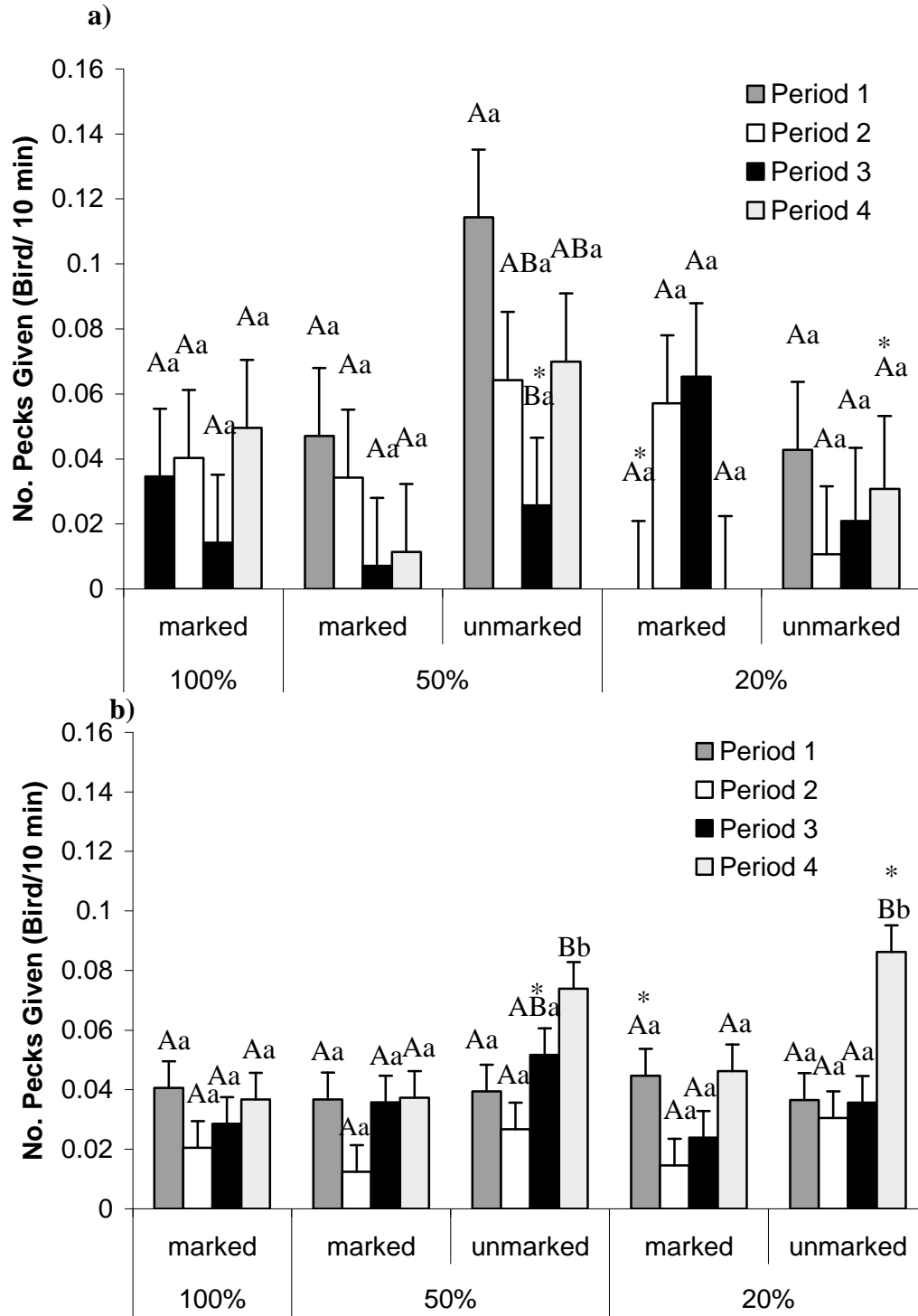
Figure 2-8 Mean threats (± SE) given for each treatment and period. AB letters represent significant differences ($P<0.05$) between periods of the same treatment; ab letters represent significant differences ($P<0.05$) within the same period across treatments.
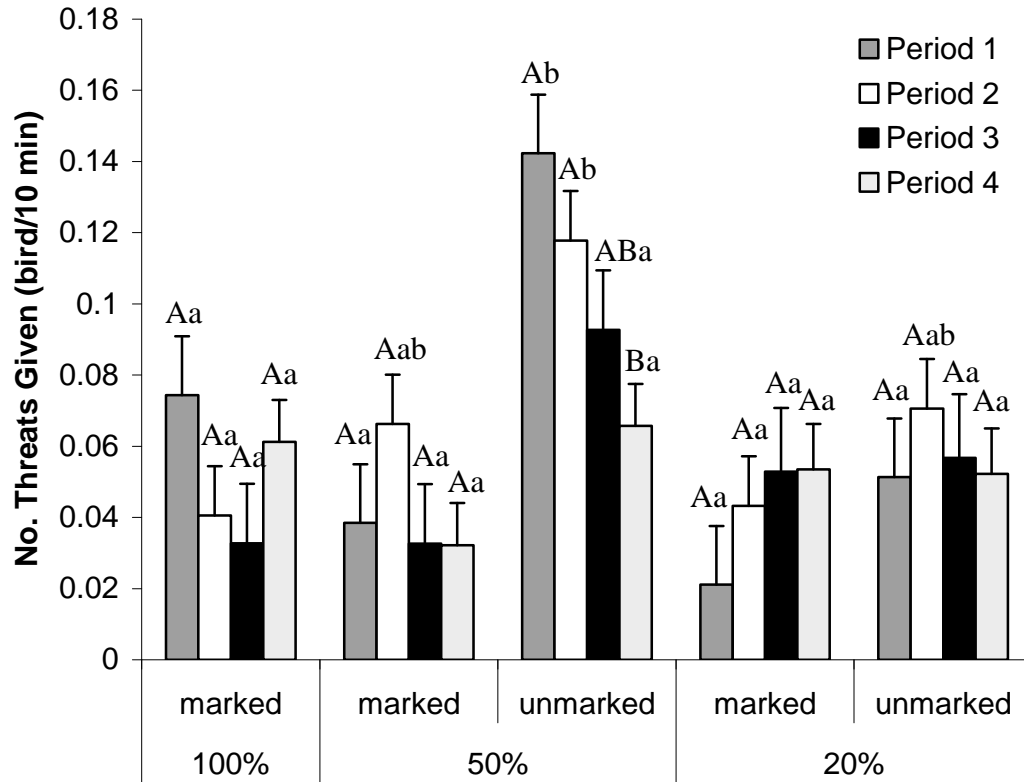
Figure 2-9 Mean times avoided (± SE) for each treatment and GS. AB letters represent significant differences (P<0.05) between GS of the same treatment; ab letters represent significant differences (P<0.05) within the same GS across treatments.

Figure 2-10 Mean pecks to tag given (± SE) for each treatment and period in (a) GS 10 and (b) GS 50. AB letters represent significant differences (P<0.05) between periods of the same treatment; ab different letters represent significant d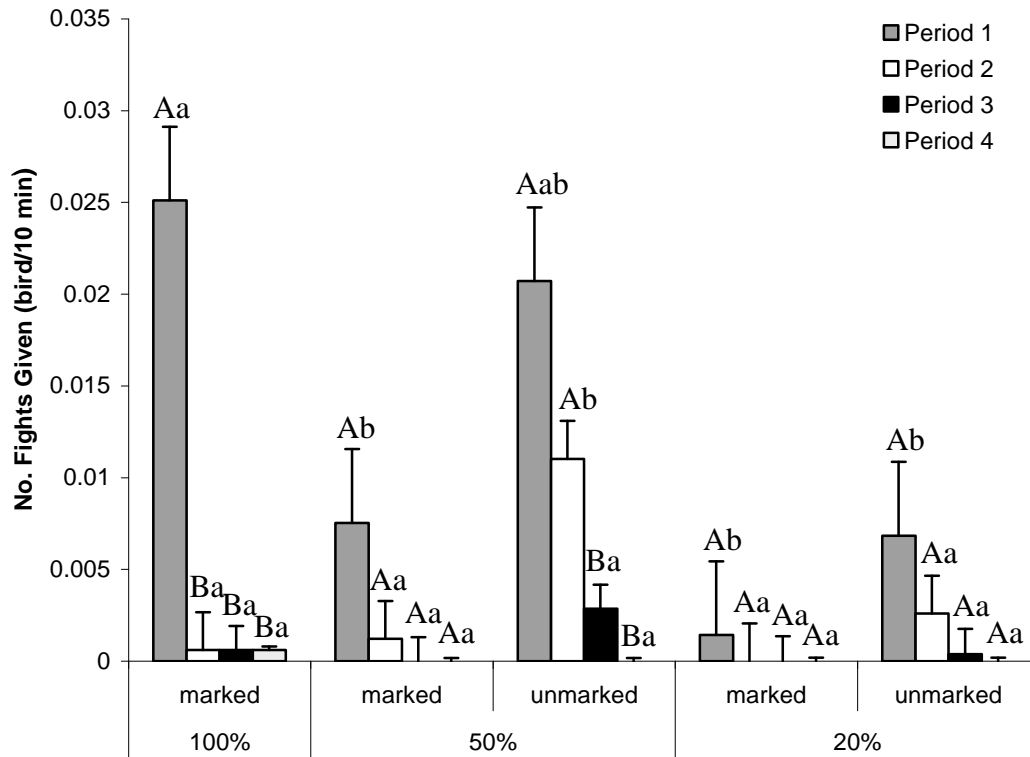ifferences (P<0.05) within the same period across treatments; (*) represents significant differences (P<0.05) with in the same period and treatment across GS.
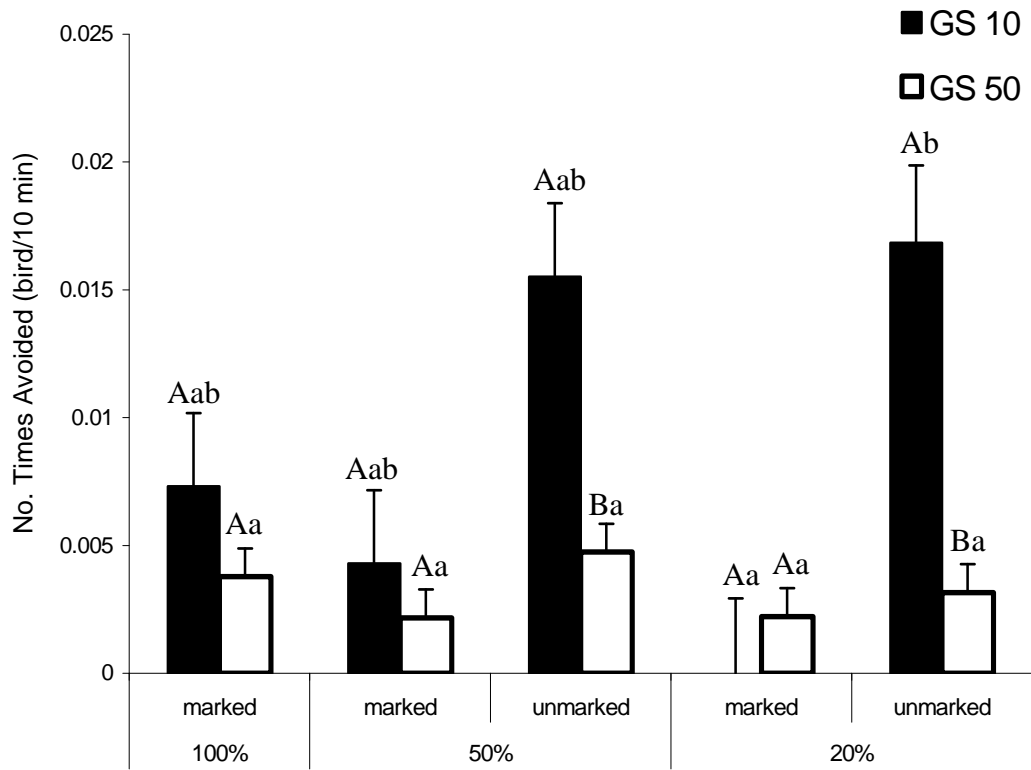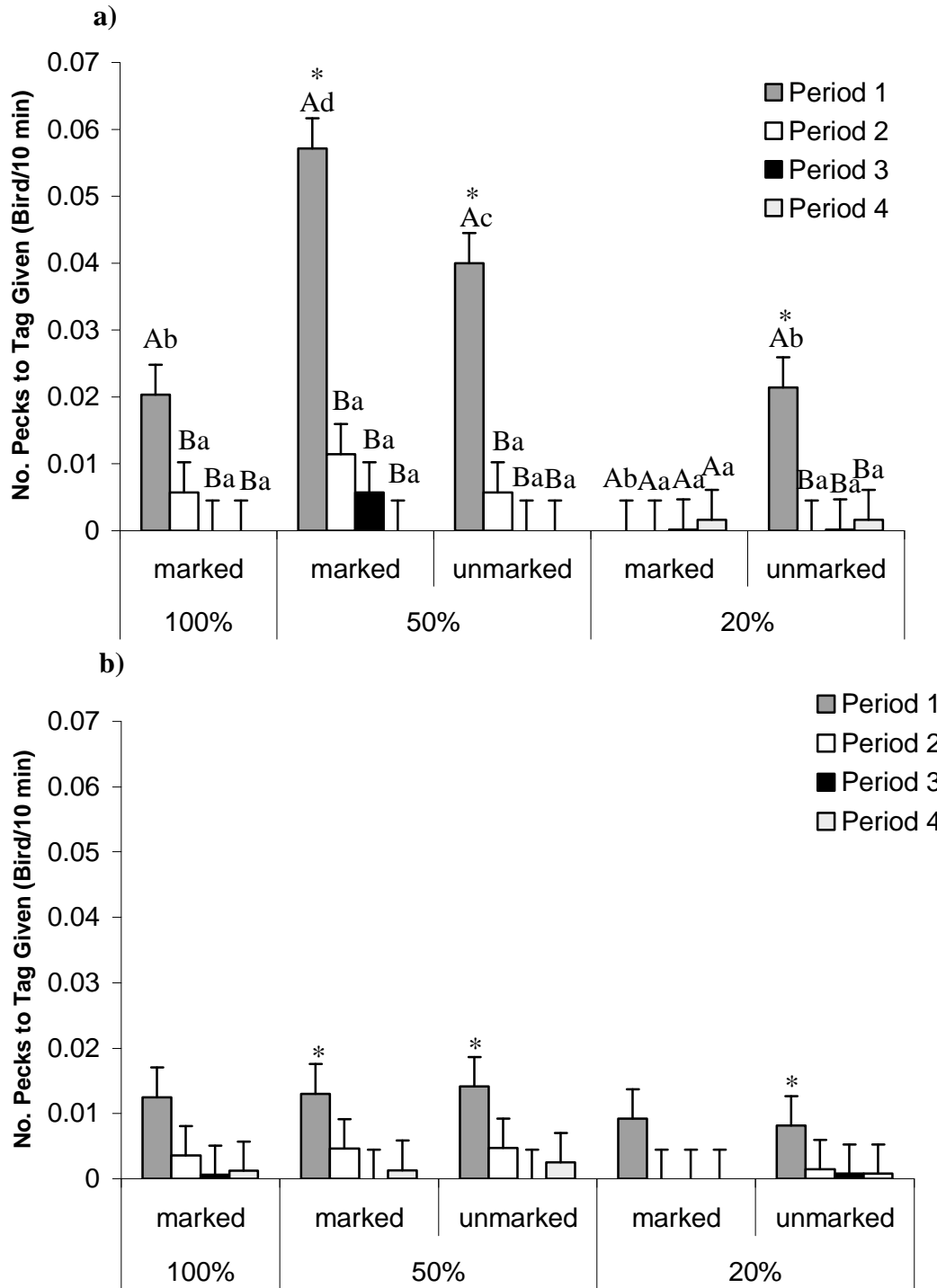
2.5 Discussion

2.5.1 Aggression received

Artificial markings have previously been used for individual recognition; however, Estevez et al. (2003) suggested that discrepancies between aggression given and received by marked focal birds in larger GS may be due to the focal birds receiving a disproportionate amount of aggression when compared to unmarked birds. No previous research has been done to examine the effect of the marks on the aggressive behavior of the birds bearing them, or of the aggression of the rest of the group toward them. Our results indicate that marked birds generally received significantly more aggression than their unmarked counterparts. In behaviors that did not show significant differences, due in part to high individual variance, there was still an obvious trend. The differences were most obvious in pens with fewer marked birds. This supports our hypothesis that marking has an effect on the amount of aggression received and thus as marked birds make up a smaller percentage of the group, the amount of aggression that they receive increases. The only exception to this pattern was seen in fights received, where fights received by marked birds were greater than for the positive control birds only in pens with an equal proportion of marked and unmarked (during period 2). Even though fights received were based on the bird that received the peck during the fight, fighting is the only aggressive behavior considered in this experiment that requires some aggressive propensity from both individuals involved. A bird confronted by an aggressive bird in a pen could

simply retreat or submit after receiving a threat or peck, or it could remain for a fight. When marked birds make up a greater portion of the group, they may be more likely to fight back. These birds still receive more aggression due to the marks. However, more time and energy is required by the unmarked birds to defeat their marked pen mates. This is supported by our finding that marked birds in 20% pens avoided conspecifics more often and more threats and pecks were received by marked birds in 20% pens than in the positive control.

Artificial marking is a clear manipulation of the physical appearance of the individual. As seen in previous studies of dubbed birds, and birds with physical alterations to the feathers and comb, modifications of the physical appearance of an individual will affect its behavior as well as the manner in which conspecifics behave toward it (Guhl and Ortman, 1953; Guhl, 1953; Marks et al., 1960; Siegel and Hurst, 1962). Artificial marking, much like dubbing, attracts increased levels of aggression from conspecifics. However, the mechanism for this increased aggression is not as clear. We have evaluated our results considering several hypothesis; handling of the birds could increase the level of aggression received by marked birds, the novelty of the marks themselves could be attracting aggression, because marks increase the physical dissimilarity causing increased aggression due to kin recognition theory, or marked birds could be receiving more aggression due to the marks as status "badges."

Handling

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Handling is confounded with the marking process for all experiments utilizing this methodology. Handling has been shown to alter stress and behavior in rats and mice (Gariepy et al., 2002; Silveira et al., 2004). Marked birds were caught and handled much more frequently than unmarked birds in order to apply and maintain the marks. The increase in stress and more gentle behavior caused by handling (Gariepy et al., 2002; Silveira et al., 2004) could have potentially caused the birds to receive disproportionately more aggression than unmarked birds. If handling was a primary factor in attracting aggression toward the marked birds, we would expect to see the positive control birds also receiving high levels of aggression. Instead we see that in most cases, marked birds of 20% and 50% pens received significantly more aggression compared to both their unmarked penmates as well as control birds. This hypothesis also does not provide an explanation for the increase in aggression received by marked birds as percent marked decreases.

Attraction to the marks

Marks could present a novel stimulus that elicits fear and eventually aggression from flockmates. Social and environmental novelties have been found to elicit fear and increase aggression in chickens in numerous studies (Lindberg and Nicol, 1996; Marin et. al., 2001; Jones et. al., 2002). To determine if the birds are more aggressive toward the marked birds because the marks are novel stimuli, we must examine the pecks directed at the identification tags. Although we did see some pecks to the tags, supporting the possibility that the birds were, to some degree,

attracted to the novel stimulus, this effect was only apparent during the first time period. If novelty was the primary factor increasing aggression we would expect to see greater frequency of pecks toward the marks and the tags and fewer threats and fights which are not directed at the marks, but instead at the bird bearing the mark. We also noted more pecks to the body received by marked birds when the proportion of marked was the lowest; clearly these attacks were not directed at the marks. If the aggression was driven by the novel stimulus we would also have expected that the level of aggression received by marked birds would decrease with time, and that is not the case for many of the behaviors considered, such as threats, pecks and avoidances. As with the kin selection theory and the handling hypothesis, we would also expect to see increased aggression in the pens with all birds marked if the aggression was due to the presence of a novel stimulus, or directed at the marks themselves.

Kin recognition

Kin recognition theory offers a good explanation for the difference in aggressive interactions received by marked and unmarked birds. Altruistic-like behaviors, including cooperation and decreased aggression, are generally linked to kin selection (Keller, 1997). Kin recognition operates on the degree of relatedness between individuals (Johnson, 1991; Keller, 1997). The obvious and intensely studied question for any given species is how relatedness is assessed. Most vertebrate species appear to use physical similarities to aid in assessing relatedness between

individuals (Hamilton, 1964; Johnson, 1991; Hancock and DeBruine, 2003). Jaisson (1991) suggested that the degree of physical dissimilarity, indicating a low degree of relatedness, might be a leading factor for increased aggression. This supports our finding that as percentage marked decreases, thereby increasing the dissimilarity between marked and the rest of the group, aggression received increases. A number of studies in birds also have shown that filial imprinting has a large impact on an individual's ability to discern relatedness of individuals in the future (Halpin, 1991; Johnson, 1991; Bukacinski et al., 2001). Filial imprinting could explain our finding that marked birds consistently received more aggression, even in groups of 50% marked, in which there is the same level of difference between any bird and the mean physical appearance of the group. Since marking was done on day one, filial imprinting for the unmarked model is not a satisfying answer. Birds would have been imprinted to whatever marked or unmarked model that they were introduced to on day one and continue to be housed with throughout the observational period. If kin selection was the only force affecting the aggressive behavior, we would not expect to see any difference in marked and unmarked birds of 50% pens. In both 100% and 50% pens no bird appears any different from the mean phenotype of the pen, however the variance is greater in the 50% pens as opposed to the 100% pens. Therefore, we might expect to see an overall increase in aggression in the 50% pens when compared to our positive control, but not between the marked and unmarked birds.

Status "badges"

Marks appear to provide a compelling subordinate status badge similar to the lower status observed when layers were dubbed (Guhl and Ortman, 1953; Guhl, 1953; Marks et al., 1960). Status "badges" have been suggested to help to identify the social status of the bird in place of active dominance relationships of birds in large GS (Pagel and Dawkins, 1997). However, in this experiment we have shown that the increase in aggression toward marked birds is similar across large and small GS. This alone does not debunk the theory; it does however, suggest that the mark presents a conspicuous status badge, attracting aggression even in small GS. As these badges occur in greater number in the group, they seem to attract less aggression. When they are rare, they may infer a larger negative status. The status "badge" hypothesis suggests that the effect of status "badges" should be seen only in large GS. However, we have shown their effect in both large and small GS. Pagel and Dawkin's model focused on dominant status "badges" and marks may confer a negative status. As negative status badges these marks may infer a decreased health or fitness, the affects of which would be regardless of GS. Differential levels of aggressive behaviors have also been linked to differential stress coping capability as well as immunocompetence (Cheng et al., 2001; Dennis et al., 2004). Marks themselves, by discolored plumage, may indicate decreased health or presence of a parasite. As suggested by Hamilton and Zuk's parasite hypothesis (1982), bright plumage of males, shown to be a dominant status badge, may infer a resistance to parasites. This same mechanism might hold true for other status badges such as marks. Artificial marks on the plumage of males may infer the existence of, or susceptibility to, parasites or disease. This would explain the increase in aggression received by marked birds in both 20%

and 50% pens over the positive control, in which all individuals were marked. If the marks were to infer susceptibility to parasites, we would not expect an increase in aggression when all birds are marked because there is no difference in the fitness of the individuals.

2.5.2 Aggression given

Our data showed that the use of artificial marks not only increased the level of aggression received by marked birds, but also decreased the amount of aggression given. In addition, for all aggressive behaviors except for fights, aggression given by unmarked birds was higher than the level observed for control birds in the 100% pens.

Decreased aggression given by marked birds is most likely due to the fact that they are the targets of considerably more aggressive encounters. Recent studies have determined that previous experience in aggressive encounters is one of the most important determinants in the outcome of future aggressive encounters (Hsu and Wolf, 1999; Cloutier and Newberry, 2000; Hsu and Wolf, 2001; Beacham, 2003). The loser effect hypothesis (Hsu and Wolf, 1999) provides an explanation for the birds that receive increased levels of aggression also giving decreased levels of aggression. Previous losing experience greatly increases the probability of losing in subsequent encounters. As the marked birds are attracting more aggression from

birds in the pen, there is an increased probability for them to lose at least some of those interactions. The losing experience will increase the probability that the bird will lose subsequent encounters and therefore it will become less likely for marked birds to be engaged in aggressive interactions.

The increased aggression of unmarked birds was most noticeable in pens with a larger percentage of marked birds. As argued earlier in fights received, groups with more equal mix of marked birds and unmarked birds may represent a more unstable group environment requiring more aggression to defeat the marked birds. Alternatively, groups with more marked birds may simply present more targets for aggression.

2.5.3 Conclusion

Artificial markings have long been used as a means by which researchers can identify specific individuals under an experimental treatment. However, it is now obvious that the marks distinguish the birds from each other as well. Marking acts as an additional treatment, increasing the level of aggression received by the individual and decreasing the level of aggression an animal will deliver to conspecifics, especially when the marked birds make up a smaller portion of the group. Our results suggest that using artificial marks to distinguish individuals introduces a serious bias into the results of any such experiment. Aggressive behaviors can be severely altered by this seemingly harmless observational methodology. In comparison with our

positive control the hypothesis of subordinate status "badges" attracting increased

aggression to the birds bearing them makes the most compelling explanation for the

differences found in levels of aggression received. As seen in studies of selection on

plumage of wild birds, status "badges" may confer information about the fitness of

the individual. Marks may infer to the group that the bird bearing the mark is

diseased or of lower fitness. Regardless of the mechanism through which marked

birds receive increased aggression, our findings indicate that the welfare of the

animals and the accuracy of results from studies utilizing marks for individual

recognition may be severely compromised.

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Chapter 3: The Effects of Artificial Marking on Stress and Catecholaminergic Reactivity

3.1 Abstract

Domestic fowl (*Gallus gallus domesticus*) marked for identification, have been shown to receive increased aggression from their unmarked counterparts, while they delivered fewer aggressive acts. Differential aggressiveness due to manipulation of physical appearance suggests that birds may also be subject to differential levels of stress and catecholaminergic reactivity. Stress and activity of the sympathetic-adreno-medullary system was measured in marked and unmarked birds in pens of group size (GS) 10 and 50 containing 20, 50, and 100% (positive control) birds marked in the group, as well as from the most and least aggressive birds in 100% pens. Body mass (BM), tonic immobility (TI) and fluctuating asymmetry (FA) were assessed and blood samples were collected for hormone analysis. BM from the second and fifth week revealed a significantly lower body mass in marked birds than unmarked in 20% and 50% pens (P=0.0062 and 0.0017, respectively). No significant difference was found in BM, TI, or FA between individuals of differential aggressiveness in 100% pens. Plasma catecholamine concentrations following manual restraint, revealed a suppressed EP response (P=0.0108) and an increase in plasma DA (P=0.0382) in marked birds of 20% pens. Birds of differential aggressiveness were found to have significantly different NE responsiveness to manual restraint, with the most and least aggressive individuals having significantly

depressed NE activity compared to the second and third most and least aggressive birds. These finding indicate a decrease in activation of the active (fight or flight) stress response, and an increase in stress on marked birds in groups with a low proportion of marked birds. Birds which are the most and least aggressive when all individuals are marked have different catecholamine reactivity. The effects of artificial marks on stress suggest that marking affects the BM, TI, and catecholamine reactivity in some groups. Our results indicate that the application of marks for identification acts as an additional treatment, increasing the stress on marked individuals, especially as marked individuals make up a smaller proportion of the group.

Key words: Domestic fowl, catecholamine, stress, marking

3.2 Introduction

As described in chapter 2, marked birds in groups of 20%, 50% and 100% marked were analyzed for differences in given and received aggressive behaviors. We found that irrespective of GS, marked birds in both 20% and 50% pens received increased aggression. These finding were most apparent in groups with a lower proportion of marked birds. We also determined that marked birds gave fewer aggressive acts than their unmarked counterparts.

Aggression and dominance order are strongly correlated with the stress of the individual (Harding and Follet 1979; Matt et. al. 1996; Hiebert et. al. 2000; Hennessy et. al. 2002). Numerous studies have shown that individuals receiving greater amount of aggression, or being of lower social status, exhibit increased levels of stress (Sgoifo et al., 1996; Matt et al., 1996; Haller et al., 1997; Hiebert et al., 2000; Hennessy et al., 2002; Wood et al., 2003). Hormonal parameters are often used to determine the effects of the physical and social environment on the animal's stress levels, glucocorticoids being most commonly used stress indicators (Korte et al., 1997; Kruk et al., 1998; Cheng et al., 2001; Mostl and Palme 2002; Wood et al., 2003). However the key hormones used in overcoming stressful situations are both glucocorticoids and catecholamines (Hashiguchi, et al., 1997; Korte et al., 1997; Mostl and Palme, 2002). A study of stressed and unstressed rats, showed that stressed individuals delivered fewer aggressive acts following exposure to an acute stressor (Wood et al., 2003).

BM is a common measure of the level of stress of an individual used in many experiments with domestic fowl (Estevez et al., 1997; Bilcik and Keeling, 1999; Nicol et al., 1999; Keeling et al., 2003). BM has also been shown to be positively correlated with rank and the frequency of double attacks (Cloutier and Newberry, 2000), possibly due to relatively less stress of the aggressors compared with those receiving the aggression.

TI is a response to a brief period of physical restraint which is characterized by a reduced responsiveness "catatonic-like" state of reduced responsiveness to stimuli (Jones, 1986). Typically the bird is laid upside down in a U- or V-shaped cradle and restrained by light pressure on the sternum for a brief period of time, usually 15 to 30 seconds. After this initial restraint the bird remains in the characteristic catatonic-like state for a period of time afterward, known as the TI duration or latency time to right itself. This reaction is thought to be an evolutionary adaptation to predation, in which the bird would enter the immobile state after it was caught until the predator lightened or let go of its grip, giving the bird a chance to escape (Jones, 1996). However, TI is also used to show the level of stress related to the fear response of an individual. Longer TI durations have been shown to be indicative of increased level of fear of an individual (Jones, 1986). An increased fear response to stress is also indicated by fewer inductions required to achieve TI (Jones, 1986).

A newer approach to determine stress is the evaluation of FA. FA is a measurement of the random deviations from symmetry in ideally bilaterally symmetric characters (Palmer and Strobeck, 1986; Allenbach et al. 1999). Both genetic and environmental stress are believed to be major factors contributing to degree of FA (Palmer and Strobeck, 1986; Parsons, 1990; Møller et al. 1995). FA has been positively correlated with an individual's low capacity to safeguard against stress (Yngvesson and Keeling, 2001; Cloutier and Newberry 2002).

Hormone levels have also been commonly used to determine levels of stress. Activity of the sympathetic-adreno-medullary system, evident by increased plasma EP and NE concentrations, has been linked to active and aggressive responses to stress (Sgoigo et al., 1996; Haller et al., 1997; Korte et al., 1997; Cheng et al., 2002). These hormones have also been shown to participate in many physiological processes related to stress such as motivations and emotions in response to stimuli (Goldstein, 1981; Cheng et al., 2001). Sgoifo *et al.,* (1996) analyzed plasma EP and NE responses in rats, which exhibited various levels of aggressiveness. Once aggressiveness of all individuals had been determined, each rat was exposed to both social stress (a defeat experience in an aggressive encounter with an alien male) and nonsocial stress (presentation of a shock-prod). Social stress was found to induce a much greater catecholaminergic effect than did the nonsocial stress. They also determined that the more aggressive the animal, the greater the chatecholaminergic reactivity to both social and nonsocial stressors. Aggressiveness was defined as the latency to attack in an inevitable agonistic encounter. Comparison of high and low aggressive animals determined that the more aggressive the animal, the greater the catecholaminergic (EP and NE) reactivity to both social and nonsocial stressors. Matt et al. (1996) determined catecholaminergic reactivity to stress in free living male tree lizards. A significant increase in EP, NE and DA was determined in response to a manual restraint stressor. Concentrations of EP and NE increased 10 to 30 times their basal levels during aggressive encounters and remained high for 4 minutes following the encounter.

Stress response has been clearly shown to differ among individuals. These

different responses have been selected for in several different lines of laying hens, by

selecting for birds of high and low feather pecking and high and low group

productivity and survivability, in attempt to obtain a greater understanding of the

different mechanisms utilized for coping with stress (Korte et al., 1997; Cheng et al.,

2001; Cheng et al., 2002). Korte et al. (1997) determined the adrenal and

neurosympathetic responsiveness of laying hens selected for high and low feather

pecking (HFP and LFP, respectively), under both resting conditions and manual

restraint. The two lines were found to differ in fear response and vocalizations. No

difference existed in baseline plasma NE or EP concentration (obtained through a

catheter). However following 1 min manual restraint, HFP chickens showed higher

plasma NE concentration. Results suggest that HFP chickens show an increase in

activation of the sympathetic-adreno-medullary axis, mediating active behavioral

response to stress.

Kojima et al. (1995) studied the active and aggressive behavior in cats and the

response of the HPA and sympathetic-adreno-medullary axes. They found that NE

increased with threats but not with restlessness, while EP and cortisol levels increased

with both threats and restlessness. EP and NE, the hormones of the "fight or flight"

response, have been linked to the regulation of motivation and emotion in response to

various stimuli, and are often used to indicate an individual's ability to cope with

stress (Dillon et al., 1992; Haller et al., 1997; Cheng et al., 2001; Cheng et al., 2002).

EP and NE are involved in the organism's preparations for a prospective fight

through metabolic changes as well as preparing the cardiovascular and nervous systems (Haller et al., 1997). Aggressiveness of an individual can be induced by either increased NE concentration or severe depletion of NE (Haller et al., 1997).

The purpose of this experiment is to test the hypothesis that marking animals for identification purposes causes stress. We expected that marked birds will be increasingly more stressed as percent marked decreases in both large and small GS. Marked birds, which received higher frequency of aggression, are expected to exhibit increased stress response, evidenced by decreased BM, and TI inductions and increased TI durations, degree of FA and plasma DA concentrations. Since marked birds also were found to deliver fewer aggressions to conspecifics we would expect to find a decreased EP and NE response in these birds.

In this experiment we also tested a second prediction that birds of lower aggressiveness, in pens with all birds marked, will also be more stressed. We expected to see BM, TI, FA, and DA concentrations to indicate increased stress in less aggressive individuals in the 100% pens. Measures of NE and EP we expected to be increased in more aggressive individuals in 100% pens as they exhibit an active and aggressive coping strategy.

3.3 Materials and Methods

3.3.1 Animals and Management

See Appendix 6.1

3.3.2 Experimental Design

This experiment was set up as an incomplete 2 x 3 x 2 factorial design. Pens were set in a randomized complete block design, with seven complete blocks, to control for location within the house. All birds were divided on day one into 42 groups of 10 or 50 chicks (21 pens per GS). Each of these groups were then assigned randomly to one of three percentages of marking in which the number of total birds marked was 20%, 50%, or 100% of the group. The group containing 100% marked birds was our positive control group in which all individuals were marked, but none was different from the other group members. The third factor was the marking or lack of marking applied as a within pen treatment. Pen size was constant for all experimental group sizes; therefore bird density varied simultaneously with group size. Rearing densities were 2.22 birds/m^2 and 11.11 birds/m^2 for groups of 10 and 50, respectively.

Birds were tagged using a dual marking system. Using the Swiftack[4] system we attached two laminated paper disks approximately 3.5 cm in diameter to either side of the neck of the bird. The disks were attached with a plastic filament. This is a common and fairly painless procedure and has been used in the past for individual

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[4] Swiftack for poultry identification system. Heartland Animal Health, Inc. Fair Play, Missouri

bird identification with good results (Cornetto and Estevez, 2001). Laminated paper disks were used due to the light weight as well as the versatility to individual experimental needs and ease of replacement. The disks were numbered to distinguish between individual birds. All tag numbers used were double digit so that all birds carried approximately the same amount of marking. Marked birds also carried an additional mark on the back of the head area. The head mark was a large black dot (approximately 5-6 cm in diameter) made with a non-toxic marker. Retagging occurred only in sporadic occasions as tags fell out, but remarking of the head mark was necessary every two weeks. Aggressive behaviors were observed and assessed until 10 wks of age as reported in chapter 2.

At week ten birds within 100% pens were ranked by number of aggressive behaviors given from three to ten wks of age. The three birds exhibiting the highest levels of aggression were labeled H1-H3 (H1 being the most aggressive in the pen and so on) and the three birds exhibiting the lowest levels of aggression were labeled L1-L3 (L3 being the least aggressive in the pen).

3.3.3 Data Collection

At week 11 BM, TI, and FA were assessed from three marked and three unmarked birds per pen taken at random. In addition, BM was also assessed at two and five wks. In pens with 10 birds and 20% marked, only a total of two birds were marked. In this case all marked birds were sampled, and four unmarked birds were

sampled in order to reduce the overall standard error of the pen. In 100% pens only three marked birds were sampled. TI was taken over two days before any other measures were taken. The additional measures were taken over two days, measuring complete blocks on the same day. BM and FA were taken immediately following blood collection so that the time restrained could be controlled for in hormone analysis.

At week 11 BM, TI, and FA were assessed from the three most (H1-H3) and three least (L1-L3) aggressive individuals per pen, blood samples were also collected from the wing vein, as described above.

Live BM at two and five weeks of age was taken from the aisle outside the pen, in order to minimize the transport stress. At 11weeks of age BM was obtained directly following blood sampling and FA. TI was conducted following the procedure as described by Jones (1986). In many cases induction required more than one attempt; the number of induction attempts was recorded. Once the bird was in the characteristic TI state, the TI duration was recorded until the bird righted itself. To reduce variation only two persons conducted the test and no other persons were allowed in the testing area during TI. We allowed each bird five minutes latency time, after which any bird still in the cradle was removed. FA was taken of the right and left shank length and width using digital calipers[5]. Individual character measurements (ICM) were taken of the right and left shank length and width. The

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[5] Digimatic Caliper, Mitutoyo Co., Ltd. Suzhou P.R. China

ICM of shank length for right and left leg were obtained by measuring the length of the metatarsus of each leg. The ICM of shank width was defined as the diameter of the shank perpendicular to the spur. Measurements were taken while bird was restrained for blood collection in order to obtain accurate measures while minimizing the number of restraint periods on the bird. Relative FA was determined from the ICM using the following standard equations (Yang et a., 1997; Møller et al., 1999),

Relative FA = Absolute FA / Character Size

Absolute FA = | Right ICM – Left ICM |

Character Size = (Right ICM + Left ICM) / 2

Sampled birds were captured and restrained manually for no less than 45 seconds before blood sampling began. The time was recorded from the moment the bird was picked up from its pen until the blood was completely drawn in order to correct for difference in length of actual restraint time between birds. Three ml of blood were collected from the wing vein of each sampled bird using a 23 gauge needle and placed into heparinized tubes for HPLC analysis of plasma catecholamine concentrations. Samples were immediately centrifuged and plasma removed. Plasma was stored at -80°C until transported in dry ice to Purdue University for analysis.

3.3.4 HPLC Assay

DA, EP, and NE were measured, in duplicate, from plasma samples using a

plasma catecholamine analysis kit[6]. Samples were deproteinized and acidified with

100μL 4 M perchloric acid. Samples were centrifuged at 13,000 x g for 10 min at

4°C, acid supernants were added and absorbed onto an alumina minicolumn with

internal standard, dihydrocybenzylamine. Minicolumns were set on a rocker to allow

catecholamines to bind to the alumina. Columns were rinsed and eluted using

solutions provided by ESA, Inc. Eluents were injected into the reverse-phase

columns where catecholamines were detected by high performance liquid

chromotagraphy (HPLC) with an ESA Coulochem II electrochemical detection[7]. The

mobile phase flow rate was 1.3 mL/min. Catecholamine concentrations were

calculated from a reference curve constructed using the provided standards.

Concentrations were obtained as picograms per ml.

3.3.5 Statistical Analysis

BM, TI, and FA, for marked and unmarked birds, were analyzed as a blocked

factorial using a Mix Model analysis of variance (ANOVA). Variance partitioning

was used to correct for heterogeneity of variance and log transformation was used

when needed to correct for normality. Data were compared across mark treatments

and GS. Treatments, for the purposes of statistical analysis, were a combination of

percentage of birds marked, and within pen treatment, whether the birds were marked

or unmarked, making a total of 5 treatment groups (marked birds from 100% marked

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[6] ESA, Inc., Chelmsford, MA
[7] INC Biomedicals, Inc., Costa Mesa, CA

pens, marked birds from 50% marked pens, unmarked birds from 50% marked pens, and marked birds from 20% marked pens, and unmarked birds from 20% marked pens). Least square means were reported for all treatment groups and GS. Contrasts were used to determine significance using the Sidak adjustment to maintain an experimental alpha of 0.05.   Main effects and interactions were tested using contrasts for marked compared with unmarked (in 20 and 50% marked groups only), and for percentage marked between 20, 50, and 100% marked pens (using marked birds only). Comparisons were made within treatment group and GS. Comparisons across treatment groups included comparisons within percent marked pens, each treatment to control, and marked across all percent mixtures as well as unmarked across all percent treatments. Catecholamine data were analyzed by analysis of covariance (ANCOVA), with the time taken to bleed the bird as the covariate. Treatment groups and contrasts used were the same as for physiological data. Data were analyzed using SAS 8.2 software[8].

BM, TI, and FA data from birds ranked for aggressiveness in 100% pens, were analyzed as a blocked factorial using a Mix Model analysis of variance (ANOVA) using partitioning of variance. Variance partitioning was used to correct for heterogeneity of variance and log transformation was used when needed to correct for normality. BM, TI and FA were compared across rank and GS. Least square means were reported for all aggressiveness ranks and GS. Contrasts were used to determine significance using the Sidak adjustment to maintain an experimental alpha of 0.05.   Catecholamine data was analyzed as an analysis of covariance (ANCOVA),

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[8] SAS Institute Inc., 1999 Version 8.2 ed. Cary, NC

with the time restrained before bleeding as the covariate. Data were analyzed using

SAS 8.2 software[9].

3.4 Results

3.4.1 Effect of Marks

No significant treatment effect was found in BM at two wks (Table 3-1).

However, main effect contrasts revealed a significant effect of the marks when

considering marked and unmarked birds in both 20 and 50% pens (Fig. 3-1a). There

was no significant main effect of percent marked. BM at five wks was found to be

significantly different according to treatment group (Table 3-1) as well as in main

effect contrasts of marking. Least square means and SEM are as follows:

1754 ± 28.18g, 1675 ± 28.18g, 1806 ± 28.18, 1695 ± 33.44, and 1742 ± 26.36 for birds

from 100% pens, marked in 50% pens, unmarked in 50% pens, marked in 20% pens,

and unmarked in 20% pens, respectively. The only significantly different contrast

across treatments was between marked and unmarked birds of 50% pens. Although

there was no significant difference between marked birds in 20% and 50% pens and

the positive control, however, they were apparently smaller than control birds

($P>0.1911$ and 0.0575, respectively). The main effect of marking revealed that

marked birds were found to be significantly less heavy, at week five, than unmarked

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[9] SAS Institute Inc., 1999 Version 8.2 ed. Cary, NC

birds across GS and both 20% and 50% pen treatments (Table 3-1, Fig. 3-1b). Final

mass, taken at eleven weeks, was not significantly different between treatment groups

(Table 3-1, Fig. 3-1c). In addition, final BM was found to differ significantly by GS

(Table 3-1, Fig. 3-2), showing that birds in GS 50 were lighter than birds of GS 10.

BM at two and five wk was not found to differ significantly by GS.

TI duration revealed no significant differences between GS or treatments

(Table 3-1). However, the number of inductions required to induce TI was

significantly different between treatments. Both the marked and unmarked birds in

the 50% marked pen required significantly fewer inductions when compared to the

positive control (Table 3-1, Fig. 3-3). There was no significant difference in relative

FA measurements of shank length or width between treatments, nor was there

between group sizes (Table 3-1).

A significant treatment effect was found in plasma EP concentration. Marked

birds in 20% pens had significantly lower EP concentrations compared to their

unmarked counterparts and the positive control (Table 3-2, Fig. 3-4). No significant

GS or time effect on EP was found. NE was greater in GS 10 compared to GS 50

(Table 3-2, Fig 3-5). A significant quadratic effect of time was found verifying our

need to analyze catecholamine data against the covariate of time restrained (Table 3-

2). However, no significant treatment effect was found in NE concentration.

A treatment by GS interaction of plasma DA concentration was found to be significant. Marked birds in 20% pens of GS 50 had significantly higher DA concentrations than their unmarked counterparts (Table 3-2, Fig. 3-6). A significant quadratic effect of time was found (Table 3-2). No significant differences were found at GS 10 or in 50% pens.

Table 3-1 Model and ANOVA results for BM,TI, and FA

	Effect	DF	F	P
Mass 2 wk	Treatment	4,54	1.86	0.1510
	GS	1,54	4.65	0.0361
	Treatment*GS	4,54	1.31	0.2933
Mass 5 wk	Treatment	4,54	3.57	0.0118
	GS	1,54	0.53	0.4687
	Treatment*GS	4,54	1.10	0.3642
Mass 11 wk	Treatment	4,46	1.02	0.4091
	GS	1,12	22.94	0.0004
	Treatment*GS	4,46	0.63	0.6438
TI Duration	Treatment	4,47	0.74	0.5717
	GS	1,13	0.01	0.9345
	Treatment*GS	4,47	1.35	0.2668
TI Inductions	Treatment	4,29	4.05	0.0100
	GS	1,28	0.26	0.6149
	Treatment*GS	4,29	0.67	0.6187
Rel FA Width	Treatment	4,23	0.34	0.8479
	GS	1,28	0.57	0.4560
	Treatment*GS	4,23	0.67	0.6218
Rel FA Length	Treatment	4,23	1.60	0.2109
	GS	1,28	0.02	0.8758
	Treatment*GS	4,23	0.22	0.9261

Table 3-2 Model and ANCOVA results for catecholamine concentrations

Hormone	Effect	DF	F	P
Epinephrine	Treatment	4,121	3.43	0.0108
	GS	1,121	1.87	0.1736
	Time	1,123	0.02	0.8822
Norepinephrine	Treatment	4,50	2.00	0.1082
	GS	1,52	4.52	0.0382
	Time	1,109	11.56	0.0009
	Time*Time	1,111	6.90	0.0098
Dopamine	Treatment	4,32	1.27	0.3017
	GS	1,25	0.52	0.4758
	GS*Treatment	4,31	2.89	0.0382
	Time	1,102	37.30	<0.0001
	Time*Time	1,100	21.30	<0.0001

Figure 3-1 Mean BM (± SE) for marked and unmarked birds (from 20 and 50% marked pens only) taken at (a) week two, (b) week five, and (c) week eleven. ab letters represent significant difference (P<0.05) between means.

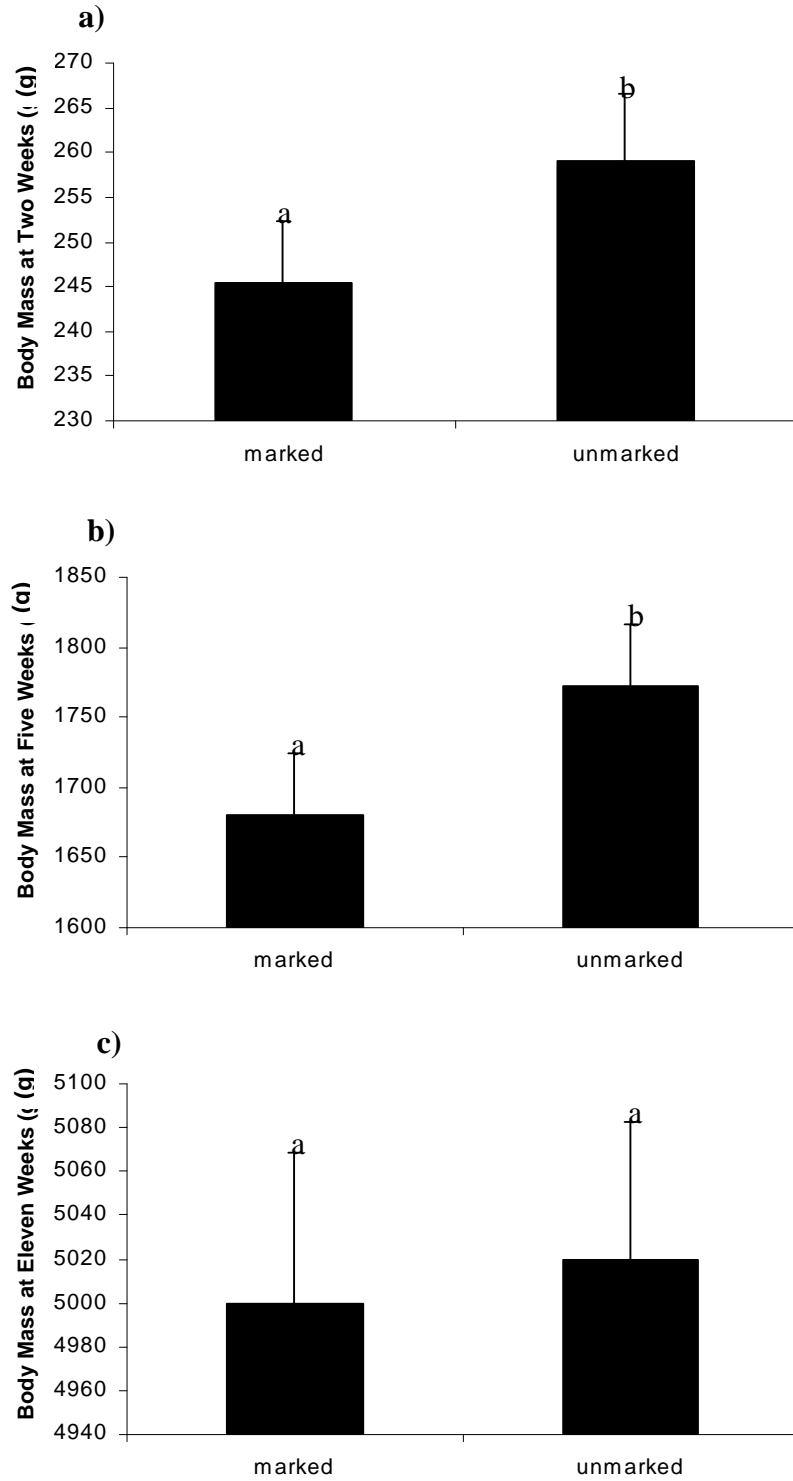**Figure 3-2** Mean 11 wk BM (± SE) for birds of size each GS. ab letters represent significant difference (P<0.05) between means.
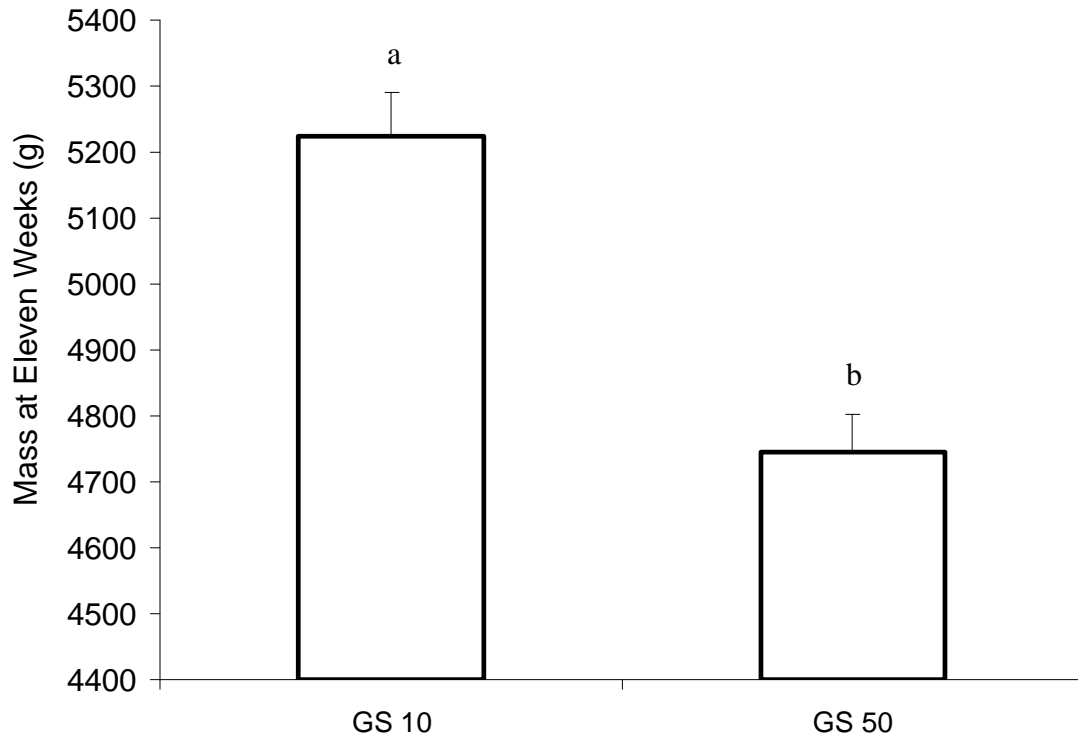
Figure 3-3 Mean number of inductions (± SE) for all marked and unmarked groups. ab letters represent significant difference (P<0.05) between means.
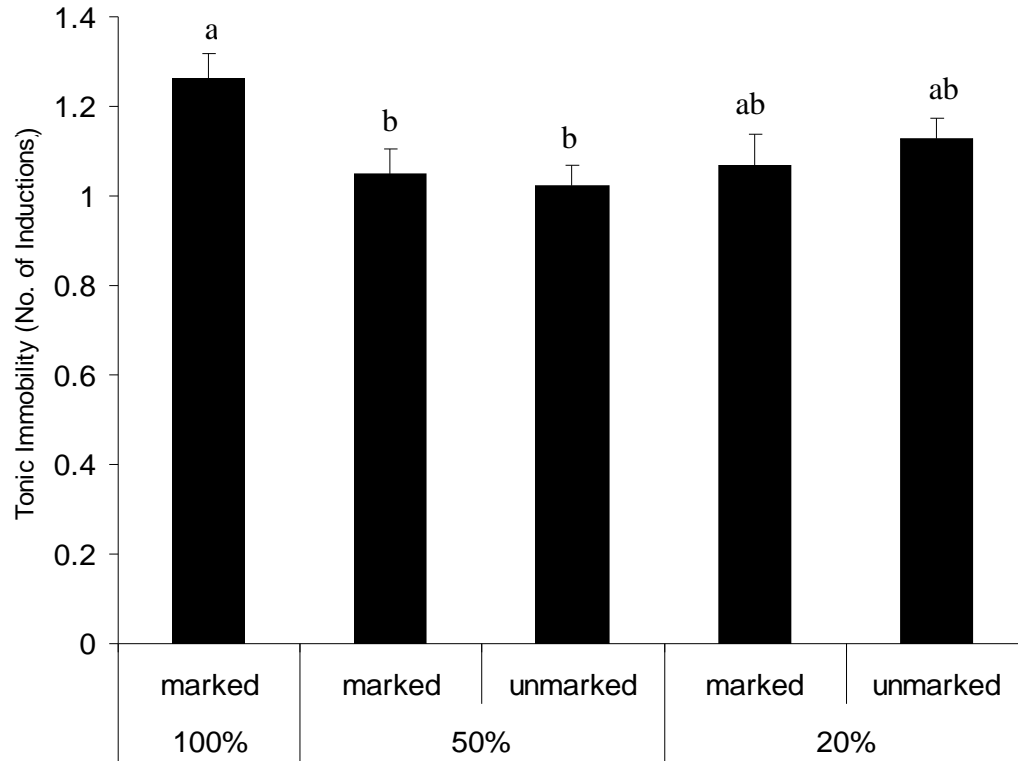
Figure 3-4 Mean plasmaEP concentration (pg/mL) (± SE) for each treatment. ab letters represent significant difference (P<0.05) between means.

Figure 3-5 Mean plasma NE concentration (pg/mL) (± SE) for birds each GS. ab letters represent significant difference (P<0.05) between means.

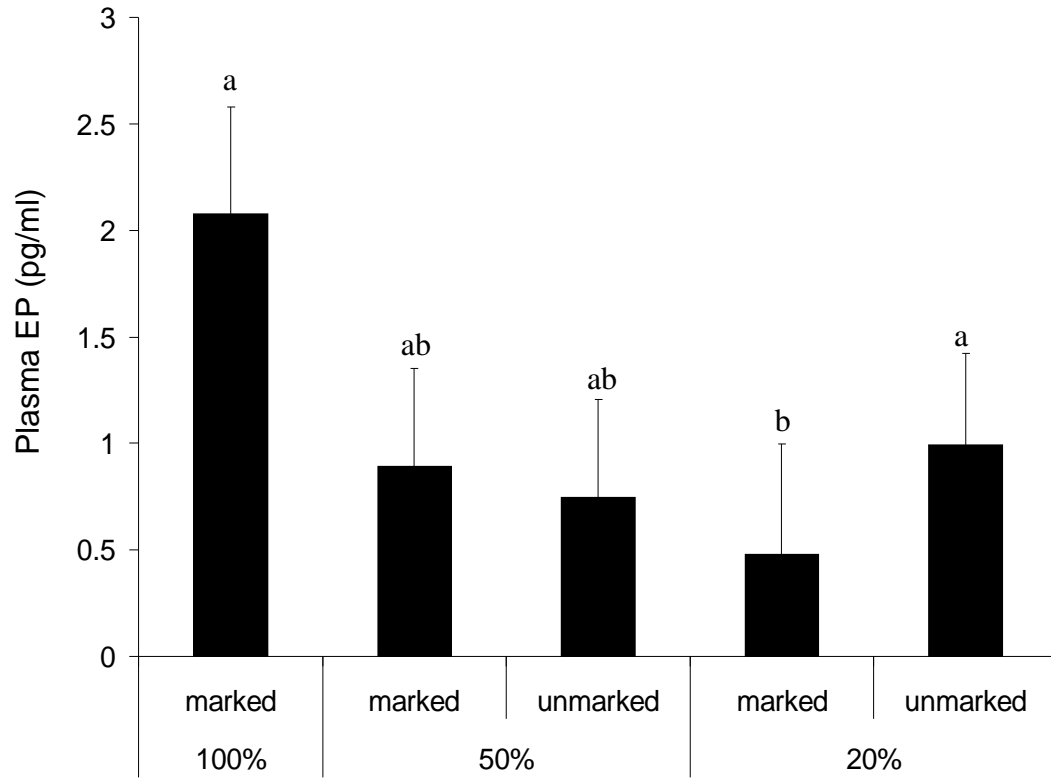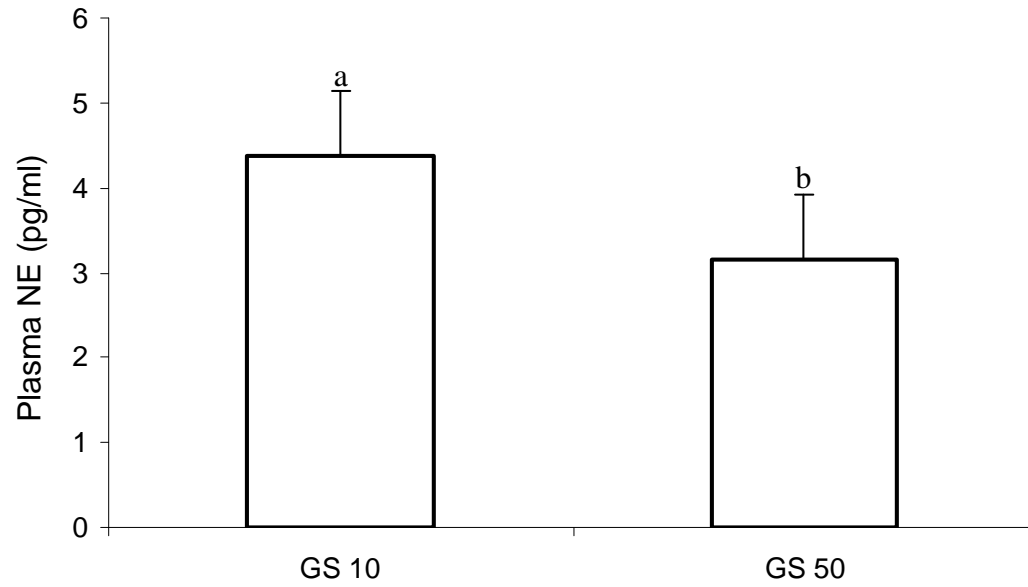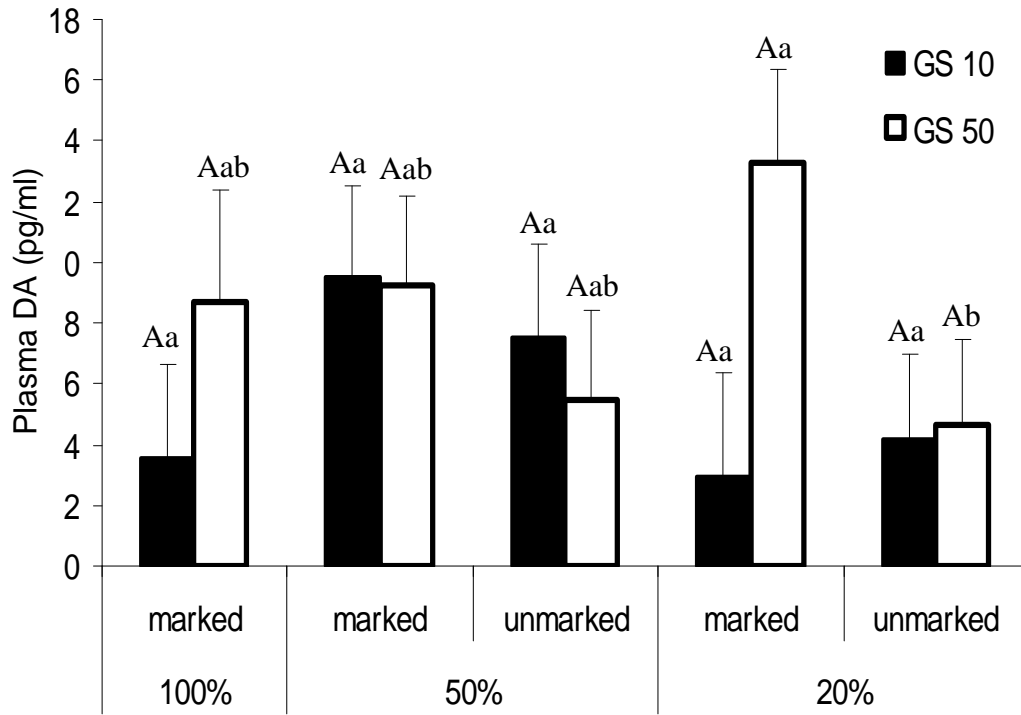**Figure 3-6** Mean plasma DA concentration (pg/mL) (± SE) for each treatment and GS. AB letters represent significant difference (P<0.05) between GS of the same treatment. ab letters represent significant difference (P<0.05) between treatments of the same GS.

3.4.2 Effect of Aggressiveness in 100% Pens

No significant differences were found in BM, FA, number of TI inductions and TI duration between birds of different aggressiveness. However, birds in GS 50 were found to have significantly lower BM compared to birds in GS 10 (Table 3-3, Fig. 3-7).

No differences were found in plasma EP or DA concentration between birds of different aggressiveness. However, NE responsiveness to time restrained was found to differ significantly dependant on the aggressiveness of the individual (Table 3-4, Fig. 3-8). NE increased logarithmically, therefore analysis and figures are represented as the log of the concentration. H1 birds showed a negative relationship between log NE and time restrained, which was found to be significantly different from both H2 and L2 birds (Fig. 3-8) both showing a positive relationship. L3 birds exhibited similar negative relationship between Log NE and time restrained (however, L3 birds were analyzed using a much shorter range of time than H1 birds). The slope of L3 was found to be significantly different from H2, H3, L1, and L2 birds, all of which had a positive slope (Fig. 3-8).

Although not significant, a strong trend was seen in plasma DA concentration between GS 10 and 50 (Table 3-4, P=0.0845).

Table 3-3 Model and ANOVA results for BM,TI, and FA

	Effect	DF	F	P
Mass	Rank	5,25	0.81	0.5539
	gs	1,66	11.68	0.0122
	gs*rank	5,25	0.84	0.5317
Length FA	Rank	5,66	0.46	0.8050
	gs	1,66	1.10	0.2985
	gs*rank	5,66	0.46	0.8057
Width FA	Rank	5,66	0.46	0.8050
	gs	1,66	1.10	0.2985
	gs*rank	5,66	0.46	0.8057
TI Duration	rank	5,62	2.01	0.0900
	gs	1,62	0.49	0.4880
	gs*rank	5,62	0.48	0.7898
TI Inductions	rank	5,59	0.88	0.5017
	gs	1,59	1.12	0.2942
	gs*rank	5,59	0.28	0.9220

Table 3-4 Model and ANCOVA results for catecholamine concentrations

Hormone	Effect	DF	F	P
Epinephrine	Rank	5,41	0.41	0.8409
	GS	1,42	0.30	0.5843
	Time	1,44	0.03	0.8533
Norepinephrine	Rank	5,37	1.91	0.1157
	GS	1,37	0.77	0.3849
	Time	1,26	2.33	0.1389
	Rank*Time	1,36	2.61	0.0413
Dopamine	Rank	5,34	1.64	0.1752
	GS	1,37	3.15	0.0845
	Time	1,40	13.02	0.0009
	Time*Time	1,40	11.93	0.0013

Figure 3-7 Mean 11 wk BM (± SE) for birds of size each GS. ab letters represent significant difference (P<0.05) between means.
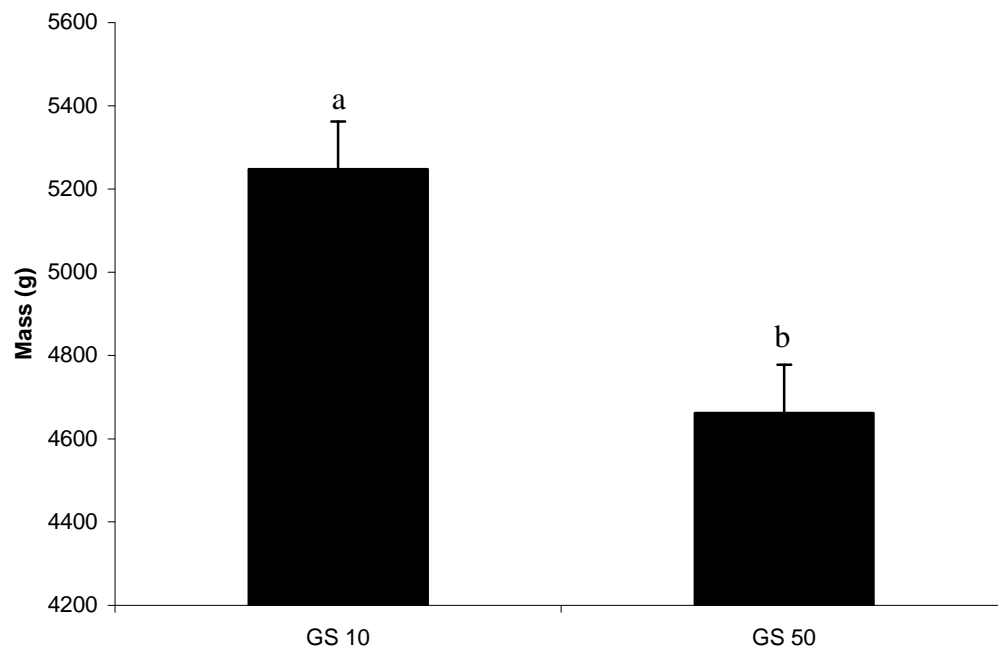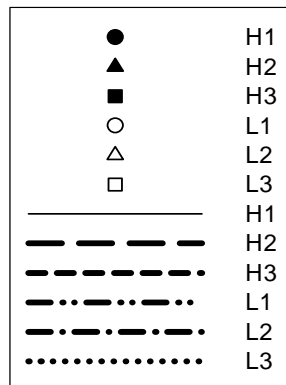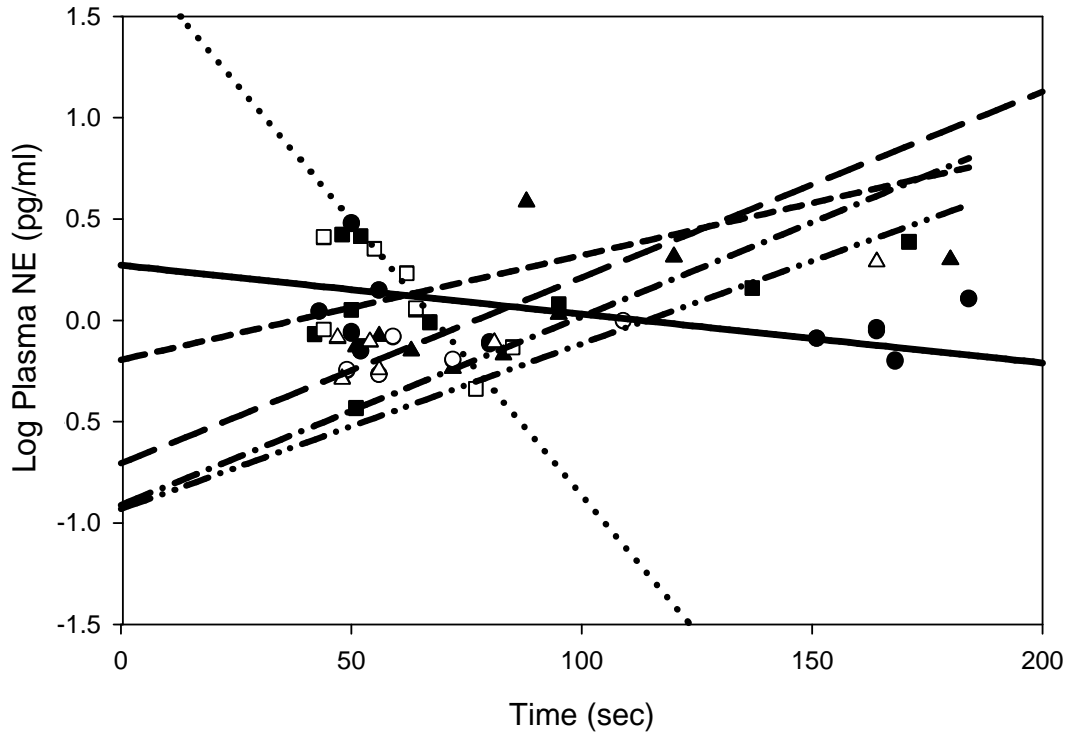
Figure 3-8 Slope plasma NE concentration over time restrained (pg/mL) for birds each aggressiveness rank. ab letters represent significant difference (P<0.05) between means.

3.5 Discussion

3.5.1 Effect of marks

BM at early ages, showing that marked birds were smaller than their unmarked penmates, indicates a decrease in growth rate of marked birds. This reduction in BM may have been related to an increase of the stress levels due to the marking and the increased aggression that marked birds received (Chapter 2). Decrease in mass has been employed in previous studies to indicate high stress (Bilcik and Keeling, 1999; Nicol et al., 1999; Keeling et al., 2003). It could also be indicative of a pattern of monopolization of the food source by the more aggressive unmarked birds. Estevez et al. (1997) determined that low body mass at larger groups was most likely not due to monopolization of resources by dominant birds, as time spent at the feeder and drinker was independent of GS. Therefore, it is unlikely that the results of BM in this experiment would be related to limitations of resource access. The lack of difference in BM seen at week 11 can be explained by the broiler growth curve (Prescott, et al., 1985; Goliomytis et al., 2003). The growth rate of broilers increases rapidly until about the sixth week at which time the growth rate begins to slow down. By week 11 BM of the broiler begins to plateau and the growth rate approaches zero (Goliomytis et al., 2003). The effect of marks decreases the growth rate of broilers but does not significantly cause birds to have lower final BM

if enough time is given to the birds to grow. GS, however, was shown in this study, in agreement with several previous studies (Estevez et al., 1997; Keeling et al., 2003), to have a significant effect on the final BM. As suggested by Estevez et al., (1997), lower BM seen in larger GS, with increased density, could be due to a number of stressors including increased levels of disturbance while resting, reduction of thermoregulatory capacity, or the deterioration of the social environment.

TI, an indicator of stress related to the fear response of an animal, showed no difference in duration between birds of different marked treatments. However, fewer inductions were required to achieve TI for both marked and unmarked birds in 50% marked pens than the control. Decreased number of inductions is indicative of increased fear related stress response (Jones, 1996). These results showed that pens with an equal proportion of marked and unmarked individuals, there was an overall decrease in number of required TI inductions, which may be indicative of an increase of the level of fear. A more equal mix of marked and unmarked birds may have a group or pen effect of increased fear and stress which may incite increased activity, as shown by increased fights. We have little data supporting this because the experiment was designed to detect differences in treatment groups of marked and unmarked birds, which may be much greater than effects on the entire pen or "mixed group". A more equal mix of marked and unmarked birds may increase the fearfulness and vigilance of all individuals. Gvaryahu et al. (1996) noted increased stress in groups in which they intermingled heavy and light strains of laying hens in a 50:50 ratio; results that appear quite similar to the results of this experiment. Valone

and Wheelbarger (1998) noted that in mixed-species flocks of varying percentages, white-crowned sparrows exhibited increased frequency of vigilant behaviors, indicative of increased anxiety.

No difference was noted in FA measurements of the shank length and width between treatments or GS. FA measurements were taken at week 11, five weeks past the normal date of slaughter. At this point birds had reached a large BM and leg problems were starting to become apparent in several birds (personal observation). These leg problems may have added to the variability found in the leg measurements, making it harder to find differences between the treatments or the GS.

In this experiment we have shown some differential catecholaminergic reactivity in birds, from similar genetic background, due to marks (as indicated by EP) and GS (as indicated by NE). Numerous studies have provided evidence of differential catecholaminergic reactivity and behavioral adaptations due to selection in the domestic fowl (Dohms and Metz, 1991; Castanon et al., 1995; Cheng et al., 2001). Difference in EP in our experiment appeared to be due to manipulation of physical appearance with artificial marking when the proportion of marked is low.

In response to manual restrain we found a depressed EP response in marked birds of 20% pens, indicating a decrease in active response to stress (fight or flight

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response) compared to both their unmarked counterparts as well as marked birds of 50% pens and the control birds. Our findings suggest that the marked birds in small proportion, which were also met with increased aggression, have a suppressed active coping strategy associated with activation of the sympathetic-adreno-medullary system, as shown by decreased EP levels.

We found no difference in NE response between birds of different marked treatment groups. Catecholamines, especially EP and NE, have been linked to motivation (Haller et al., 1997). A lack of difference in NE levels accompanying the differential EP response may suggest that NE is not involved in aggressions motivated by the presence of subordinate status "badges." It is also possible that the level of replication used in this experiment was not sufficient to detect potential low level differences among our treatments.

DA was found to be greater in marked birds in 20% pens of GS 50, suggesting an increase in stress on these birds. No difference was found in 20% groups of GS 10, however, fewer marked birds were available in these groups for sampling. This reduced number of birds per pen may have reduced the sensitivity, decreasing the power of our analysis. It is also possible that the stress was not as great on marked birds of 20% pens in GS 10 due to the reduced stocking densities. The increased DA concentration seen in birds of 20% pens in GS 50 may be due to an additive effect of two stressors: 1) lower percent of marked birds, and 2) increased GS with increased

stocking density. Our findings from Chapter 2 suggest that marked birds from 20%

pens may receive more aggression than marked birds from 50% pens. GS and density

has also been shown to have an effect on the stress of birds. As shown in our BM

data, birds from GS 50 are more stressed than birds of GS 10. Studies of dominant

and subordinate animals have determined an increase in stress in subordinate animals

as indicated by BM, as well as differential activity of the HPA and sympathetic-

adreno-medullary axes (Shively, 1998; Fano et al., 2001).

Birds bearing artificial marks were found to receive more aggression from

their unmarked counterparts (Chapter 2). Our results suggest that these markings

may also cause an increase in stress on the animals bearing them especially in large

GS when they make up a smaller portion of the group, as evident by lower BM in

marked birds and increased DA response in marked birds of GS 50 and 20% groups.

The increase in stress is likely caused by increased defeats as indicated by increased

pecks received by marked birds of both 20% and 50% pens and increased threats

received by marked birds in 20% pens only (Chapter 2). The elevated stress of the

animal may in turn make it a target for further aggression from their unmarked

counterparts. This elevated stress was not seen in our positive control pens in which

all birds were handled and marked. The suppressed EP response observed in marked

birds of 20% pens suggests a strategy of decreased active and aggressive response to

stress in birds when they make up a smaller portion of the group.

3.5.2 Effect of Aggressiveness in 100% Pens

Our findings of BM, TI, FA and DA levels in high and low aggressive birds in 100% pens show no evidence of increased stress on birds of lower aggressiveness when all birds are marked. This suggests that when all birds are marked, the normal levels of aggression exhibited by broilers, does not seem to have an impact on the stress levels. The lack of differences in stress indicators was found across both large and small GS. These findings are opposed to our hypothesis that differential levels of aggressiveness between high and low aggressive birds within a group would significantly affect the level of stress on the birds. Our results suggest that differences in levels of aggression in broilers do not have an effect on stress levels as measured by BM, TI, FA, and DA.

Birds with differential aggressiveness, when all birds were marks, showed no difference in EP concentrations but did exhibit different NE responsiveness over time dependent on aggressiveness rank. The most aggressive bird (H1) per pen had a decreased NE response to manual restraint, while the second and third most aggressive birds (H2 and H3) had increased NE response. In an extensive review of catecholamine involvement in aggression (Haller et al., 1997), NE in excess, or depletion, has been suggested to mediate increased aggressiveness, although far fewer models suggest that NE can mediate aggressive response in depletion. H1 birds had a

more suppressed NE response than any other ranked bird except for the least

aggressive bird (L3), which showed a uniquely depressed response. It must be noted

that the depressed NE response of L3 birds, however, was estimated over a severely

truncated time of manual restraint due to a lack of viable samples from longer

bleeding times. Therefore, the calculated response of L3 birds is not as extrapolated

to later times for comparison with other bird rankings. Our results suggest a

distinctly different activation of the sympathetic-adreno-medullary system by the

most aggressive individual and the least aggressive individual in a group, while the

remaining ranks all seem to have similar, catecholaminergic response to manual

restraint. Interestingly, the distinctive NE responses held true across both large and

small GS. These findings suggest that birds with very high or very low levels of

aggressiveness within a group may have different sympathetic-adreno-medullary

responses. Unique catecholaminergic responses may mediate aggression differently

dependent on both the motivation for aggression and the degree of aggressiveness in

relation to the rest of the group.

Conclusion

Our findings from the effects of GS 50 and in 20% pens, showed that the less

aggressive marked birds had higher DA levels and decreased BM, suggesting that

they may have been more stressed. The increase in stress seen in 20% pens of GS 50,

could be due in part to stress due to intermingling marked and unmarked birds in

unequal proportions. Stress could be caused by the manipulation itself or from a possible increased intensity of the aggressions from greater perceived differences.

EP may have more of a role in aggressions mediated by feedback from behavior such as the winner and loser hypothesis models, in which levels of aggressiveness are due to previous experience. It is possible that lack of a depressed EP response in the less aggressive birds of this study, could be indicative of a different strategy for coping with stress in birds which are naturally less aggressive. Artificial marks, when used on a small proportion of the group, have an impact on the stress and catecholamergic activity of the birds that bear them.

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Chapter 4: Summary and Conclusion

Our results showed clear evidences that the aggressive profile of an individual can be manipulated by the presence of marked birds in the group. As hypothesized by Estevez et al., (2003), marked birds receive increased aggression from flockmates when housed with unmarked birds. Increased aggression received by marked birds was most significant when the portion of marked birds in the pen was low. Subsequently, unmarked birds delivered significantly more aggression than their marked penmates, or the control birds, especially when the marked and unmarked birds were in equal proportions.

We also found evidence that suggests marked birds may be under more stress than unmarked birds, especially in larger GS with a small proportion of marked birds. Birds in larger GS were also found to be more stressed than birds in smaller GS, as indicated by our BM data. The catecholaminergic "fight or flight" response as indicated by EP response to manual restraint, revealed a depressed response in marked birds when they were housed in 20% pens. However, a NE response was not different between marked and unmarked birds, different responses were only evident in birds of different aggressiveness ranks in control pens. Birds in larger GS were also found to have significantly decreased NE responses compared to birds of smaller GS, possibly due to an overall reduction in aggressiveness in birds of larger GS.

Our finding that marked birds received aggressive interactions from conspecifics with greater frequency than did the birds in pens with all birds marked suggests that the marks themselves were not attracting the aggression, nor is the handling having a great effect on the level of aggression received. Rather, these results suggest that the difference in appearance between marked birds and their unmarked counterparts seems to fuel this increase in aggression. At first glance then, kin selection theory seemed to provide a reasonable explanation for increased aggression toward marked birds in pens with 20% and 50% marked. However, in altruistic-like behaviors resulting from kin selection we would expect that only marked birds in 20% marked pens would receive increased aggression from conspecifics. In both 100% and 50% pens no one bird is any more different from the mean marked "phenotype" than any other. In the case of 100% pens all birds were marked and the mean and median phenotype was marked. In the case of 50% marked pens half of the birds were marked and half are not. Therefore all birds were presented equal number of birds that were different from and similar to itself. Under kin selection theory we would predict that this situation would present a paradigm of overall increased aggression received by both marked and unmarked birds due to their apparent lack of relatedness. However, this did not agree with our findings. Kin selection may have an effect on the increased aggression received by marked birds, but it alone cannot explain the differences we observed in this study.

The tolerance hypothesis (Estevez et al., 1997) and Pagel and Dawkin's model (1997) suggest that in larger GS dominance hierarchies are not established because it

may not be beneficial or because the chance of meeting the same bird again is low. Status "badges" were suggested as an explanation of how a bird's status might be recognized without the need for individual recognition of the bird itself. This explanation is most fitting for our findings of the effects of marks. However, contrary to the model proposed by Pagel and Dawkins (1997), in our experiment we found that even in small GS an artificial mark may relegate its bearer to an apparent low status as determined by the amount of aggression received. Additionally, Hamilton and Zuk (1982) proposed that differences in plumage in wild birds might infer resistance or susceptibility to parasites. These mark "badges" may infer lower fitness, increased susceptibility to disease or the presence of parasites. Regardless of what information the marks may infer about the birds bearing them, the effect of the marks as status badges" is evident in both large and small group sizes.

Our finding that marked birds gave less aggression than their unmarked penmates supports the loser hypothesis that birds that were attacked more often (and probably lose some encounters) will be more likely to lose in subsequent encounters. Therefore, these birds would be less likely to initiate aggressive interactions with conspecifics.

Our results also suggest that marked birds were more stressed than their unmarked pen mates as indicated by decreased growth rate in marked birds across GS and percent marked pens. Stress levels of marked birds increased as their representation in the group decreased, as indicated by increased DA concentrations in

marked birds of 20% pens in GS 50. The increase in stress may be attributed to the increase in aggression they received. However, birds in our positive control group, ranked for aggressiveness, had no notable differential stress response between high and low aggressive individuals. When high levels of aggression received are due to marked birds intermingled with unmarked birds, the marked birds become more stressed, as evident by decreased BM, unlike the less aggressive birds in pens where all birds are similarly marked. BM was also found to be affected by the GS. This finding supports the finding of Estevez et al. (1997), that BM was lower in larger GS. They propose that this phenomenon could be due in part to increased frequency of disturbances, reduced thermoregulatory capacity, and deterioration of the environment.

The hormones responsible for the "fight or flight" response, being EP and NE would be expected to be greater in the more aggressive unmarked birds compared to their marked pen mates. They would also be expected to increase in the more aggressive ranked birds compared to their low aggressive counterparts. Marked birds in 20% pens showed a decreased EP response, while no NE response was noted across the treatment groups. Our results suggest that a decrease in EP levels, was induced only when the marked birds make up a small proportion of the group. NE has been more commonly noted as being linked to the aggressiveness of an individual (Haller et al., 1997), but it is necessary to point out that our data were collected from plasma samples and hypothalamic expression was not obtained. Ranked birds H1 and L3 in 100% pens, however, exhibited differential NE responses, but showed no

difference in EP response. The highest ranked birds (H1) had a decreased NE response compared with all other ranks, except for the lowest ranked birds (L1). However, the remaining high aggressive birds H2 and H3 were all increased over L1, suggesting that aggressiveness can be mediated by NE both in excess and in depletion.

Our findings suggest that catecholamine activity might be induced differentially dependent on the motivation of aggressiveness. Birds with a natural aggressive tendency may mediate their aggressive behaviors through different mechanisms than birds that become aggressive when presented with a bird bearing a subordinate status "badge" that may infer the presence of disease or parasite. NE response was also found to be depressed in birds from larger GS. This is indicative of a suppression of the "fight or flight" response of the sympathetic-adreno-medullary system. A decreased responsiveness of the "fight or flight" reaction seems to support the findings of Estevez et al. (1997) and Pagel and Dawkin's model (1997) that larger group sizes exhibit relatively lower levels of aggression. However, the difference observed in NE levels of birds from different GS may also be related to the different stocking densities in which they were housed.

Marking not only affected the aggressive behavior of the marked birds, it had additional physiological ramifications including increased stress and depressed responsiveness of the sympathetic-adreno-medullary system. Marking a bird not only eased in the identification of the bird, it increased the aggression that was directed

toward the marked bird, decreased the amount of aggression that bird was likely to give, and created an archetype of high stress and low active or aggressive responsiveness, as noted reduced aggressive behaviors given, especially when marked birds make up only a small portion of the group, as seen by severely reduced aggression given and depressed EP response. Marks appear to be inferring additional information to the birds nearby. Whether this information confers susceptibility to disease, parasites, or simply information about status is unclear from these data.

Appendices

6.1 Animals and Management

One thousand two hundred and sixty, one-day old male broiler chickens (*Gallus gallus domesticus*) were obtained from a commercial hatchery. The birds were transported to the University of Maryland's Applied Poultry Research Facility in Upper Marlboro, where they were housed and observed. A total of forty-two 1.88m x 2.5m (4.5 m^2 of floor space) pens, were utilized for this experiment. Each pen was initially equipped with two brooder lamps that were removed after the brooding period at 2 wks of age. One tube feeder and a line of nipple drinkers were installed in each pen. Feeders were restricted by the use of PVC corrugated tubing so that a constant amount of feeder space per bird was provided to birds at all group sizes. A three phase commercial diet was provided ad libitum. The starter diet (19.00% crude protein (CP) and 2800.0 kcal/kg metabolizable energy (ME)) was fed from 1 to 21 days of age. The grower diet (17.00% CP and 2801.7 kcal/kg ME) and finisher diet (19.00% CP and 3251.7 kcal/kg ME) were fed from 21 to 35 and 35 to 84 days of age respectively. An artificial lighting program (14L:10D) was followed. A standard temperature program was followed (Table 6-1). Automatic wall curtains opened to allow circulation with fresh air and natural lighting when set temperature was exceeded in the house.

Table 6-1 Temperature schedule followed during the experiment.

Age (Days)	Temperature (F)
1-3	93-90
4-5	90-88
6-7	88-87
8-9	87-86
10-11	85-84
12-13	84-83
14-15	83-82
16-17	81-80
18-19	79-78
20-21	78-77
22-23	77-76
24-25	75-74
26-28	74-72
29-84	72-71

6.2 SAS Sample Programs

Model 1. The following sample program is an ANOVA with
repeated measures for Pecks to the head in Marked and
Unmarked treatment groups. Similar programs were used
for all aggressive behavior analysis. Each behavior was
fit to the appropriate matrix. Below are explanations of
the variables and abbreviations used in this program.
BM, FA, and TI were analyzed with similar programs,
without the repeated measures.

```
Chase – chase
Threat – threat
Ph – peck (or peck to the head)
Pb – peck to the body
Pt – peck to the tag
Fightph – fight (or fight with a peck to the head)
Fight pb – fight with a peck to the body
Avoid - avoid
block - block within the house (1-7)
trt - five treatments (100%marked, 50%marked,
50%unmarked, 20%marked, 20%unmarked)
gs - two group (10 and 50)
perc - percent marked (100, 50, 20)
pen - pen number (1-38 -- some missing)
mark - marked or unmarked
birdid – lab id for bird
timep – the time period in which the observation was
taken (there were four time periods of two weeks each)
```

```
Title1 'Rachel Dennis';
dm 'log;clear;out;clear;';
Title2 'Aggressions Given';

options ls=76 ps=49 pageno=1;

data one;
input perc$ trt$ gs block timep pen mark$ chase threat ph
pb pt fightph fightpb avoid;

DATALINES;
(Data lines omitted for sample)
run;
```

```
Title3 'Fight w/ peck head';
Proc print uniform;
quit;


proc means data=one n;
class timep;
var mph;
quit;

proc means data=one n;
class block gs perc;
var mph;
quit;

proc means data=one n;
class block gs perc mark;
var mph;
quit;


proc sort data=one;
by block gs perc mark timep;
quit;
proc print data=one uniform;
quit;

Title4 'Proc mixed and resids Aggression Given';
Title5 'As a 2 way factorial (complete)';

Title3 'Pecks to the Head Given';

data one;
set one;
mph= ph /5;
if mph >0 then bin=1;
else bin=0;
run;


proc mixed data=one covtest;
class  block trt gs timep perc mark pen;
model mph=gs trt gs*trt
          timep timep*trt timep*gs timep*trt*gs/ddfm=kr
outp=resids;
random block block*gs*perc;
```

```
repeated timep / group=gs subject=block*gs*perc*mark
type=cs r rcorr;


contrast 'Main Effects';
contrast '  GS'              GS      1  -1;
                                                    *100M 20M
20U 50M 50U;
contrast '  Mark_2050 (20&50 only)'      trt    0    1  -1
1  -1;
contrast '  Perc_2050 (20&50 only)'          trt        0
    1    1  -1  -1;
contrast '  Perc_M   (Marked only)'      trt    1  -1    0
0    0,
                                                         trt
1    0    0  -1    0;


contrast '2 Factor Interactions';
contrast '  Mark_2050*Perc_2050'          trt    0    1  -1
-1    1;
contrast '  GS*Mark_2050'              GS*trt  0  0   1 -1 -1
1  1 -1 -1  1;
contrast '  GS*Perc_2050'              GS*trt  0  0   1 -1  1
-1 -1  1 -1  1;
contrast '  GS*Perc_M'                 GS*trt  1 -1 -1  1  0
0  0  0  0  0,
                                       GS*trt  1 -1  0  0  0
0 -1  1  0  0;


contrast '3 Factor Interaction';
contrast '  GS*Mark_2050*Perc_2050' GS*trt  0  0  1 -1 -1
1 -1  1  1 -1;


                                         *100M 20M 20U 50M
50U;
estimate '20m 50m 100m vs 20u 50u' trt  -2  -2    3  -2
3;
estimate '20m vs. 20u'                   trt    0  -1    1    0
0;
estimate '50m vs. 50u'                   trt    0    0    0  -1
1;
estimate '20m vs. 50m'                   trt    0  -1    0    1
0;
estimate '50m vs. 100m'              trt    1    0    0  -1
0;
```

```
estimate '20m vs. 100m'              trt    1  -1    0    0
0;

lsmeans timep*gs/ pdiff;
quit;

proc print data=resids;
quit;

data resids;
set resids;
resid = resid/stderrpred;

proc plot data=resids;
plot resid*pred
resid*perc
resid*mark
resid*gs
resid*trt
/vref=0;
quit;
```

Model 2. The following sample program is an ANCOVA for DA
in Marked and Unmarked treatment groups. Similar
programs were used for all catecholamine analysis. Below
are explanations of the variables and abbreviations used
in this program.

day is the day the assay was run (5 days)
number is the lab number given to that vial (numbers 13-
100 each day, not unique)
run is the replication -- each blood sample was run in
duplicate (2 runs per sample)
NE - norepinephrine concentration
EPI - epinephrine concentration
DA - dopamine concentration
block - block within the house (1-6)
trt - five treatments (100%marked, 50%marked,
50%unmarked, 20%marked, 20%unmarked)
gs - two group (10 and 50)
perc - percent marked (100, 50, 20)
pen - pen number (1-38 -- some missing)
mu - marked or unmarked

```
sample - bird from treatment per pen (between 1 and 4 per
trt gs block combo)
birdid - lab id for bird
time - time in seconds it took to bleed bird -- covariate

Title1 'Rachel Dennis';
dm 'log;clear;out;clear;';
Title2 'Dopamine concentration of Marked and Unmarked
Treatments';

options ls=96 ps=33 pageno=1;

data one;
input day number run NE EPI DA block trt$ gs perc$ pen
mu$ sample birdid$ time;

DATALINES;
(Data was omitted for sample)
run;

proc sort data=one;
by block pen gs perc mu trt day birdid sample time run;
quit;

proc print data=one uniform;
var block pen gs perc mu trt day birdid sample time run
NE EPI DA;
quit;

data one;
set one;
lDA=log(DA);
run;


Title4 'Proc mixed and resids cat';
proc mixed data=one covtest;
class block pen gs perc mu trt day birdid sample run;
model lDA=     gs trt gs*trt
               time
               time*time
         /*  time*trt    0.1260 */
         /*  time*gs     0.1102 */
         /*  time*gs*trt    0.3534 */
          / ddfm=kr outp=resids solution;
random block block*gs*perc block*gs*perc*mu
birdid(block*gs*perc*mu)day;
```

```
estimate 'intercept trt 100m'  intercept 1 trt 1 0 0 0 0;
estimate 'intercept trt 20m'   intercept 1 trt 0 1 0 0 0;
estimate 'intercept trt 20u'   intercept 1 trt 0 0 1 0 0;
estimate 'intercept trt 50m'   intercept 1 trt 0 0 0 1 0;
estimate 'intercept trt 50u'   intercept 1 trt 0 0 0 0 1;
estimate 'intercept gs 10'     intercept 1 gs 1 0;
estimate 'intercept gs 50'     intercept 1 gs 0 1;

lsmeans trt*gs /pdiff;
quit;



proc plot data=resids;
plot resid*pred
resid*gs
resid*perc
resid*mu
/vref=0;
quit;

proc univariate data=resids normal plot;
var resid;
quit;
```

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