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**INFLUENCES OF MATERNAL CORTICOSTERONE ON  
INCUBATION LENGTH AND HATCHABILITY OF EGGS LAID BY  
QUAIL HENS SELECTED FOR DIVERGENT ADRENOCORTICAL  
STRESS RESPONSIVENESS**

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

in

The Interdepartmental Program in Animal,  
Dairy and Poultry Sciences

by

Jason Berante' Schmidt  
B.S., Louisiana State University, 2003  
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## ABSTRACT

Unstressed and stressed quail hens selected for exaggerated (HS, high stress) rather than reduced (LS, low stress) plasma corticosterone (B) response to brief restraint deposit more B into their eggs than do their LS hen counterparts. HS hens implanted with B also show reduced egg lay when compared to LS- and HS-control and LS-B-implanted hens. Herein, the effects of stress line on length of egg incubation (LEI) and chick body weight at emergence (BWTE) (Exp. 1) and the interactive influences of line with maternal B-treatment (sub-Q control, CON-, or B-implants) on LEI (Exp. 2) and on egg fertility (FERT), total (TOTHATCH) and fertile (FRTHATCH) egg hatchability, and the percentages of early (ED) and late (LD) dead embryos and pipped (PIP) eggs (Exp. 3) were determined. In Exps. 1 ( $P < 0.0003$ ) and 2 ( $P < 0.0001$ ), mean LEI was shorter for eggs laid by HS than LS hens, while chick BWTE was unaffected by line (Exp. 1). In Exp. 2, B-implanted hen eggs also hatched sooner ( $P < 0.0001$ ) than did CON eggs and line\*hen B-implant treatment affected ( $P < 0.05$ ) the LEI as follows: LS-CON > LS-B > HS-CON > HS-B. In Exp. 3, FERT and TOTHATCH were dramatically reduced ( $P < 0.0001$ ; both cases) in eggs of HS compared to LS hens and in eggs of B-implant compared to CON hens ( $P < 0.0001$  and  $P < 0.0002$ , respectively). Line\*implant treatment FERT and TOTHATCH means differed ( $P < 0.05$ ) as follows: LS-B = LS-CON > HS-CON > HS-B and LS-CON = LS-B = HS-CON > HS-B, respectively. Although FRTHATCH and ED was unaffected by the main treatments, HS-B-implanted hen eggs had more ( $P < 0.05$ ) EDs. LD embryo and PIP egg percentages were unaffected. The stress line\*maternal B findings are important to avian geneticists as they further emphasize the benefits that selection for reduced adrenocortical responsiveness has on

hen reproductive performance and they warn poultry producers that stress in the laying barn may abbreviate egg incubation periods and negatively affect egg FERT, TOTHATCH, and ED embryos, particularly in hens genetically predisposed towards high stress responses.



# CHAPTER 1

## INTRODUCTION

In birds, the effects of the major adrenal glucocorticoid hormone, corticosterone (B), on egg hatching processes are important to study because: 1) B plays many roles in the metabolic regulations and other physiological events that underlie adaptation to stress in embryos, and 2) it is well known that heightened and persistent adrenocortical responses (B releases) are associated with many deleterious effects on poultry production and animal well-being (See **Chapter 2.1.2: HPA Axis Control of Corticosterone Release, Production Performance and Animal Welfare**; below). In commercial fowl, the effects of B on the length of egg incubation (LEI), egg fertility (FERT) and hatchability (both total hatchability, TOTHATCH, and fertile hatchability, FRTHATCH), embryonic mortality (early dead, ED, and late dead, LD, embryos and pipped, PIP, eggs) and chick body weight at emergence (BWTE) are particularly relevant variables to study because certain unavoidable husbandry practices used in modern-day confinement housing systems for breeder birds can be quite stressful and can therefore negatively impact these parameters. Furthermore, because a chick's weight at hatch is positively associated with early body weight gain (Moran, 1990) and with body weight at harvest age (Goodwin, 1961; Merritt and Gowe, 1965), producing large numbers of heavy day-old chicks is an important consideration in poultry hatcheries as their outputs (chicks) become the starting points (inputs) for broiler grow-out farmers. In other words, since the vast majority of worldwide poultry production businesses embrace the vertical commodity system approach, a prime goal of hatchery managers is to generate as many chicks that are of the highest quality possible in order to insure the greatest success downstream in broiler grow-out enterprises.

Satterlee and Johnson (1988) have selected divergent lines of Japanese quail for either reduced (low stress, LS) or exaggerated (high stress, HS) plasma B response to brief immobilization. These lines provide an excellent model to study the interactive influences of B derived from maternal genomic and/or supplemental sources (the primary subjects of this thesis), on hen reproductive performance parameters that reflect embryonic development (LEI and BWTE) and egg hatching processes (FERT, TOTTHATCH, FRTHATCH, ED, LD and PIP) for the following reasons. Regarding embryonic development, only a few studies on the effects of maternally-derived B or *in ovo* B treatment on the LEI in birds (and other oviparous animals) exist and no studies on the effect of maternally derived B on LEI could be found. Furthermore, the studies that address these treatments and their outcomes conflict in many ways. For example, Rubolini et al. (2005) demonstrated that yellow-legged gull eggs treated with B hatched later than did eggs treated with a vehicular control. In contrast, in an oviparous lizard species, *in ovo* B-treated eggs hatched before both positive (vehicle treated) and negative (untreated) control eggs (Weiss et al., 2006). On the other hand, in chicken eggs, Tona et al. (2007) found that, depending upon early incubator ventilation treatment (ventilation vs. non-ventilation during the first 10 d of egg incubation) and the age at which near term developed chick embryos were challenged with the powerful synthetic glucocorticoid dexamethasone (16 vs. 18 d of incubation), the LEI either decreased, did not change or increased. Furthermore, De Smit et al. (2008), in using the same incubator ventilation treatments as Tona et al. (2007) on eggs from two different broiler breeder strains, found that non-ventilation (a presumably stressful treatment) shortened LEI, an effect that was associated with heightened embryonic levels of plasma B from 11 – 17 d of egg incubation. Finally, it deserves brief mention here (and will be reviewed in more detail later) that a voluminous literature exists that addresses the effects of maternal stress on preterm delivery

(PTD), defined as birth prior to 35 wk of gestation, in humans (Copper et al., 1996; Hobel et al, 1999; Ruiz et al., 2003; Dole et al, 2003, 2004; Glynn et al., 2008). These studies have invariably demonstrated that a host of psychological factors, such as anxiety, psychosocial events, and general maternal stress, predict and likely bring about the majority of the observed instances of PTD. Indeed, all of these studies have concluded that limiting maternal stress responses during pregnancy may alleviate the adrenal-related mechanisms that appear to be associated with the etiology of PTD.

In regards to chick BWTE as a final component of embryonic development, the body weights of hatchling chicks from the gull eggs of Rubolini et al. (2005) were found to be unaffected by B-treatment when compared to their vehicular controls. Similar to these findings in gulls, Tona et al. (2007) found their two differently timed late stage egg dexamethasone injection treatments (see above) to be ineffective in altering embryo body weight at internal pipping. Eriksen et al. (2003) in chickens and Hayward and Wingfield (2004) in quail also reported that *in ovo* and maternal B treatment, respectively, had no effect on day-1 chick body weights. However, in the Hayward and Wingfield (2004) study, wherein more B deposition into the yolks of eggs laid by B-implanted mothers was confirmed, it is important to note that, while Day 1 chick hatch weights were unaffected by implant treatments, reduced growth rates were nevertheless evident during the first 7 d of life in chicks hatched from eggs derived from B-implanted hens. Interestingly, and in agreement with the four avian chick body weight studies just cited above, in the oviparous tree lizards studied by Weiss et al. (2006), hatchling body weights were also found to be unaffected by *in ovo* B treatment. In contrast, reductions in neonate body weight due to exaggerated B concentrations in developing embryos produced by *in ovo* B

treatments have been demonstrated in both barn swallows (Saino, et al., 2005) and in chickens (Mashaly, 1991; Heiblum et al., 2001).

Regarding egg hatching processes, it has recently been shown that not only do both unstressed and stressed HS hens deposit more B (62 and 96% more, respectively) into their eggs than do their LS hen counterparts (Hayward et al., 2005), but HS hens implanted with B also show a dramatically reduced rate of egg production when compared to LS- and HS-control and LS-B-implanted hens (Satterlee et al., 2007). These line\*maternal B interactive effects on egg lay suggest that such treatments are likely to affect egg FERT and hatchability as well since hen-day egg production rates are well known to be highly positively correlated with egg fertility and hatchability in genetically unremarkable (non-selected) chickens (North, 1990). Moreover, *in ovo* B treatment has clearly been associated with a reduction in egg hatchability in a host of avian species (Mashaly, 1991; Eriksen et al., 2003; Heiblum et al., 2001; Rubolini et al., 2005; Saino et al, 2005) as well as in the tree lizards of Weiss et al. (2006). And, there is some limited, although as the researchers freely admit not overly convincing, evidence that turkey hens selected for low (LL) as opposed to high (HL) plasma B response to cold stress showed “superior” percent fertility and percent hatch of fertile eggs “whenever significant differences (in these variables) occurred in between the lines” (Brown and Nestor, 1973, 1974). In reality, line differences (LL > HL) were detected amongst the 9 initial yearly generations of selection only twice (at G<sub>4</sub> and G<sub>7</sub>,) and only once (at G<sub>7</sub>) for egg fertility and hatchability, respectively.

Despite the propagation of the LSU quail stress response lines (the LS and HS lines of Satterlee and Johnson, 1988; described above) for more than 30 generations over the past 20 years, line differences in the LEI, chick BWTE, egg FERT, TOTTHATCH, and FRTHATCH, ED and LD embryos, and PIP eggs have never been determined. However,

subjective impressions during chick pulls at hatch have always been that hen reproductive performance differs in regard to several of these parameters between quail of the LS and HS lines. Furthermore, because *in ovo* B treatment clearly affects several of these parameters in random bred avians (and controversially so in many cases; see discussion above), the present experiments were conducted to investigate the influences of quail stress line, maternal B-implant treatment, and their interaction on the aforementioned variables. The effects of quail stress line on LEI and BWTE are discussed in Chapter 3, Experiment 1. The interactive influences of line with maternal B-treatment (either sub-Q implants filled with no-B (controls, CON) or B) on LEI are described in Experiment 2 of Chapter 3. In a third experiment (described in Chapter 4), the interactive effects of stress line and maternal B-treatment, the same four treatments used in the Experiment 2 of Chapter 3, on egg FERT, TOTHATCH, and FRTHATCH, ED and LD embryos, and PIP eggs are examined.

## CHAPTER 2

### REVIEW OF LITERATURE

#### 2.1 Hypothalamic-Pituitary-Adrenal Axis (HPA) Control of Corticosterone Release and the Role of Corticosterone in Avian Production Performance and Well-Being

##### 2.1.1 General Adaptation Syndrome, Stressors, and Stress

Hans Selye is considered to be the father of modern-day stress biology by virtue of his very early postulation of the General Adaptation Syndrome (GAS; see Selye, 1936, 1949, 1950, 1976) which has stood the test of time. Selye originally explained his choice of the GAS terminology as follows: "I call this syndrome **general** because it is produced only by agents which have a general effect upon large portions of the body. I call it **adaptive** because it stimulates defense . . . I call it a **syndrome** because its individual manifestations are coordinated and even partly dependent upon each other." The GAS is thought to manifest itself in three incremental stages: an alarm reaction (AR), a stage of resistance (SR; adaptation), and the stage of exhaustion (SE). The AR, often referred to as the "fight or flight" reaction, is an acute response characterized by tissue catabolism, hyperglycemia, and the release of adrenal glucocorticoids. If a stressor (defined below) progresses from being an acute to chronic stimulus, an organism will theoretically transition into the SR wherein it will attempt to adapt to the negative stimulus, thereby alleviating some, if not all, of the physiological states (including elevations of the glucocorticoids) elicited during the AR. The SE may arise if a stressor is too robust or malingers in such a way that exhaustion of physiological resources occurs. During the SE, the aforementioned AR physiological reactions may reappear (in birds, most notably the major avian glucocorticoid, corticosterone or B, will again be released and in massive amounts) in order to make more metabolic resources available in

a last ditch effort to survive. Unfortunately, such overly heightened and/or prolonged releases of B can be very detrimental to animal production performance and well-being as described below.

As Selye further pointed out in his also now somewhat dated, but notably still relevant, 1976 review: a number of “problems and misconceptions” concerning the use of the terms “stressors” and “stress” as they relate to the GAS remained back then (statements made by Selye 40 years after he first postulated the GAS and more than 30 years ago). Unfortunately, these terminology problems continue to persist today despite the fact that nearly 75 years have now passed since Selye first introduced the GAS. Indeed, as Zanchetti (1972) warned: “Stress is a dangerous and useless word. It may seem useful because it is a unifying word, but it unifies our ignorance rather than our knowledge.” Thus, it remains important at the outset of any review of stress biology to delineate the differences between the terms, stressors and stress, and how these terms will presently be used.

In his 1976 review, Selye emphasized several fundamental concepts that he considered important in attempting to understand stress and research in the field of stress biology that I believe are important to reiterate here. First, he emphasized that one must have the “correct definition of **stress**, **stressors** (addressed below) and the **GAS**” (briefly described above) and that scientists must understand “the concept of **nonspecificity** in [stress] biology.” Nonspecificity is an important concept that underlies the genomic result of selection for contrasting adrenocortical responsiveness of the quail lines used in the present studies (see **2.1.3 LSU Quail Stress Lines**, below). Selye (1976) went on to state that biological systems are susceptible to “the conditioning of stress responses by diverse endogenous (mainly **genetically** determined) and exogenous (**environmental**)

factors.” Both of these factors (genetic and environmental) are also main subjects of this thesis considering the treatments used; i.e., maternal B treatment of the quail stress lines during egg formation; see **Chapters 3 and 4**). Selye (1976) concluded by discussing “the relation between general and local adaptation syndromes, the difference between direct and indirect pathways, the mode of action of syntoxic and catatoxic hormones, drugs and behavioural attitudes and the so-called **first mediator of the stress response [release of stress hormones]**, which carries the message that a state of stress exists from the directly affected area to the neurohormonal regulatory centres” (yet another concept important to the present studies).

Thus, over the past thirty plus years (since Selye’s 1976 review), useable and workable definitions of what best defines animal stress responses (i.e., activation of the hypothalamic-pituitary-adrenal, HPA, axis) and how such responses might best be measured (e.g., via measurements of adrenocortical glucocorticoid hormones) have not changed much. In agreement, in the recent review of Cockrem (2007), entitled *Stress, Corticosterone Responses and Avian Personalities*, “stress” is defined as “the state of HPA axis activation which leads to an increase in secretion of glucocorticoids in response to the particular stressor.” If this “classic” pure and simple definition of stress is accepted, then only the questions of exactly what are “stressors” and how do stressors differ from “stress” remain. Unfortunately, as mentioned above, the two terms have been and are continued to be used interchangeably as synonyms, when they are clearly not, thus producing much confusion. Stressors should be considered as physical or psychological traumas (forces) that bring about the “state” of stress. Thus, stressors are “external happenings” that bring about “internal events” (stress states) in the body. For example, stressors commonly encountered in the poultry industry would include:



temperature extremes, inappropriate stocking densities (e.g., crowding), disruptions of the peck order, human-animal interactions (e.g., capture, handling and restraint to affect determinations of body weight), unexpected harsh sounds, inappropriate lighting, heavy parasite loads, etc. Coming more from a behavioral (predator-prey) viewpoint, Cockrem (2007) further stated in his review- a “stressor” is a stimulus that can only be called a stressor if “it is considered a threat by an animal.” Cockrem (2007) asserts that only in that instance is the HPA axis activated and glucocorticoids released from the adrenal gland (i.e., conditions indicative of stress). Thus, for the purposes of this thesis, the definitions of stressors and stress put forth by Cockrem (2007) will be used with the caveat that “threats” to the animal can and do go beyond emotional events to include lots of physical traumas as well that are not necessarily perceived by the animal as predatory, e.g., inclement weather.

### **2.1.2 HPA Axis Control of Corticosterone Release, Production Performance and Animal Welfare**

The HPA axis consists of three component parts: the hypothalamus, the hypophysis (or pituitary gland), and the adrenal glands. Each component is part of a sophisticated neuroendocrine control system that regulates the release of the avian major glucocorticoid hormone, corticosterone (B; see reviews of Siegel, 1971, 1976, 1980, 1995; Harvey et al., 1984; Carsia and Harvey, 2000), the so-called “avian stress hormone equivalent” of the perhaps more familiar mammalian glucocorticoid, cortisol (see Hadley, 2000). Reduced to simplicity, the neuroendocrine system basically works as follows. When presented with a stressor stimulus (see above), the avian hypothalamus is neuronally signaled to release corticotrophin-releasing hormone (CRH) into the hypothalamo-hypophyseal portal blood system that connects the hypothalamus with the anterior lobe of the pituitary gland (Carsia and Harvey, 2000). Once CRH reaches the

pituitary, it stimulates the release of adrenocorticotrophic hormone (ACTH) into the general circulation. ACTH then travels to the avian adrenals where this peptide hormone stimulates the release of B that, in turn, can travel back to the brain and serve as a negative feedback inhibitor of further ACTH release or travel to numerous target tissues throughout the body and bring about the hormone's stress adaptation functions and other biological actions (see **2.1.1: General Adaptation Syndrome, Stressors, and Stress;** above). Upon release, B appropriately redirects energy (via altering carbohydrate, protein and fat metabolism) and alters vasomotor tone, water and electrolyte balance, and certain behaviors to help the animal best adapt (short term) to the stressful situation (Carsia and Harvey, 2000). Thus, in a short-term adaptive sense, B release is considered to be beneficial. However, in stress situations wherein fear and distress cause B releases that are overly heightened and/or persistent, serious negative consequence can occur. For example, in poultry, exaggerated and prolonged stress responses have been associated with the following deleterious effects on production performance and animal well being: energy wastage, feather damage, reduced growth, poor feed conversion, declines in egg production and eggshell quality, impaired male and female reproductive function, developmental instability, injury, pain, and higher death rates (Mills and Faure, 1990; Jones, 1996, 1997; Jones and Hocking, 1999; Carsia and Harvey, 2000; Satterlee et al., 2000, 2002, 2007, 2008; Satterlee and Marin, 2004).

### **2.1.3 LSU Quail Stress Lines**

Classic environmental physiology principles teach two strategies to enhance production performance and animal welfare (and thereby maximize profitability) when attempting to manage and rear livestock under modern-day intense farming conditions wherein many stressful but necessary (unavoidable) husbandry techniques are employed

to optimize success. Simply put, these are: one can “alter the animal to fit the environment” (i.e., via genetic selection, administering appropriate vaccinations, etc.) and/or “alter the environment to fit the animal” (e. g., in poultry production, providing environmentally controlled dark-out housing conditions for the relief of heat stress and to better control light, practicing ‘all-in/all-out’ principles to aid in biosecurity, etc.). Early on, Satterlee and Johnson (1988) employed the former principle in genetically selecting two Japanese quail lines for divergent stress responsiveness. Many studies of these stress response lines over the last 20 years have shown that selection for reduced (low stress, LS), as opposed to exaggerated (high stress, HS), plasma B response to brief mechanical restraint is associated with many intuitively desirable physiological and behavioral traits in the LS line that make the LS quail more suitable to rear. For example, LS quail exhibit an apparent “non-specific” stressor reduction in adrenal stress responsiveness to a wide variety of stressors in addition to the genetic selection stressor of manual restraint (e.g., handling, cold, crating, feed and water deprivation, social tension, and presentation with a novel object; Jones et al., 1992b, 1994, 2000; Jones, 1996; Cockrem et al., 2008a,b). This is the ever-important concept of “nonspecificity” that Selye described in his 1976 review (see **2.1.1: General Adaptation Syndrome, Stressors, and Stress**, above). The concept is important because it teaches that activation of the HPA axis and subsequent releases of glucocorticoid hormones (B in avians) with their potential to induce negative results is a shared consequence of all physical and psychological stressors, regardless of the nature of the potential stressors, provided that a given stressor represents a potent enough stimulus to induce the stress state. Thus, it is logical as well to expect that quail of the LS line, when compared to their HS counterparts, would have genetically-controlled reduced plasma B releases to many other yet untested stressors that

they might typically encounter in their routine (day-to-day) poultry production settings. And, in theory, these reduced stress responses should translate into improvements in production performance and animal well being. Indeed, this seems to be exactly the case for differences detected between the lines in literally dozens of animal performance and welfare variables tested to date. For example, quail of the LS line, in comparison to HS quail, show: improved growth (Satterlee and Johnson, 1985); less cortical bone porosity (Satterlee and Roberts, 1990); reduced developmental instability (Satterlee et al., 2000, 2008); reduced fear (Jones et al., 1988, 1992a,b, 1994, 1996, 1999; Satterlee et al., 1993; Jones and Satterlee, 1996; Kembro et al., 2008; Davis et al., 2008); increased sociality (Jones et al., 2002; Guzman et al., 2008); and, accelerated puberty and enhanced reproductive performance in both males (Satterlee et al., 2002, 2006, 2007; Marin and Satterlee, 2004; Satterlee and Marin, 2004) and females (Marin et al., 2002; Satterlee et al., 2007).

It is important to note the specifics of what is known thus far about quail stress line differences in female reproductive performance since the focus of this thesis is to further knowledge in that broad area. Firstly, broiler chicks that navigate a T-maze quickly (HP, high performers) to socially reinstate with live conspecifics were shown to exhibit a reduced plasma B response to acute stress than their slower (LP, low performer) counterparts (Marin and Jones, 1999). This led to a follow-up study by Marin et al. (2002) that showed the average ages at first egg lay and at 25% HDEP were reduced in LS quail adults that were categorized as HP in a T-maze as chicks when compared to the intermediate and similar responses found for these two reproductive milestones in LP-LS and HP-HS quail and the yet further declines in them found in LP-HS quail hens. In addition, mean cumulative HDEP during the first 8 wk of lay reverse mirrored these

puberty differences across the four interactive T-maze performance by line treatments (i.e., for HDEP,  $HP-LS > LP-LS = HP-HS > LP-HS$ ). It was thus speculated in the Marin et al. (2002) study that: T-maze identification of HP individuals in the LS quail line likely picked the lowest plasma B stress-responders in the LS line, while T-maze identification of LP individuals in the HS quail line was likely associated with identification of the highest plasma B stress-responders in the HS line. Identification of these two extreme cases within each of the two lines was further hypothesized to be what underlied why the HS-LP hens showed a compromised onset of puberty and reduction in egg lay in comparison to the LS-HP hens. HS hens implanted with B also show a dramatically reduced rate of egg production when compared to LS- and HS-control and LS-B-implanted hens (Satterlee et al., 2007). These line\*maternal B interactive effects on egg lay suggest that such treatments (maternal genomic and supplemental B influences) are likely to affect egg FERT and hatchability (TOTATCH and FRTHATCH) as well since HDEP rates are well known to be highly positively correlated with egg fertility and hatchability in genetically unremarkable (non-selected) chickens (North, 1990). Changes in egg FERT, TOTATCH and FRTHATCH associated with maternal B-treatment during egg formation in quail hens of the LS and HS lines are three variables investigated in the present studies described in Chapter 4.

## **2.2 Development and Functioning of the Adrenal Glands in Avian Embryos**

The avian adrenals are a set of paired glands located anterior and medial to the cephalic lobes of the kidneys. Generally, the adrenal glands are flattened, lie close together and may become fused in some bird species (Hartman and Brownell, 1949). In chickens, around Day 4 of egg incubation, precursor adrenal cells develop from the dorsal celomic epithelium and by Day 6 of incubation these cells form “paired solid

masses on each side of the aorta” (Bohus et al., 1965; Adjovi, 1970; Domm and Erickson, 1972). These precursor cells begin to secrete small amounts of B around Day 4 of egg incubation, but sustainable and HPA-regulated levels of B apparently do not arise until about Day 14 of incubation (Wise and Frye, 1973; Kalliecharan and Hall, 1974, 1976; Scott et al., 1981) even though pituitary ACTH is detectable by Day 8 of incubation (Pedernera, 1972). Once regulated, embryonic blood levels of B rise dramatically throughout the remainder of egg incubation (post-Day 14; Scott et al., 1981) as these higher levels of B are believed necessary for organ differentiation and maturation (Siegel and Gould, 1976). B is believed to play a direct role in the transition of the chick embryo from cardiovascular to pulmonary respiration via B-driven lung maturation and surfactant production late in embryogenesis (Decuypere, 1990). A role for B in the initiation of the hatching process has also been hypothesized (Scott et al., 1981).

### **2.3 Maternal and *In Ovo* Corticosterone Effects on the Length of Egg Incubation, Chick Body Weight, and the Fertility and Hatchability of Eggs**

It was originally thought that the embryos of avian species (which are oviparous) produced all the steroid hormones needed for proper embryogenesis. More recently, however, there have been numerous reports in birds (Schwabl, 1993, 1996a,b; Adkins-Regan et al., 1995; Schwabl et al., 1997; Gil et al., 1999; Lipar et al., 1999; Lipar and Ketterson, 2000; Sockman and Schwabl, 2000; Eising et al., 2001, 2003; Royle et al., 2001; Wittingham and Schwabl, 2002; Eising and Groothuis, 2003; Hayward and Wingfield, 2004; Andersson et al., 2004; Hayward et al., 2005; Groothuis and Schwabl, 2008) that have shown maternal steroids are not only present in eggs at oviposition, but these parentally-derived steroids can also affect both the development of the embryo and subsequent hatchling long before embryonic tissue *per se* initiates the production of steroid hormones.

Theoretically, in mammals and birds, a maternally-derived prenatal “embryonic stress response” likely occurs whenever a stressor activates the HPA axis of the dam sufficiently enough to elevate maternal levels of blood glucocorticoids such that levels of circulating embryonic glucocorticoids are also increased above those derived from the embryos themselves. In fact, avian embryos not only appear to be susceptible to maternal stress-induced elevations of yolk B (Saino et al., 2005) but also, exposure of birds (all of which are oviparous animals) to stressful stimuli during or slightly before egg formation seems to affect embryogenesis similarly to what has been seen in prenatally stressed viviparous animals (Janczak et al., 2006). But, more importantly, birds provide unique models to study the influences of maternal stress-induced and *in ovo* B effects on embryonic, juvenile and adult offspring development, physiology, and behavior (e.g., alterations in embryonic vocalizations; body weight; plumage development; aggression, fear and food drive behaviours; HPA axis responsiveness; cell-mediated immunity; length of egg incubation and hatchability; rate of lay; and, cloacal gland size and foam production; see Heiblum et al., 2001; Lay and Wilson, 2002; Hayward and Wingfield, 2004; Love et al., 2005; Saino et al., 2005; Rubolini et al., 2005; Janczak et al., 2006; Satterlee et al., 2007; Schmidt et al., 2008). This is because: in avians, the hormonal link between the dam and her offspring is severed post-oviposition as opposed to the continual shared blood communication that occurs between mothers and their fetuses throughout gestation in placental (viviparous) animals. Thus, the severed dam-offspring relationship in birds allows researchers to examine two gestational periods independently. Firstly, the maternal-fetal endocrine milieu up to the point at which oviposition occurs can be examined; and secondly, hormonal effects on embryogenesis can be further investigated from that point onward (i.e., during egg incubation) without the influence of

the varying glucocorticoid levels of the dam. The dynamic interactions within the dam-placental-fetal triangle throughout gestation are thus removed when studying maternal stress effects on avian offspring.

Unfortunately, however, only a scant and controversial avian literature exists that examines the effects of *in ovo* B-treatment on the LEI and chick BWTE, and no studies on the effect of maternally derived B on the LEI (a treatment used in the present studies; see below) could be found. For example, Rubolini et al. (2005) have shown that yellow-legged gull (*Larus michahellisi*) eggs treated with B hatched later than eggs treated with vehicle alone, and *in ovo* B-treatment did not affect hatchling body weight. In contrast to the LEI findings of Rubolini et al. (2005), in an oviparous lizard species, *in ovo* B-treated eggs hatched before both positive (vehicle treated) and negative (untreated) control eggs (Weiss et al., 2006). On the other hand, in chickens eggs, Tona et al. (2007) found that, depending on early incubator ventilation treatment (ventilation vs. non-ventilation during the first 10 d of incubation) and egg incubation age at injection (16 vs. 18 d) with dexamethasone (a powerful synthetic glucocorticoid), their late stage *in ovo* challenges resulted in a decrease, no change, or an increase in the LEI. Using eggs from two broiler breeder hen strains, the same lab workers (De Smit et al., 2008) used the incubator ventilation treatments that were employed by Tona et al. (2007; see above) in a second experiment and found that early non-ventilation treatment shortened the LEI, an effect associated with heightened embryonic levels of plasma B from 11 – 17 d of egg incubation. In addition, and in support of the similar findings of Rubolini et al. (2005) in gulls, Tona et al. (2007) found both of their late stage egg dexamethasone injection treatments to be ineffective in altering embryo body weight at internal pipping.



It is also worthy of note here that a voluminous literature exists that addresses the effects of maternal stress on preterm delivery (PTD; or birth prior to 35 wk of gestation) in humans. These studies demonstrate that anxiety (Glynn et al., 2008), psychosocial factors (Dole et al., 2003) and maternal stress in general (Copper et al., 1996; Hobel et al., 1999; Dole et al., 2003; Ruiz et al., 2003) are all predictive factors for PTD in women; and, all of these studies have concluded that limiting stress responses during gestation may alleviate the mechanisms that cause PTD.

Eriksen et al. (2003) in chickens and Hayward and Wingfield (2004) in quail also reported that *in ovo* and maternal B treatment, respectively, had no effect on day-1 chick body weights. However, in contrast, reductions in neonate body weight due to exaggerated B concentrations in developing embryos produced by *in ovo* B treatments have been demonstrated in both barn swallows (Saino, et al., 2005) and in chickens (Mashaly, 1991; Heiblum et al., 2001).

Many studies show *in ovo* B treatment reduces egg hatchability in a host of genetically unremarkable (non-selected) avian species (Mashaly, 1991; Eriksen et al., 2003; Heiblum et al., 2001; Rubolini et al., 2005; Saino et al., 2005) as well as in the oviparous tree lizards (Weiss et al., 2006). In addition, the very early studies of Brown and Nestor (1973, 1974) that examined the reproductive physiology of eggs laid by turkey hens from lines selected for either low (LL) or high (HL) plasma B response to cold stress deserve mention here. Although their data were not overly convincing, a case was made by the authors of these papers that female turkeys from their LL line showed some limited “superior” reproductive performance. For example, as the authors readily admitted, “whenever significant differences occurred [in FERT and FTRHATCH] between the lines, the LL line was superior to the HL.” This language referred to the fact

that, over the initial nine yearly generations of selection of their turkey adrenal stress response lines, egg FERT was greater in the LL line only at G<sub>4</sub> and G<sub>7</sub>, while FRTHATCH was shown to be better in the same line solely at G<sub>7</sub>. Presently, other than this very limited work by Brown and Nestor (1973,1974), there are no reports on the effects of maternal B on egg FERT, TOTHATCH and FRTHATCH.

#### **2.4 Rationale for the Present Studies**

As discussed above, B plays many roles in important metabolic regulations and other physiological events that underlie adaptation to stress. Moreover, during chick embryogenesis, B is involved in numerous vital developmental roles outside adaptation to stress. For example, B is thought to be necessary for organ differentiation and maturation throughout embryogenesis plus transition of the chick embryo from cardiovascular to pulmonary respiration via lung development coupled with increased surfactant production late in embryonic development. It has also been suggested that B may also serve to trigger of the hatching process itself.

Unfortunately, little is known about the effects of *in ovo* B treatment on the LEI and chick BWTE, and what is known is controversial. In addition, no studies on the effect of maternally derived B on the LEI could be found. Many studies have also shown that *in ovo* B treatment reduces egg TOTHATCH in a host of genetically unremarkable (non-selected) avian species and there is some marginal data that suggests turkey hens from lines selected for low (LL), as opposed to high (HL), plasma B response to cold stress show “superior” egg FERT and FRTHATCH. HS hens implanted with B during egg formation also show a reduced rate of egg production when compared to their LS- and HS-control and LS-B-implanted hen counterparts and it is well known that highly

positive associations exist between hen-day egg production rates and egg FERT and hatchability.

Thus, because genetically unremarkable quail hens implanted with B during egg formation deposit significantly more B into the yolks of their eggs and produce chicks with a reduced growth rate than do control hens, and because unstressed and stressed HS hens deposit more B into their egg yolks than do their LS hen counterparts, the present studies were conducted to determine whether the LS and HS quail genomes would interact with maternal B treatment to: 1) alter the LEI and chick BWTE from the egg, and 2) alter FERT, TOTHATCH, FRTHATCH, and embryonic mortality (EDs, LDs, and PIP eggs). Chapter 3 (Experiment 1) describes a preliminary study that solely assessed the effects of quail stress line on the LEI and chick BWTE. Chapter 3 (Experiment 2) used a larger number of eggs to confirm the line effects on LEI found in the first experiment reported in Chapter 3 and added the further study of the effects of hen B-implant treatment during egg formation and its interaction with line on the LEI. In a third and final study (reported in Chapter 4), the effects of quail stress line, maternal B treatment and their interaction on FERT, TOTHATCH, FRTHATCH, ED, LD, and PIP were determined.

## CHAPTER 3

### **INFLUENCES OF MATERNAL CORTICOSTERONE ON EGG INCUBATION LENGTH AND CHICK BODY WEIGHT AT EMERGENCE IN EGGS LAID BY QUAIL HENS SELECTED FOR DIVERGENT ADRENOCORTICAL STRESS RESPONSIVENESS**

#### **3.1 Introduction**

In birds, the effects of maternal corticosterone (B) on the length of egg incubation (LEI) and hatchling body weight at emergence (BWTE) are important variables to study for many reasons. Firstly, B plays many roles in the metabolic regulations and other physiological events that underlie adaptation to stress (Siegel, 1971, 1976, 1980, 1995; Carsia and Harvey, 2000; Cockrem, 2007)- hormonal influences that theoretically could alter embryonic development. Secondly, B is thought to be generally needed for embryonic organ differentiation and maturation (Siegel and Gould, 1976), transition of the chick embryo from cardiovascular to pulmonary respiration via B-driven lung maturation and surfactant production late in embryogenesis (Decuypere, 1990), and perhaps to trigger the hatching process itself (Scott et al., 1979).

Furthermore, it is important to identify and understand factors beyond the classic effects that pre-incubation egg storage and egg incubation temperature, humidity and turning conditions are known to have on LEI so that hatchery managers can better recognize the optimum time to conduct chick pulls from hatches. Because a chick's weight at hatch is also positively associated with early body weight gain (Moran, 1990) and with body weight at broiler harvest ages (Goodwin, 1961; Merritt and Gowe, 1965), optimizing the production of high quality day-old chicks is vital to poultry hatchery managers because their output (saleable chicks) becomes the starting point (input) for

broiler grow-out farmers in the vertical commodity systems that underlie the majority of modern-day poultry production enterprises.

In Section **2.3: Maternal and In Ovo Corticosterone Effects on the Length of Egg Incubation, Chick Body Weight, and the Fertility and Hatchability of Eggs** of this thesis, the avian literature that addresses the effects of *in ovo* B treatment on the LEI and chick BWTE was reviewed in detail. Therefore, further review of these studies will not be repeated here. It is important, however, to remind the reader that this literature was, in sum, both brief and conflicting and that no studies on the effect of maternally derived B on the LEI (a treatment used in the present studies; see below) were found. These facts provided further impetus to conduct the present studies.

It has also been suggested for reasons stated earlier that an excellent model to examine the effects of maternal B on the LEI and chick BWTE is the quail stress lines of Satterlee and Johnson (1988) who selected their lines based on either a reduced (LS; low stress) or exaggerated (HS; high stress) plasma B response to brief immobilization. Because of the controversial literature that addresses B-influences on LEI and hatching body weight, and because: 1) genetically unremarkable quail hens are known to transfer B to their egg yolks and to produce chicks of reduced body weight when mothers are implanted with B (Hayward and Wingfield, 2004), 2) egg yolks from unstressed and stressed HS quail hens contain B concentrations that are 62 and 96% higher, respectively, than what's found in the egg yolks of LS hens (Haywood et al, 2005), and 3) subjective impressions for years have been that HS eggs hatch sooner than do LS ones, presently, the influences of quail stress line (LS vs. HS) on LEI and chick BWTE were assessed in a preliminary study (Experiment 1). Because line was found to influence LEI (i.e., LEI was reduced in eggs laid by HS hens) without affecting BWTE in Experiment 1, a second

study (Experiment 2) was conducted to: 1) confirm the results of the preliminary stress line/LEI study using a larger number of eggs, and 2) additionally determine if quail stress line genome interacts with maternal B treatment in affecting LEI.

## **3.2 Materials and Methods**

### **3.2.1 Experiment 1**

#### **3.2.1.1 Genetic Stocks and Animal Husbandry**

Female Japanese quail from generation (G)<sub>38</sub> of two lines selected for either a low (LS, low stress) or high (HS, high stress) plasma B response to brief mechanical restraint (Satterlee and Johnson, 1988) were studied. The lines' most recent genetic history, up to G<sub>36</sub>, is discussed in detail elsewhere (Satterlee et al., 2000; Marin and Satterlee, 2004; Satterlee et al., 2006; Satterlee et al., 2007). It should also be noted that, while line differences in levels of plasma B were not directly measured herein, recent findings in the stress lines attest to the maintenance of divergent adrenocortical responsiveness to a variety of non-specific systemic stressors, e.g., restraint and handling (G<sub>32</sub>; Cockrem et al., 2008a) and treatment with a novel object (G<sub>34</sub>; Cockrem et al., 2008b). Moreover, Hayward et al. (2005), in a study of G<sub>32</sub> quail, found egg yolk B concentrations to be greater in yolks collected from eggs of HS hens than in yolks from LS hens by 62 and 96 %, respectively, when hens were undisturbed or cooped and socially stressed during egg formation, and mean fecal B was found to be higher ( $P < 0.0003$ ) in colony-caged mixed-sex adult HS (101.9 ng of B/g of feces) compared to LS (85.9 ng of B/g of feces) quail of the same generation (G<sub>32</sub>; Cockrem et al., 2008c).

Egg incubation and chick brooding, feeding, and lighting procedures were similar to those described elsewhere (Jones and Satterlee, 1996). Post-brooding, juvenile quail (28 d of age) were housed in two three-tier breeder cage battery units with a within line

10:5 female to male ratio in each cage. Each cage battery contained 12 cages with individual cages measuring 60 cm x 51 cm x 25.5 cm. Breeder birds were fed a breeder ration (21% CP; 2,750 kcal ME/kg) with feed and water continued *ad libitum*. The daily photostimulatory cycle was 14:10 L:D (approximately 280 lux during the lighted portion of the day); lights-on was at 06:00 h and lights-off was at 20:00 h daily. Daily maintenance and feeding chores were done at the same time each day (09:00 h).

### **3.2.1.2 Treatments and Variables Measured**

At 68 d of age, all eggs laid by G<sub>38</sub> LS (n = 297) and HS (n = 278) breeder colony hens were collected over a four day period. All eggs laid were identified by pencil markings as to their origin by line. Eggs collected during the first three days of egg collection were first stored at 18 C until being set along with the freshly laid eggs (non-stored ones) on day 4 of egg collection. Eggs were set in a Nature Form NMC 2000 incubator. As best as possible, LS and HS eggs were equally distributed within and between two tiers of the incubator to minimize potential effects that incubator temperature and humidity gradients may have had on embryogenesis and subsequent egg hatchability. During the first 14 d of incubation, eggs were turned six times a day and subjected to 37.5 C and 62% RH. Upon transfer of the eggs to a second NMC 2000 hatcher unit on day 14, eggs were no longer turned and incubation conditions were changed to 37.2 C and 69% RH.

Beginning at 400 h of incubation and every 2 h thereafter, the hatcher was checked for the presence of hatched chicks. At each observation period, all hatched chicks were removed from the incubator. The identity of the length (h) of egg incubation (LEI) associated with each eggs' line (i.e., a hatched chick) was recorded for each hatchling that was removed from the hatcher. Chicks were also weighed at this time to

determine body weight at emergence (BWTE). The above process was repeated every 2 h until three consecutive checks of the hatcher (a 6 h interval) resulted in no further hatching or the observation of no additional pipped eggs. Thus, the LEI variable served to roughly estimate, within 2 h of emergence, the number of hours necessary for each chick to hatch or, simply put, the length of the egg incubation period.

In addition to the determination of mean differences in the LEI between eggs laid by LS and HS hens, cumulative percent hatching by LEI curves for both lines adjusted for the numbers of eggs that hatched within each line were constructed to allow examinations of hatching spreads across time of egg incubation.

### **3.2.1.3 Statistical Analyses**

The GLM procedure (SAS, 2000) was used to statistically analyze both the LEI and BWTE variables using a one-way ANOVA that incorporated a completely randomized design with quail stress line (LS vs. HS) as the main effect. Individual eggs and hatched chicks were used as the experimental units for the LEI and BWTE variables, respectively. The results are reported as means  $\pm$  SE for the effects of quail stress line and a P value  $\leq$  0.05 was used to determine significant differences in treatment means.

## **3.2.2 Experiment 2**

### **3.2.2.1 Genetic Stocks and Animal Husbandry**

The information on the genetic history underlying the generation used ( $G_{38}$ ) of the quail stress lines for Experiment 2 is identical to what was described earlier for Experiment 1 and thus it will not be repeated here. Test subjects for Experiment 2 (see below) were taken from a hatch of approximately 600 quail per stress line. At 266 d of age, 96 females (48 LS + 48 HS) were randomly selected for pairing with same-line, same-age adult males reared from the same hatch. Care was taken to insure that each of



the breeding pairs selected, while randomly selected from larger family populations within each line, constituted, as nearly as possible, equal representation of the 12 different families that make up each line. Also, within each line, pairing of full-siblings was avoided. Once selected, each breeder pair was then randomly housed in a single cage of one of two Alternative Cage Designs four-tier cage batteries (Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR). Each battery contained 48 pedigree-style breeder cages with individual cage dimensions of 50.8 x 15.2 x 26.7 cm (length x width x height). Breeder birds were fed a breeder ration (21% CP; 2,750 kcal ME/kg) with feed and water provided ad libitum. The daily photostimulatory cycle was 14:10 L:D (approximately 280 lux during the lighted portion of the day); lights-on was at 06:00 h and lights-off was at 20:00 h daily. Daily maintenance and feeding chores were done at the same time each day (09:00 h).

### **3.2.2.2 Hen Treatments and Variables Measured**

At 280 d of age, half of the hens in each line (n = 24 hens/line) were surgically implanted with either an empty 16 mm silastic tube (Dow Corning Corp., Midland, MI, Cat. No. 508-006; controls, CON) or with a same length silastic tube filled with B (Sigma-Aldrich Co., Atlanta, GA; Cat. No. C2505; B-implant). Implants were placed s.c. in the back of the neck using a No. 10 biopsy needle (Becton Dickinson, Franklin Lakes, NJ). Silastic tube implants were sealed on one end with a silicone sealant while the other end remained open.

All hens were allowed one week to acclimate to the implant treatments before collecting any eggs. This was done to allow sufficient time for maternal B deposition into the eggs of B-implanted hens (Hayward et al., 2004). Eggs were then collected daily for the next 28 d of lay. All eggs laid during this period were identified by pencil

markings as to their origin by hen line and implantation treatment, and they were stored at 18 C until incubation. All eggs from the first 2 wk of egg collection were set together into an incubator (NatureForm NMC 2000; NatureForm Hatchery Systems, Jacksonville, FL) as Replication 1 of the experiment. Eggs gathered from the second 2 wk of egg collection were stored under identical conditions and then set as Replication 2 of the experiment. During the first 14 d of incubation in each rep, eggs were turned 6 times a day and subjected to 37.5 C and 62% RH. Upon transfer of the eggs to a second NMC 2000 hatcher unit on Day 14, eggs were no longer turned and incubation conditions were changed to 37.2 C and 69% RH.

For each of the two egg hatch replications, beginning at 370 h (15 d and 10 h) of incubation, and every 2 h thereafter, the hatcher was checked for the presence of hatched chicks. At each of these observation periods, all hatched chicks were removed from the incubator. The identity of the LEI associated with each eggs' (hatched chick) treatment combination (LS-CON, LS-B-implant, HS-CON, HS-B-implant) was recorded for each chick that was removed from the hatcher. The above process was repeated every 2 h until three consecutive incubator checks (a 6 h interval) resulted in no further hatching or the observation of no additional pipped eggs. Thus, as in Experiment 1, the LEI variable served to roughly estimate, within 2 h of emergence, the number of hours necessary for each egg to hatch or, simply put, the length of the egg incubation period.

### **3.2.2.3 Statistical Analyses**

The GLM procedure (SAS, 2000) was used to analyze the LEI variable. The statistical design incorporated a randomized block design with the eggs set from each of the two 2-wk egg collection periods comprising the blocks (experimental replications). Thus, the analysis was an ANOVA that incorporated a 2 x 2 x 2 factorial arrangement of

treatments, with replications, quail stress line (LS vs. HS) and maternal implantation treatment (CON vs. B-implant) used as the factorials. Post-hoc testing using Duncans New Multiple Range Tests (DNMRT) to partition line\*treatment means was also performed. The LEI parameter is reported as mean  $\pm$  SE outcomes for all ANOVA effects and a P value  $\leq 0.05$  was used to determine significant differences in means.

### **3.3 Results**

#### **3.3.1 Experiment 1**

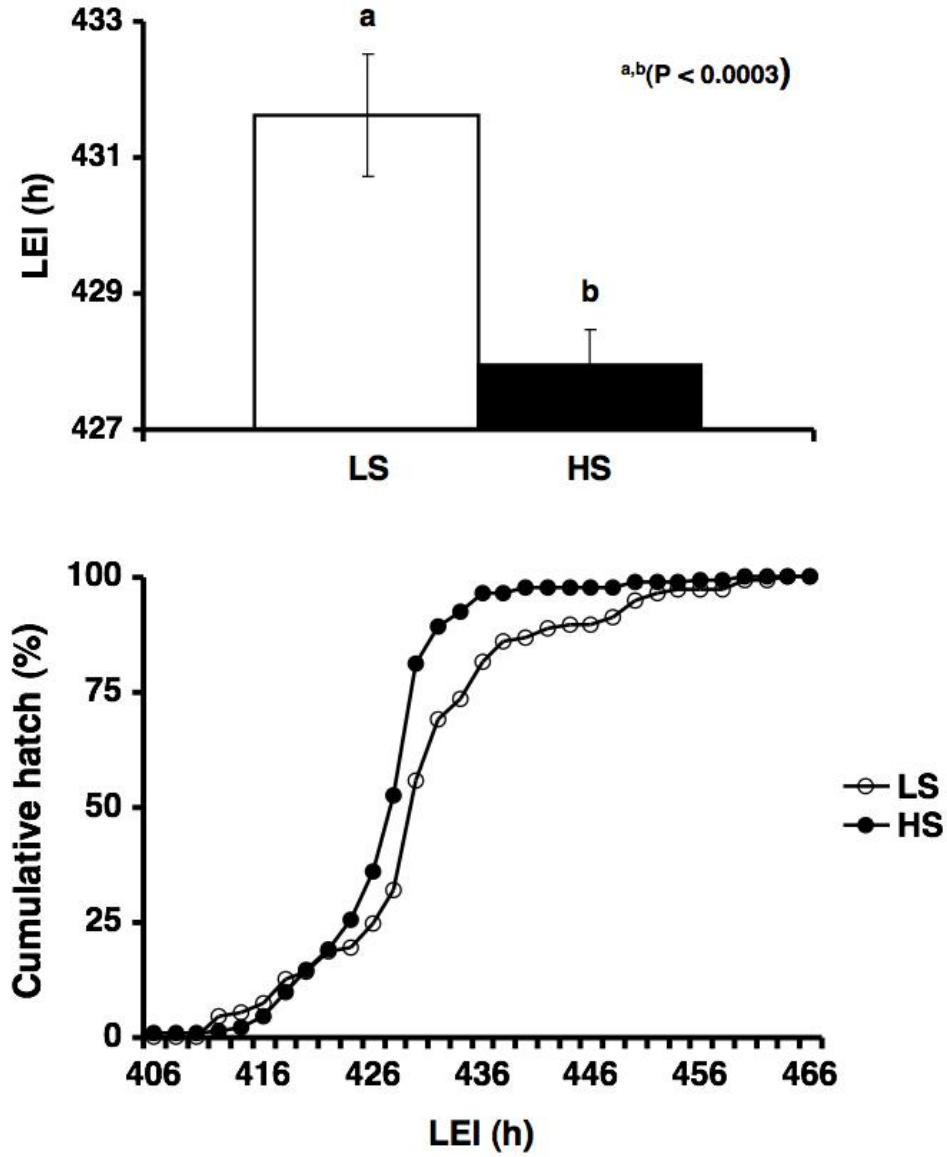
Figure 1 depicts the mean ( $\pm$  SE) LEI for the LS and HS quail lines. HS eggs (n = 158) hatched sooner, and markedly so ( $P < 0.0003$ ), than did LS eggs (n = 136). Figure 1 also shows cumulative percent hatching by LEI curves for the LS and HS lines adjusted for the numbers of eggs that hatched within each line. Figure 2 depicts mean ( $\pm$  SE) chick BWTE for the LS and HS quail lines. Chick BWTE did not differ by line.

#### **3.3.2 Experiment 2**

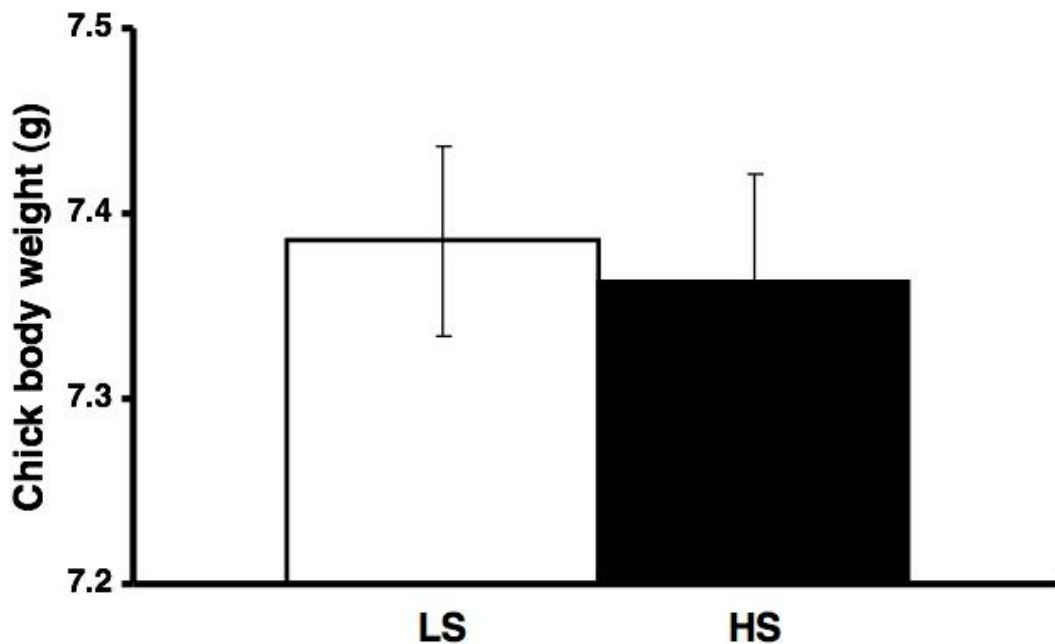
Figure 3 depicts differences in the main effect on the LEI means for eggs laid by the LS and HS quail hens (top panel), maternal CON- and B-implantation treatments (middle panel), and their interaction (bottom panel). On average, eggs from HS hens hatched 4.4 h sooner ( $P < 0.0001$ ) than did eggs from LS hens and eggs from B-implanted hens hatched 2.9 h sooner ( $P < 0.0001$ ) than did eggs from the CON hens. Differences in line\*implant treatment effects on mean LEI partitioned by DNMRT ( $P < 0.05$ ) were as follows: LS-CON > LS-B-implant > HS-CON > HS-B-implant.

### **3.4 Discussion**

For many years during the reproduction of dozens of generations of the LSU quail stress response lines, a subjective, but nevertheless distinct, impression has stood the test of



**Figure 1.** Effect of quail stress line (LS, low stress vs. HS, high stress) on mean ( $\pm$  SE; vertical bars) length of egg incubation (LEI; top panel). Cumulative percent hatching by LEI curves adjusted for the numbers of eggs that hatched within each line are depicted in the bottom panel.

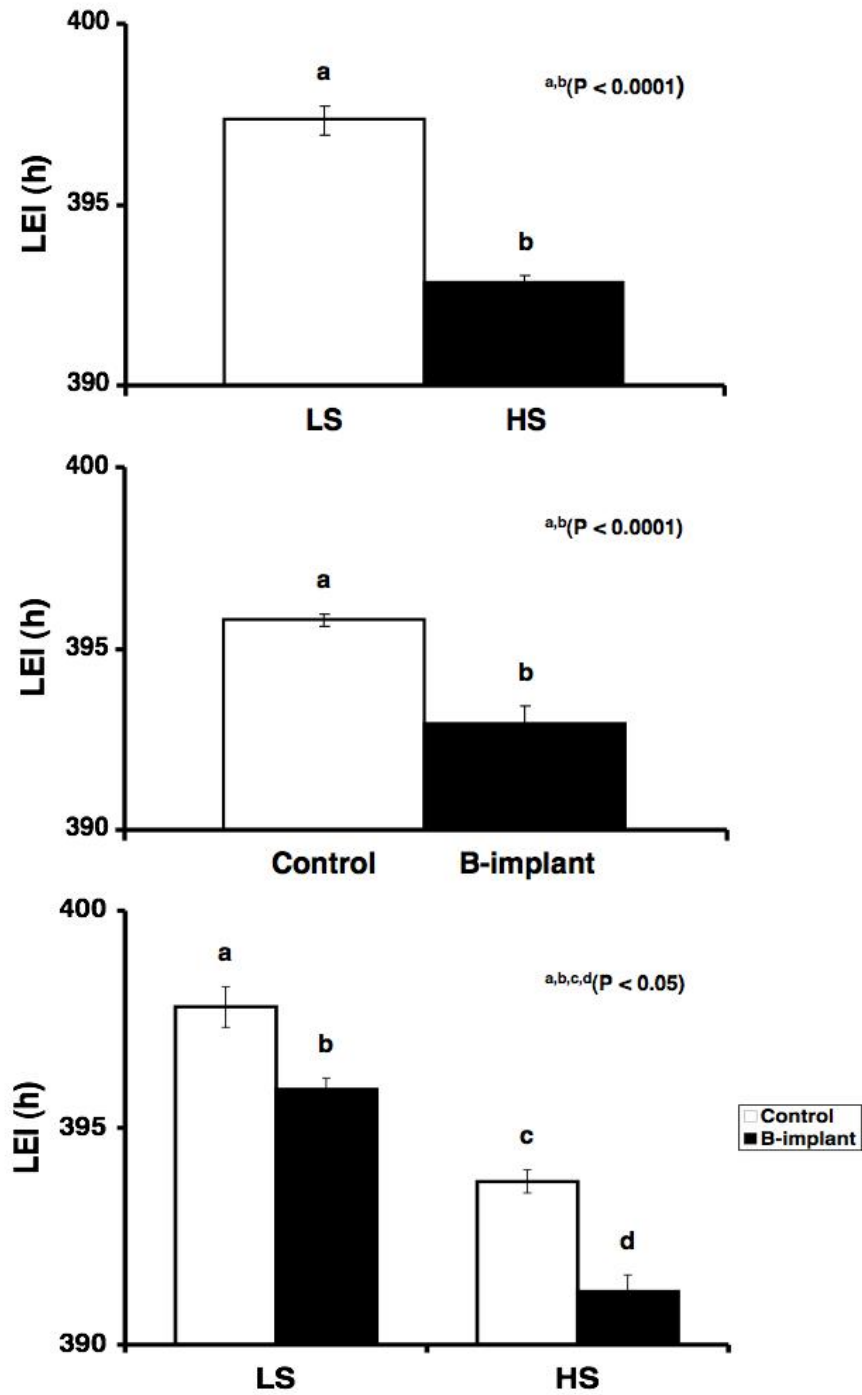


**Figure 2.** Effect of quail stress line (LS, low stress vs. HS, high stress) on mean ( $\pm$  SE; vertical bars) chick body weight at emergence (BWTE).

time- that HS quail eggs hatch sooner than do eggs laid by LS quail. However, whether this impression was real or not has not been experimentally tested until now.

Furthermore, the line differences (HS > LS) in egg yolk B deposition recently reported by Hayward et al. (2005), coupled with the recently emerging controversial literature on the effects of *in ovo* B on the LEI, provided further impetus to conduct the present studies.

In a preliminary study (Exp. 1), but with a very high degree of statistical confidence ( $P < 0.0003$ ), eggs from HS hens were found to hatch on average approximately 3.7 h sooner than did eggs laid by LS quail, thus verifying subjective lab suspicions. Furthermore, in Exp. 2 that used a much larger number of eggs, the results of Exp. 1 were confirmed in that the LEI of eggs from the HS-CON treated birds hatched about 4.4 h sooner (also a statistically robust finding;  $P < 0.0001$ ) than did the eggs from LS-CON treated birds.



**Figure 3.** Effects of quail stress line (LS, low stress vs. HS, high stress; top panel), maternal implant treatment (Control vs. corticosterone (B)-implant; middle panel), and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) length of egg incubation (LEI).

Also in Exp. 2, when compared to the CONs, eggs derived from hens implanted with B showed a mean LEI that was shortened ( $P < 0.0001$ ) by nearly 3 h and the line\*hen implant treatment effects on mean LEI partitioned ( $P < 0.05$ ) as follows: LS-CON > LS-B-implant > HS-CON > HS-B-implant.

Although line differences in hen plasma and egg yolk B concentrations were not measured presently, strong cases can be made that circulating B was higher in the HS than LS hens of Exp. 1 and in the HS-CON than LS-CON hens of Exp.2, and that higher levels of yolk B were present in the eggs laid by the HS than LS hens of Exp. 1 and in eggs laid by the HS-CON than LS-CON hens of Exp. 2. Hayward and Wingfield (2004) have demonstrated that, in comparison to control responses, B-implanted genetically unremarkable quail showed higher levels of plasma B that were, in turn, associated with higher deposition of egg yolk B. The implant silastic tube lengths, source of B, and procedures that underlied Hayward and Wingfield's (2004) study were identical to those used presently. Thus, it is reasonable to suspect that levels of B were also elevated in the plasmas of the present B-implanted LS and HS hens and in the yolks of their eggs owing to the B-implant treatments alone. However, Hayward et al. (2005) have also found non-maternal B-treated unstressed and stressed HS quail hens deposit 62 and 96 %, respectively, more B into their egg yolks than do their LS counterparts. Thus, when considering the combined potential influences of implant treatments and quail stress line genome, it is possible that levels of maternal yolk B deposition in eggs laid by the present four quail stress line by maternal implant treatment groups may have partitioned (greatest to least) consistent with the following treatment categories: HS-B-implant > HS-CON > LS-B-implant > LS-CON hens. If so, it is further tempting to speculate that, in Exp. 2, the observed partitioning of stress line\*maternal implantation treatment effects on LEI of LS-

CON > LS-B-implant > HS-CON > HS-B-implant hens simply reflects an inverse relationship between egg yolk B concentrations and LEI. In other words, the more B present in the yolks of eggs, the shorter the LEI.

In addition, the genetic selection done in the quail of the HS line may have caused a higher expression of B in the HS-embryos as well- a situation that, in turn, could have independently or further contributed to the earlier hatching seen in the eggs laid by HS hens. It is also important to note that, in non-selected (genetically unremarkable) Japanese quail, Hayward and Wingfield (2004) found B-implant treatment of hens during egg formation results in offspring that possess a heightened HPA axis responsiveness to brief mechanical restraint at 8 wk of age (in terms of measured levels of B). If heightened HPA axis responsiveness exists earlier than the reported 8 wk of age in the quail of Hayward and Wingfield (2004) (i.e., during embryogenesis in the eggs from their B-treated hens; a reasonable assumption), then yet further contributions to the embryonic pool of total B might be expected from the embryos themselves beyond that directly being received from hen B-treatment. Therefore, assuming such influences also existed herein, it is entirely possible that the total B pool available to the present HS compared to LS embryos was increased via three separate sources: 1) higher HS maternal B contributions to the egg yolks of HS embryos, 2) greater HS embryo B release during embryogenesis due to their genome (genetic selection contributions of the HS embryos), and 3) more B from enhanced HPA axis responsiveness of HS embryos due to greater B deposition into their egg yolks by their HS mothers (number 1, above).

Glucocorticoids serve many roles within the developing embryo, including the induction of gluconeogenesis and lipolysis, as well as lung maturation and surfactant production late in embryonic development to facilitate the embryonic switch from



cardiovascular to pulmonary respiration (Decuypere, 1990). Therefore, in the eggs of our HS hens (in both Exps. 1 and 2), augmented B-driven enhancement of gluconeogenesis to free up energy stores needed to support embryonic development and hastened lung development may explain why HS eggs hatched sooner than did LS ones. This same explanation can be offered to explain why hen B-implant treatments produced similar effects on LEI in Exp. 2- i.e., maternal B treatment during egg formation further shortened the LEI in both quail lines.

It is important to note here that while considerable care was taken to avoid disturbing the quail hens during egg formation, they were, unavoidably, likely routinely stressed by daily management activities (e.g., manual feeding and egg collection, sanitization procedures, drip-nipple waterer adjustments, etc.). Conceivably then, such activities would have resulted in periodic contrasting circulating adrenocortical responses in the two lines in accordance with their altered genomes (HS > LS; Satterlee and Johnson, 1988; Jones et al., 1994; Jones, 1996); which, in turn, would have resulted in line differences (HS > LS) in yolk B deposition during egg formation (similar to what was found by Hayward et al. (2005) in the egg yolks from intentionally “unstressed” LS and HS quail hens; see above).

Two additional outcomes of the present line\*implantation treatment partitioning of the LEI means are also worthy of note here. Firstly, while B-implant treatment in LS quail hens shortened the LEI when compared to the LS-CON hatching response, this reduction was not enough to equal the yet further abbreviated LEI found in the eggs laid by the HS-CON hens. Furthermore, maternal B treatment was yet even more potent in reducing the LEI in eggs laid by HS-B-implanted mothers who had the shortest LEI of all four-treatment combinations. Collectively, these results suggest that LS hens are much

more capable of tolerating a significant chronic supplemental B challenge during egg formation than HS hens in terms of the effect maternal B apparently has in shortening the LEI.

The present maternal B-induced reductions in the LEI disagree with the findings of Rubolini et al. (2005) who found yellow-legged gull eggs treated with *in ovo* B hatched later than did control eggs. However, gulls are a wild avian species wherein egg thermoregulation, managed by parental investment, is a factor that was not present in our artificially incubated quail eggs. Indeed, Rubolini et al. (2005) noted that approximately 3 d prior to hatching, gull chicks solicit parental incubation by initiating embryonic vocalizations and embryos of B-treated gull eggs vocalized less frequently and loudly than did their control embryos. Thus, the authors hypothesized that this reduced intensity of signalling by the offspring may have resulted in parental egg neglect (i.e. periodic decreases in egg nest warmth) and, therefore, a delay in hatching.

On the other hand, while the present quail stress line and maternal B-treatment differences in the LEI are consistent with some of the chicken egg findings of Tona et al. (2007), it is difficult to make direct comparisons to these studies as well. These workers found eggs that were non-ventilated (NV; a presumably stressful situation) during the first 10 d of incubation coupled with injection of the powerful synthetic glucocorticoid dexamethasone (DEX) late in incubation (at 18 d) resulted in eggs that hatched sooner than NV controls. However, it seems unlikely that their early NV egg incubation treatment would have been effective in causing an embryonic adrenal stress response that would have resulted in added embryonic B production since the HPA axis in chicken embryos is believed not to function until around Day 14 of incubation (Wood et al., 1971; Wise and Frye, 1973; Kalliecharan and Hall, 1974). Thus, it is tempting to speculate that

the late DEX challenge at Day 18 of egg incubation used by Tona et al. (2007) was solely responsible for accelerating hatch in the early NV-DEX-18 treated eggs. However, such a conclusion should also be guarded, since these workers also found eggs incubated using normal early ventilation (V) along with DEX challenge on the same day of egg incubation (Day 18) did not differ in their LEI when compared to V controls. Thus, collectively, Tona et al.'s (2007) findings suggest that a powerful glucocorticoid surge late in egg incubation affects LEI differently depending upon whatever effects early ventilation treatment has on altering the rate of embryonic development that is likely independent of B intervention. Furthermore, Tona et al. (2007) found early NV-treated eggs that were given DEX only two days earlier (at 16 d of incubation; DEX-16), as well as V-DEX-16 treated eggs, took longer to hatch than NV- and V-control eggs, respectively.

De Smit et al. (2008) also used the same NV and V treatments as Tona et al. (2007) in two broiler chicken strains known to differ in susceptibility to ascites syndrome, Cobb (less prone) and SAS (more prone), in measuring differences in LEI. De Smit et al. (2008) found that, regardless of strain, the NV treatment shortened LEI and, within each strain (and pertinent to the present study), NV was also associated with heightened embryonic levels of plasma B from 11 – 17 d of egg incubation. Thus, although the HPA axis in chicken embryos is thought not to function until around 14 d of incubation (see above references), and therefore the idea of early (i.e., the first 10 d of incubation) NV-induced stress being able to increase embryonic B levels was dismissed, NV treatment does somehow apparently produce late-stage elevations in embryonic levels of plasma B. Interestingly, changes in temporally measured blood gases (pCO<sub>2</sub> and pO<sub>2</sub>) caused these workers to hypothesize that their shortened LEI in eggs of the NV

treatment “were most probably the result of the higher metabolic rate of the NV compared with the V chicks.” It is also worth noting that consistent with our present findings, *in ovo* B-treatment also shortened the LEI in tree lizards (a non-avian oviparous species; Weiss et al., 2007).

Presently, in Exp. 1, quail stress line did not affect chick BWTE. At first blush, it would seem that because eggs of the HS line hatched sooner, then their chicks’ body weight would by necessity have to be altered. However, we submit two possibilities to explain our combined LEI and chick BWTE results of Exp. 1. Firstly, because chick BWTE was not significantly different between the two quail stress lines, which suggests that somatic development was similar between the HS and LS hatchlings by the time of emergence, then the rate of growth for a HS embryo must have been accelerated in order for the HS chicks to hatch sooner but yet achieve the same body weight as the LS chicks at hatch. Secondly, while HS eggs hatched sooner than did LS ones, the 3.7 h difference in average LEI may have been an insufficient time to be reflected by changes in chick body weight at hatch. Interestingly, the body weights of chicken embryos subjected to the incubator ventilation treatments of Tona et al. (2007) were greater in their NV group (eggs that hatched sooner) than in their V group at 12, 14, 16 and 18 d of incubation, but not at internal pipping. This finding supports our first hypothesis that embryos that develop faster than others during early incubation can ultimately still share a similar body weight by the end of egg incubation.

The hatchling body weight findings in the chickens of Eriksen et al. (2003), quail of Hayward and Wingfield (2004), gulls of Rubolini et al. (2005), and tree lizards of Weiss et al. (2006) are also similar to the BWTE findings herein in that their hatchlings did not differ in body weight with respect to either direct maternal or *in ovo* B treatment.

However, in chickens (Mashaly, 1991; Heiblum et al., 2001) and in barn swallows (Saino et al., 2005), eggs treated with B produced Day 1 hatchlings of a reduced body weight when compared to controls. No readily apparent explanations can be offered for the emergence body weight outcome discrepancies between these latter three studies (that associated elevated egg B to reduced hatchling weight) with the other five studies cited above and the results of Exp. 1 (that all found B not to affect hatchling weight).

Chick BWTE was not measured in Exp. 2 since this variable was unaffected by line in Exp. 1. However, in hindsight, it is unfortunate that chick BWTE was not measured in the second experiment as it may have proved interesting to assess the effects of maternal B treatment *per se* and its interaction with line on hatchling body weight.

## CHAPTER 4

### **INFLUENCES OF MATERNAL CORTICOSTERONE ON THE FERTILITY, HATCHABILITY AND EMBRYONIC MORTALITY OF EGGS LAID BY QUAIL HENS SELECTED FOR DIVERGENT ADRENOCORTICAL STRESS RESPONSIVENESS**

#### **4.1 Introduction**

While the major adrenal glucocorticoid hormone in birds, corticosterone (B), plays many important roles in the metabolic regulations and other physiological events that underlie adaptation to stress, when heightened adrenocortical responses persist, many deleterious effects on poultry production and animal well-being can occur. These negative outcomes have been discussed in detail previously in Section **2.1.2: HPA Axis Control of Corticosterone Release, Production Performance and Animal Welfare** of this thesis. In commercial fowl, the effects of B on egg fertility (FERT), total and fertile hatchability (TOTATCH and FRTHATCH, respectively), and related hatching parameters (e.g., embryonic mortality related to stage of egg incubation; early dead, ED, and late dead, LD, embryos and pipped, PIP, eggs) are important variables to study because some of the husbandry practices used in modern-day confinement housing systems for breeder birds can be stressful. Moreover, learning how to reduce hen stress and thereby optimize the production of high quality day-old chicks in poultry hatcheries helps insure the success of chick grow-out farmers.

As argued previously in making the case for the study of B effects on the LEI and chick BWTE in Chapter 3, the same reasonings are offered again here to justify why the Japanese quail LS and HS adrenocortical stress response lines of Satterlee and Johnson (1988) represent a most useful model to study B effects on egg FERT, TOTATCH, FRTHATCH, ED and LD embryos, and PIP eggs as well. The reader is reminded here that, although these lines were selected for either a reduced (LS, low stress) or

exaggerated (HS, high stress) plasma B response to brief mechanical restraint, the lines also show contrasting adrenocortical responses to a host of “non-specific” systemic stressors and quail of the LS line possesses many intuitively desirable traits as well (see Section **2.1.3: LSU Quail Stress Lines**). However, despite the propagation of these lines for more than 30 generations, differences in egg FERT, TOTHATCH, FRTHATCH and embryonic mortality during egg incubation between them have never been determined, although subjective impressions have always been some of these reproductive parameters in the HS line have been compromised. Assessment of the genomic influences on egg FERT, hatchability, and embryonic mortality in the quail stress lines is justified for several other reasons as well. For example, many studies have shown *in ovo* B treatment reduces egg hatchability in a host genetically unremarkable (non-selected) avian species (Mashaly, 1991; Heiblum et al., 2001; Eriksen et al., 2003; Rubolini et al., 2005; Saino et al., 2005) as well as in tree lizards (Weiss et al., 2006). In addition, albeit of only marginal significance (as the authors readily admit), the very early studies of Brown and Nestor (1973, 1974) that examined the FERT and FRTHATCH of eggs laid by turkey hens from lines selected for low (LL) or high (HL) plasma B response to cold stress deserve mention again. These researchers found that, during their initial 9 generations of selection, “whenever significant differences occurred [which was only at G<sub>4</sub> and G<sub>7</sub> for FERT and solely G<sub>7</sub> for FRTHATCH] between the lines, the LL line was superior to the HL.”

The effects of supplemental maternal B on egg FERT and hatchability parameters in the LS and HS quail stress lines are also unknown, although it was presently found that eggs laid by HS hens hatch sooner than do eggs from LS hens (see Chapter 3, Experiment 1); and, while maternal B-treatment in these lines decreases the LEI in eggs laid by hens of both lines,

the effect that supplemental B has on shortening incubation time is most profound in eggs laid by HS-B-implanted hens (see Chapter 3, Experiment 2). A study of the effects of maternal B treatment of LS and HS hens during egg formation on egg FERT and hatchability is justified because not only do non-selected quail hens implanted with B show elevated levels of plasma B that are consistent with the deposition of increased B into their egg yolks (Hayward and Wingfield, 2004), but egg yolks from unstressed and stressed HS quail hens also contain B concentrations that are 62 and 96% higher, respectively, than what is found in the egg yolks of LS hens (Haywood et al, 2005). Furthermore, HS hens implanted with B during egg formation show a reduced rate of egg production when compared to their LS- and HS-control and LS-B-implanted hen counterparts (Satterlee et al., 2007) and it is well known that highly positive correlations exist between hen-day egg production rates and egg FERT and hatchability (North, 1990).

Thus, in the present study, during egg formation, LS and HS hens were implanted with either silastic tubes that were empty (controls) or filled with B to assess genomic, maternal B challenge, and their interactive influences on egg FERT, TOTTHATCH, and FRTHATCH. The percentages of embryonic mortality (ED and LD embryos) in broken out unhatched eggs and PIP eggs were measured as well to aid in interpretation of any treatment-induced differences found in FRTHATCH.

## **4.2 Materials and Methods**

### **4.2.1 Genetic Stocks and Animal Husbandry**

Japanese quail from generation (G)<sub>37</sub> of two lines selected for either a low (LS, low stress) or high (HS, high stress) plasma corticosterone (B) response to brief mechanical restraint (Satterlee and Johnson, 1988) were studied. The lines' most recent genetic history, up to G<sub>36</sub>, that attests to the fact that differences between the lines (HS



>LS) in plasma B responses to various non-specific systemic stressors has been maintained has been discussed in detail in Section **3.2.1: Experiment 1**. Therefore, for the sake of brevity, this information will not be repeated here.

Test subjects were taken from a hatch of approximately 600 quail per stress line. At 25 d of age, 72 females (36 LS + 36 HS) were randomly selected for pairing with same-line, same-age adult males reared from the same hatch. Care was taken to insure that each of the breeding pairs selected, while randomly selected from larger family populations within each line, constituted, as nearly as possible, equal representation of the 12 different families that make up each line. Also, within each line, pairing of full-siblings was avoided. Once selected, each breeder pair was then randomly housed in a single cage of one of two Alternative Cage Designs four-tier cage batteries (Alternative Design Manufacturing and Supply, Inc., Siloam Springs, AR). Each battery contained 48 pedigree-style breeder cages with individual cage dimensions of 50.8 x 15.2 x 26.7 cm (length x width x height). Breeder birds were fed a breeder ration (21% CP; 2,750 kcal ME/kg) with feed and water provided *ad libitum*. The daily photostimulatory cycle was 14:10 L:D (approximately 280 lux during the lighted portion of the day); lights-on was at 06:00 h and lights-off was at 20:00 h daily. Daily maintenance and feeding chores were done at the same time each day (08:00 h).

#### **4.2.2 Hen Treatments and Variables Measured**

At 62 d of age, half of the hens in each line (n = 18 hens/line) were randomly selected and surgically implanted with an empty 16 mm silastic tube (Dow Corning Corp., Midland, MI, Cat. No. 508-006; controls, CON) while the remaining half of the hens were each fitted with a same length silastic tube implant filled with B (Sigma–Aldrich Co., Atlanta, GA; Cat. No. C2505; B-implant). Implants were placed s.c. in the

back of the neck using a No. 10 biopsy needle (Becton Dickinson, Franklin Lakes, NJ). Silastic tube implants were sealed on one end with a silicone sealant while the other end remained open.

All hens were allowed 1 wk to acclimate to the implant treatments before collecting any eggs. This was done to allow sufficient time for maternal B deposition into the eggs of B-implanted hens (See Hayward et al., 2004). Eggs were then collected daily for the next 28 d of lay. All eggs laid during this period were identified by pencil markings as to their origin by hen line and implantation treatment and they were stored at 18 C until incubation. All eggs from the first 2 wk of egg collection were set together into an incubator (NatureForm NMC 2000; NatureForm Hatchery Systems, Jacksonville, FL) as Replication 1 of the experiment. Eggs gathered from the second 2 wk of egg collection were stored under identical conditions and then set as Replication 2 of the experiment. During the first 14 d of incubation, eggs were turned 6 times a day and subjected to 37.5 C and 62% RH. Upon transfer of the eggs to a second NMC 2000 hatcher unit on Day 14, eggs were no longer turned and incubation conditions were changed to 37.2 C and 69% RH.

On Day 17 of incubation, chicks were pulled from the hatcher and counted relative to their line\*maternal B-treatment. All unhatched eggs were also broken-out at this time to enable determination of egg fertility (FERT), total hatchability (TOTTHATCH), and fertile hatchability (FRTHATCH), as well as make estimates of embryonic mortality (early and late dead embryos: ED and LD, respectively). The numbers of unhatched eggs that had pipped eggshells (PIP) were also recorded.

### 4.2.3 Statistical Analyses

The FERT, TOTHATCH, FRTHATCH, ED, LD and PIP data were subjected to a randomized block design that incorporated a two-way ANOVA. Blocking was done on the two 2 wk egg collection periods that served as experimental replications. The main effects considered in the ANOVAs were partitioned along with experimental replications within a 2 x 2 x 2 factorial arrangement of treatments- the two blocks or replications, the two stress lines (LS vs. HS), the two implantation-treatments (CON vs. B-implant groups), and their interactions. Duncan's tests were used to partition differences in the line\*implantation treatment means. A P value  $\leq 0.05$  was used to determine significant differences in means.

### 4.3 Results

Figure 4 depicts the differences found in mean ( $\pm$  SE) percent FERT of eggs laid by LS and HS CON- and B-implanted hens. The FERT of eggs laid by LS hens was dramatically higher (by almost 16%;  $P < 0.0001$ ) than that found in eggs laid by HS hens (top panel) and the FERT of eggs laid by CON hens was also considerably higher (by about 7%;  $P < 0.0001$ ) in comparison to the eggs obtained from B-implanted hens (middle panel). Post-hoc partitioning of the interactive effects of line with hen implantation treatment on mean egg FERT resulted in the following order (highest to lowest) of differences ( $P < 0.05$ ): LS-B = LS-CON  $>$  HS-CON  $>$  HS-B (bottom panel).

Statistically robust differences in mean TOTHATCH were also detected for the main effects of quail stress line and hen implantation treatment (Figure 5). Average percent TOTHATCH was nearly 12 % higher ( $P < 0.0001$ ) in LS hen eggs in comparison to the TOTHATCH of eggs from their HS counterparts (top panel), while eggs from the CONs hatched at a nearly 8% better rate ( $P < 0.0002$ ) than those from hens implanted

with B (middle panel). The ranked order (highest to lowest) of differences ( $P < 0.05$ ) between the line by implantation treatment means on TOTHATCH was as follows: LS-B = LS-CON = HS-CON > HS-B (Figure 5, bottom panel).

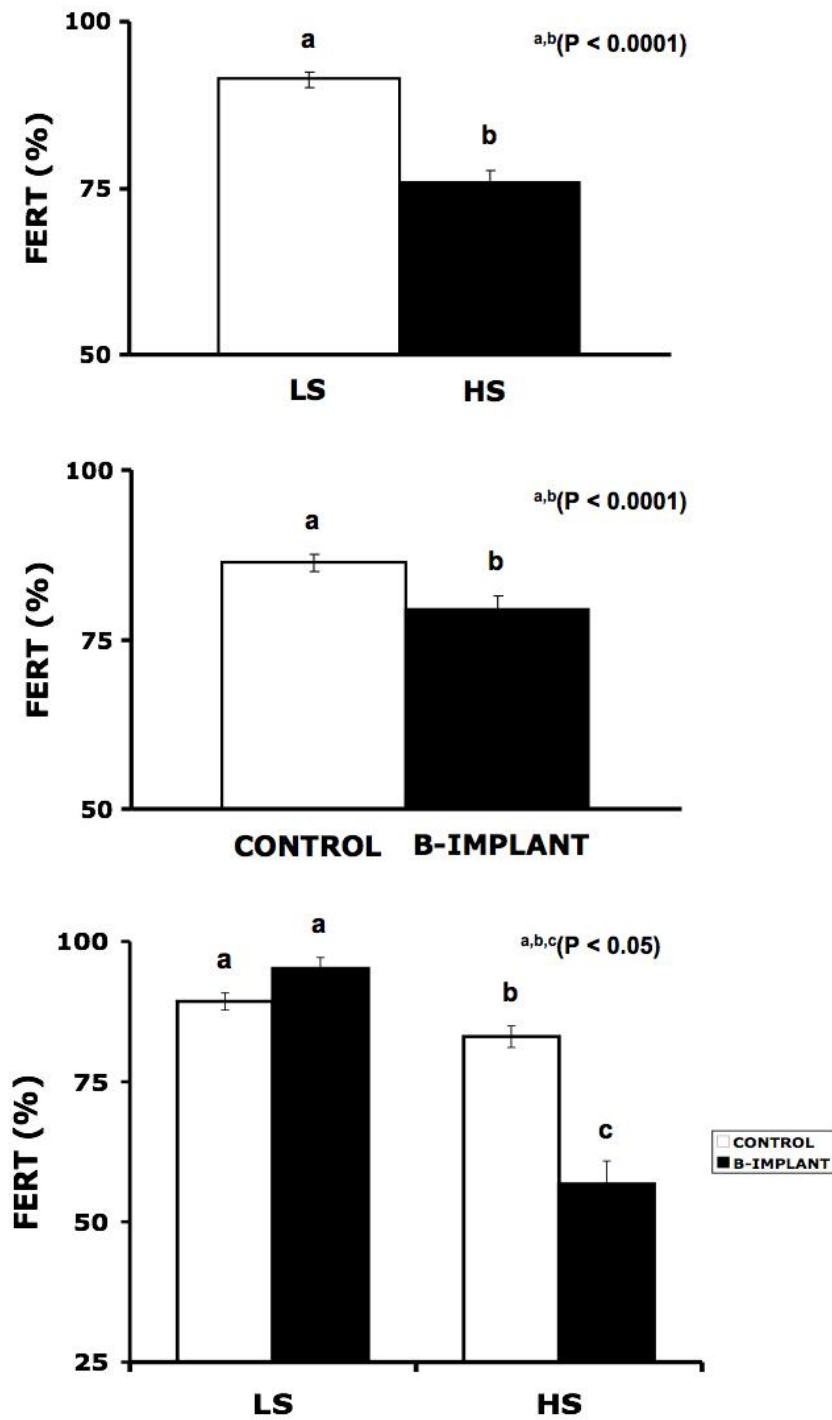
The percent FRTHATCH means are shown in Figure 6. FRTHATCH did not differ by line (top panel) or implantation treatment (middle panel). However, multiple ranked orders (highest to lowest) of differences ( $P < 0.05$ ) in FRTHATCH of eggs laid by the four treatment combinations were detected as follows: HS-CON = LS-B = LS-CON, LS-CON = HS-B, and HS-CON = LS-B > HS-B (Figure 6, bottom panel).

Figure 7 depicts line (top panel), hen implantation treatment (middle panel) and line\*implantation treatment (bottom panel) effects on mean percent ED embryos. The percentages of ED embryos in broken-out unhatched eggs that were fertile did not differ by line or implantation treatment. However, the combined influences of line with hen implantation treatment affected ( $P < 0.05$ ) mean ED percentages (in rank order, lowest to highest) as follows: LS-B = LS-CON = HS-CON < HS B (Figure 7, bottom panel).

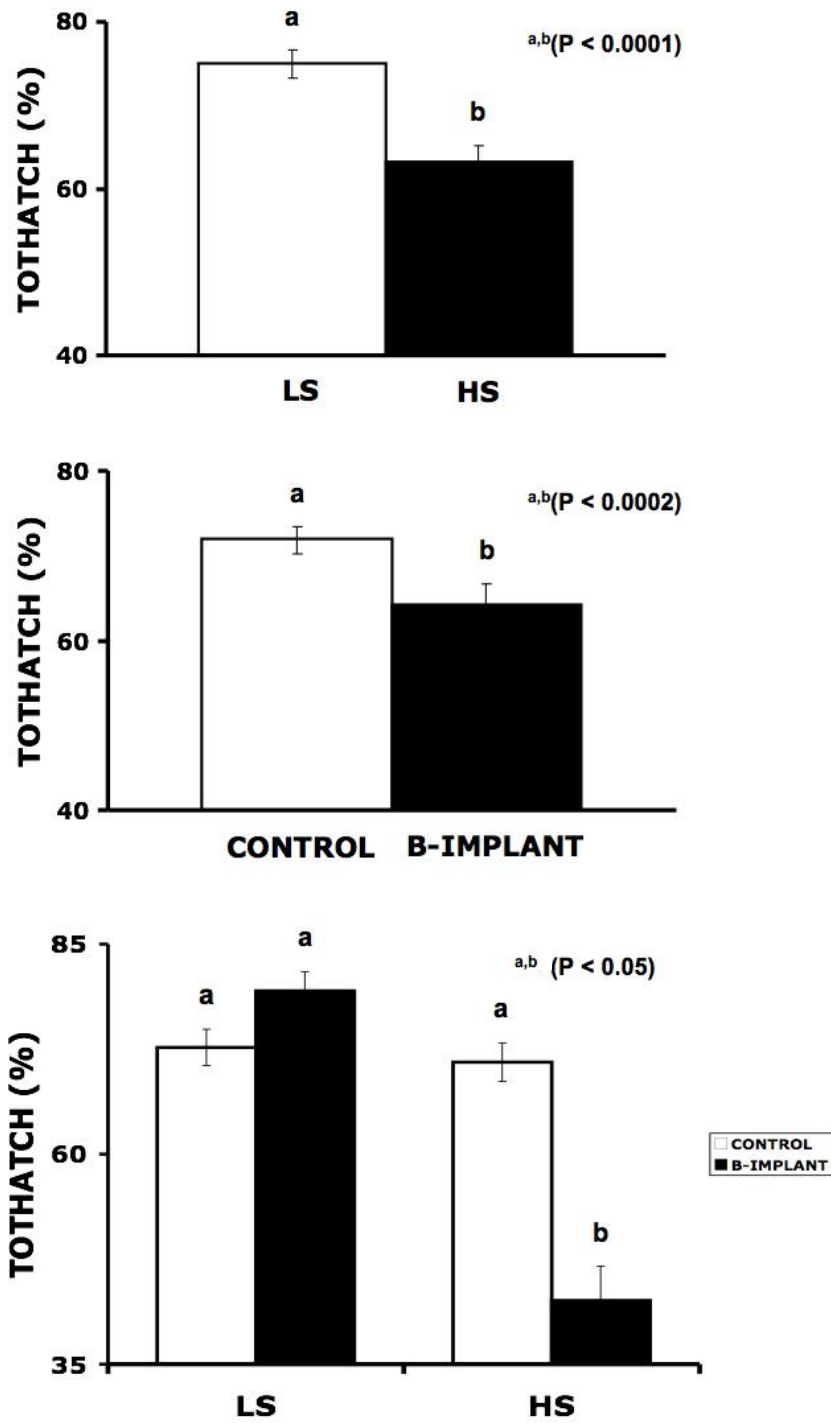
The mean ( $\pm$  SE) percentages of LD embryos (Figure 8) and PIP eggs (Figure 9) were unaffected by quail stress line, hen implantation treatment, or their interactive influences.

#### **4.4 Discussion**

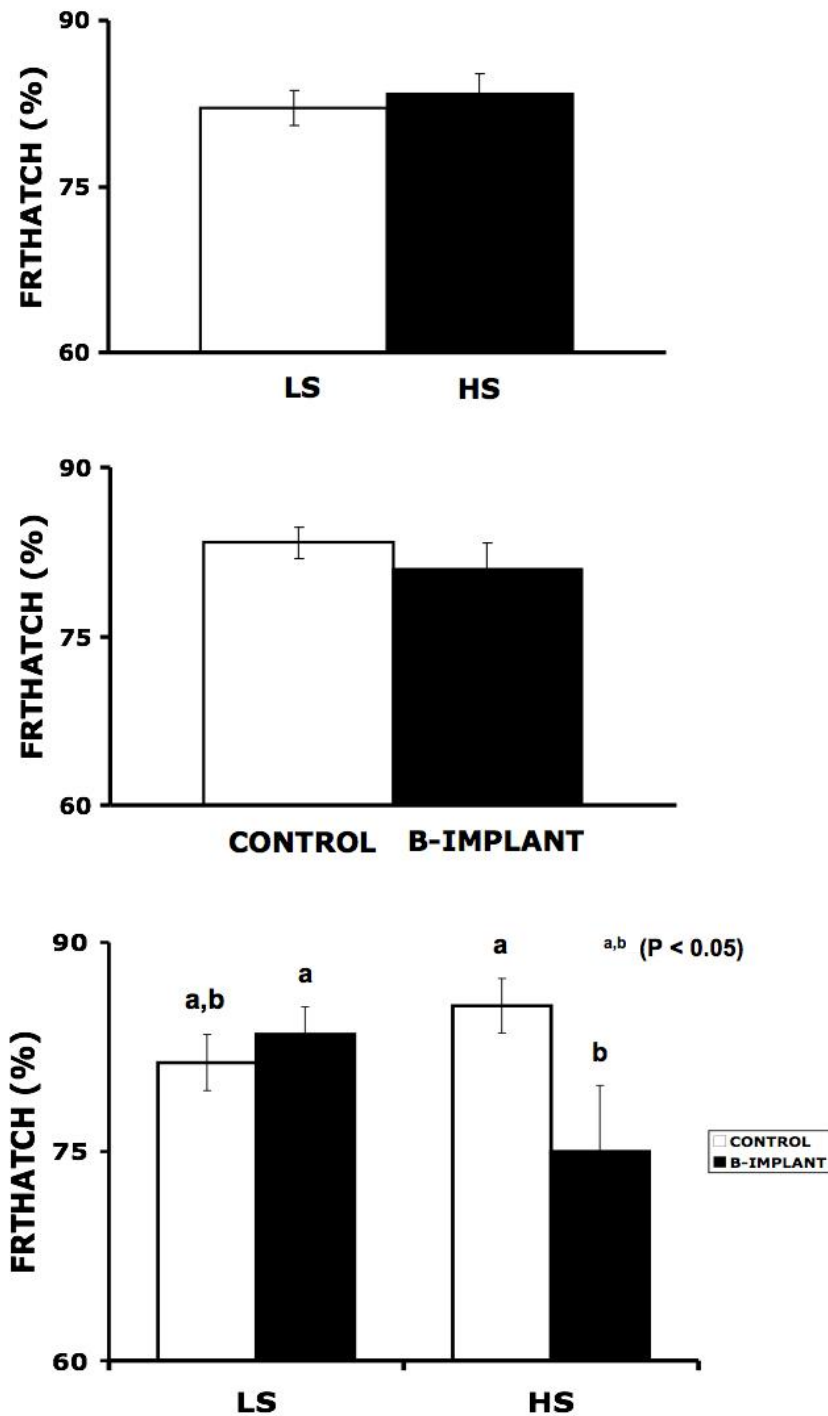
Regardless of maternal implant treatment, LS hens were better than HS ones at producing viable embryos as evidenced by the similar and greater FERT seen in eggs laid by both LS-CON and LS-B-implanted hens when compared to the decreased egg FERT observed in the other two HS hen groups. Furthermore, implanting HS hens with B during egg formation further exacerbated the decline in egg FERT seen in eggs of the HS-CON hens when compared to either of the other two LS hen groups. In other words,



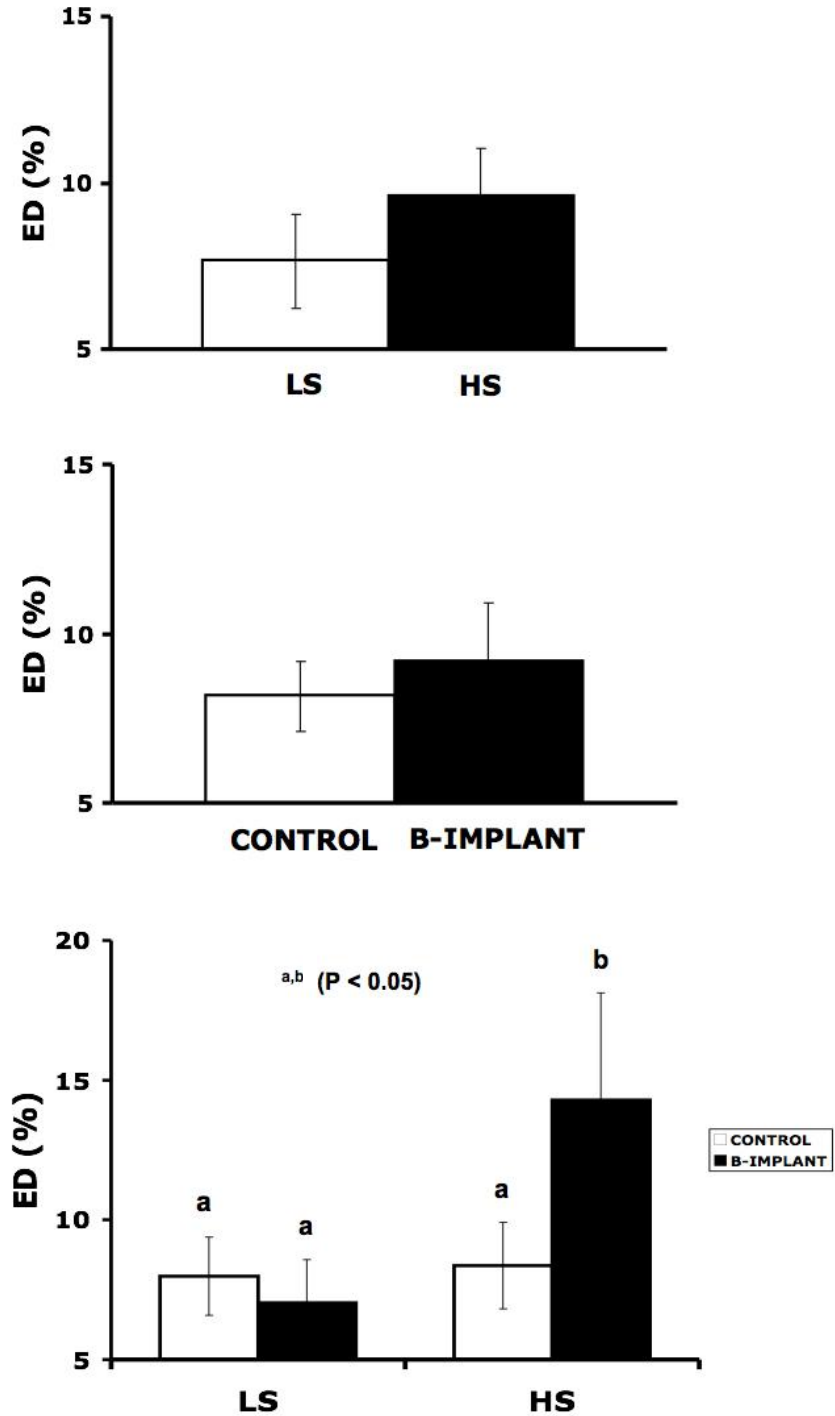
**Figure 4.** Effects of line (LS, low stress vs. HS, high stress; top panel), implantation treatment (Control vs. corticosterone (B)-implant; middle panel) and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) percentages of fertility (FERT) of eggs laid by implanted hens.



**Figure 5.** Effects of line (LS , low stress vs. HS, high stress; top panel), implantation treatment (Control vs. corticosterone (B)-implant; middle panel) and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) percentages of total hatchability

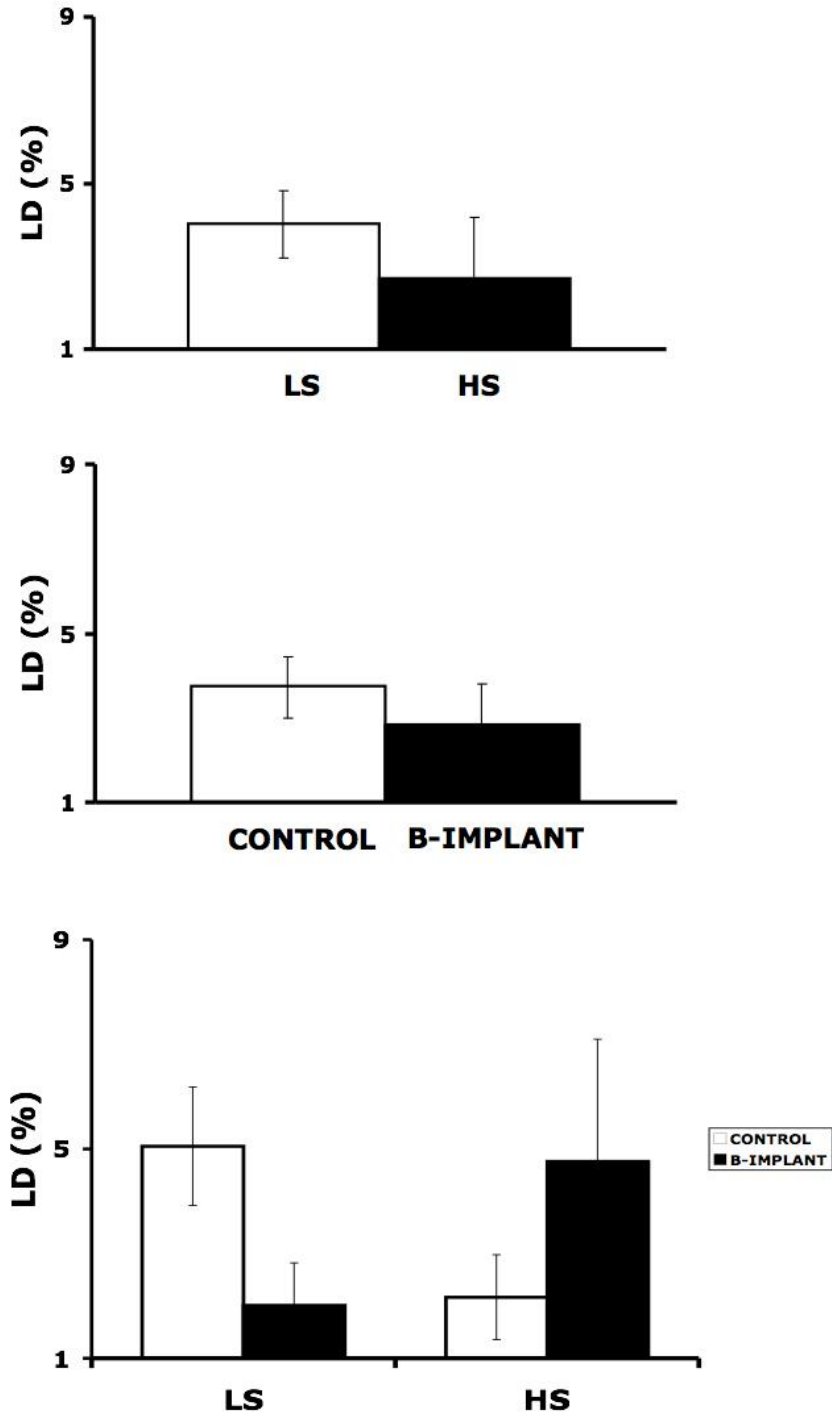


**Figure 6.** Effects of line (LS, low stress vs. HS, high stress; top panel), implantation treatment (Control vs. corticosterone (B)-implant; middle panel) and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) percentages of fertile hatchability (FRTHATCH) of eggs laid by implanted hens.

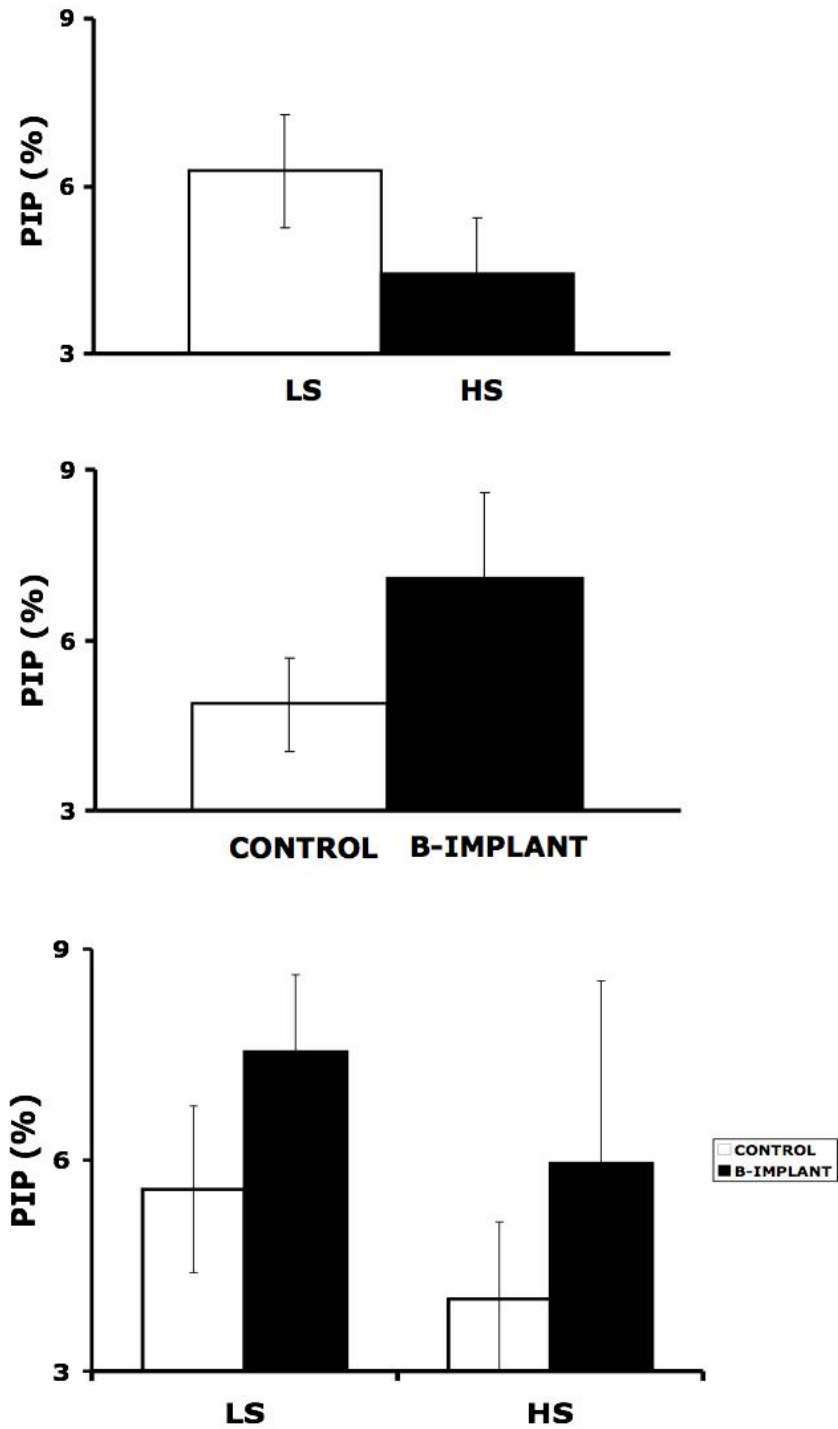


**Figure 7.** Effects of line (LS, low stress vs. HS, high stress; top panel), implantation treatment (Control vs. corticosterone (B)-implant; middle panel) and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) percentages of early dead (ED) embryos in broken out unhatched eggs laid by implanted hens.





**Figure 8.** Effects of line (LS, low stress vs. HS, high stress; top panel), implantation treatment (Control vs. corticosterone (B)-implant; middle panel) and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) percentages of late dead (LD) embryos in broken out unhatched eggs laid by implanted hens.



**Figure 9.** Effects of line (LS, low stress vs. HS, high stress; top panel), implantation treatment (Control vs. corticosterone (B)-implant; middle panel) and their interaction (bottom panel) on mean ( $\pm$  SE; vertical bars) percentages of pipped eggs laid by implanted hens.

egg FERT was unaffected and remained very high in eggs laid by B-implanted LS hens, while the HS genome *per se* (HS-CON hen effects) negatively impacted egg FERT that was even further reduced in eggs from HS-B-implanted hens. This suggests that the main effect of maternal implant treatment on egg FERT (B-implant < CON) was due exclusively the very dramatic reduction seen in the FERT of eggs from HS-B-implanted hens.

Because of the greater FERT found in eggs laid by hens of the LS line, the finding of a greater TOTHATCH in this line as well is understandable since these variables are linked by the fact that calculation of TOTHATCH includes both infertile and fertile egg effects. Furthermore, since incubational effects on egg hatchability, which are best measured by FRTHATCH, showed no differences by line, it is most likely that a greater number of the total number of LS eggs set hatched at least partially so as a direct result of these eggs having a higher rate of FERT. Indeed, this belief is borne out by the reduced FERT seen in eggs of the HS-CON compared to LS-CON hens. But, what truly underlies the statistically robust reduced TOTHATCH seen in both the main effects of line (HS < LS) and maternal implant treatment (B-implant < CON), is the interactive finding that TOTHATCH was markedly reduced solely in eggs laid by HS-B-implanted hens when compared to the other three treatment groups. It should also be noted that FRTHATCH was lowest in eggs laid by HS-B-implanted hens and significantly lower in comparison to the FRTHATCH of eggs of two (LS-B and HS-CON) of the other three groups.

Collectively, the above findings suggest that: 1) genetic selection for reduced adrenocortical responsiveness to stress has resulted in increased reproductive efficiency in birds of the LS line that is primarily driven by enhanced fertility, and 2) the hens of the LS line are more resistant to reductions in reproductive efficiency than are their HS

counterparts when hens are challenged with B during egg formation. These conclusions are particularly important to poultry producers as they serve to warn breeder flock managers to take care to limit stress in the laying barn else reductions in egg FERT and TOTHATCH may result, especially in hens genetically predisposed to exaggerated adrenocortical responsiveness to stress.

Herein, the B-implants that were utilized were identical to those used by Hayward and Wingfield (2004) in random bred quail hens who showed such treatment increased B deposition into the yolks of their hen's eggs. Egg yolks from non-implanted unstressed and stressed HS quail hens also contain B concentrations that are 62 and 96% higher, respectively, than what is found in the egg yolks of non-implanted LS hens (Haywood et al., 2005). Thus, it is possible in the present study that maternal B treatment of LS hens could have made their levels of egg yolk B more resemble those of the HS-CON hens, while the known to be higher levels of egg yolk B in HS hens may have been even further increased by hen B-implant treatment. If such outcomes were operative, then it may be that the postulated largest increases in levels of egg yolk B in eggs laid by HS-B-implanted quail hens were the cause of the dramatic reductions in the FERT, TOTHATCH, and FRTHATCH seen in these eggs. The reader is reminded here that in barn swallows (Saino et al., 2005), chickens (Eriksen et al., 2003; Heiblum et al., 2001), gulls (Rubolini et al., 2005), and tree lizards (Weiss et al., 2007), *in ovo* B reduces egg hatchability which is consistent with our TOTHATCH findings. Also, Japanese quail embryos cultured with the powerful synthetic glucocorticoid, dexamethasone, show retarded development (Kaltner et al., 1993) and dipping chicken eggs in low doses of B increases mortality and reduces embryonic weights of surviving embryos (Mashaly, 1991). However, unlike our present maternal B study, the hatchability results of the

avian *in ovo* B studies cited above may also represent outcomes beyond just the effects of *in ovo* B *per se* because techniques for injecting B into eggs may not be free from phenomena that cause increased embryonic mechanical tension, dehydration, friction, and neural tube defects (Fineman et al., 1987). In addition, increases in embryonic levels of dopamine and epinephrine (Epple et al., 1992) have been associated with the trauma of opening eggs as well.

While neither line nor hen implant treatment affected the percentages of ED embryos, statistical partitioning of the line\*implant treatment interaction means showed a sharply increased ED embryo mortality in the eggs laid by HS hens implanted with B when compared to the similarly lower percentages of ED embryos observed in the other three treatment groups. Because FRTHATCH (see above) essentially reverse-mirrored the ED embryo results, we submit that the decrease in FRTHATCH in eggs laid by HS-B-implanted hens was due to an increase in the number of embryos that died during early egg incubation in that treatment group.

There are many plausible explanations as to why a higher percentage of ED embryos were found in eggs from HS-B-implanted hens. For example, Kaltner et al. (1993) have shown that cultured Japanese quail embryos treated with dexamethasone respond to challenge as early as Day 5 of incubation with depressed protein synthesis, uric acid excretion and retarded development of kidney tubules and glomeruli, outcomes that were associated with a marked decrease in embryonic survival rate. *In ovo* B treatment of chicken eggs also results in dermal hemorrhaging, abnormal feathering and increased embryonic mortality (Heiblum et al., 2001) and, in chicken embryos, high glucocorticoid levels apparently have teratogenic effects (e.g., alterations in face and limb bud cell division; Pavlik et al., 1986) as well. Therefore, using the logic advanced

previously that the highest levels of egg yolk B likely occurred in eggs laid by HS-B-implanted hens, the B threshold needed to produce embryonic toxicity may have been breached in these eggs thereby compromising embryonic homeostasis sufficiently enough to cause heightened mortality.

In developing chicken embryos, regulation of B secretion is thought to be initially under autonomous adrenal control, becoming regulated by HPA axis negative feedback inhibition mechanisms around Day 14 of egg incubation (Kalliecharan and Hall, 1974; Scott et al., 1981). Therefore, while the embryos of the present study would have been unable to initially cope with (i.e., regulate) elevated levels of B early in embryogenesis that presumably would have been present from genomic (HS line) and/or supplemental maternal (B-implant) sources, after development of the HPA axis, they would have been able to regulate their circulating levels of B in response these stimuli. This may explain why in the present study, a higher percent of ED embryos was found in unhatched eggs laid by HS-B-implanted hens, but no affects of line, hen implantation treatment, or their interaction on the percentages of LD embryos and PIP eggs.

## CHAPTER 5

### SUMMARY AND CONCLUSIONS

The present studies determined the interactive influences of maternal B-treatment (control, CON or B-implant) during egg formation and quail line genome (i.e, quail selected for divergent adrenocortical stress responsiveness) on female reproductive efficiency. The study animals used differ in their adrenocortical responsiveness by virtue of having either a reduced (low stress, LS) or exaggerated (high stress, HS) plasma B response to brief mechanical restraint. Experiment 1 was a preliminary study performed to examine solely the effects of quail stress line on the LEI and chick BWTE. Experiment 2 used a larger number of eggs to confirm the LEI results of Experiment 1 and to further study the interactive effects of line with maternal B-treatment on this variable. Experiment 3 assessed the interactive influences of quail stress line with maternal B-treatment on egg FERT, TOTHATCH, and FRTHATCH, ED and LD embryos, and PIP eggs.

In Experiment 1, eggs laid by HS hens hatched sooner than did eggs collected from LS hens while chick BWTE was unaffected by line. The Experiment 1 LEI result was confirmed in Experiment 2 wherein a shorter LEI was also found in eggs laid by HS-CON than LS-CON hens. In Experiment 2, eggs laid by B-implanted hens also hatched sooner than did the CON eggs and line\*hen B-implant treatment effects on mean LEI differed as follows: LS-CON > LS-B > HS-CON > HS-B.

In Experiment 3, FERT and TOTHATCH were both dramatically reduced in eggs of HS compared to LS hens and in B-implant compared to CON-treated hens.

Differences found in line\*hen implant treatment FERT and TOTHATCH means were as follows: LS-B = LS-CON > HS-CON > HS-B and LS- CON = LS-B = HS-CON > HS-B;

respectively. Although the percentages of FRTHATCH and ED embryos were unaffected by the main treatments, more ED embryos were found in eggs laid by HS-B-implanted hens than in eggs from any of the other three treatment groups. The percentages of LD and PIP eggs were unaffected by any treatment.

In summary, the stress line\*maternal B findings are important to avian geneticists and poultry producers as they further emphasize the benefit that selection for reduced adrenocortical responsiveness would have on the reproductive performance of hens. In addition, the findings warn layer, breeder farm and hatchery managers that unless stress in hens during egg formation is avoided, abbreviated egg incubation periods and negative consequences in egg FERT, TOTHATCH, and ED embryos can also result, particularly in hens genetically predisposed towards high stress responses.



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## VITA

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Upon graduation with his Bachelor of Science degree, Jason accepted the position of Research Farm Assistant at the LSU AgCenter Reproductive Biology Center. Two years later (in the fall of 2005), he accepted a Research Associate position with the School of Animal Sciences in their poultry division under the direction of Dr. Dan Satterlee. In January of 2006, he began his pursuit of a Master of Science degree in the Interdepartmental Program of Animal, Dairy, and Poultry Sciences at Louisiana State University under the mentorship of Dr. Satterlee.