STRESS RESPONSE AND HABITUATION AS INFLUENCED BY NUTRITIONAL
AND THERMAL FACTORS IN DWARF AND NORMAL CHICKENS

by

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(ABSTRACT)

Experiments were conducted to explore physiological, pathological, and immunological aspects of stress in chickens. Mechanisms of habituation and their association with adrenal steroids were also studied.

Degree of stress experienced during perinatal and neonatal stages was evaluated. In both stages, heterophilia and lymphopenia were noted and post-hatching heterophil to lymphocyte (H/L) ratios declined in a quadratic manner with age.

Feed restriction elicited a similar stress response, as indicated by H/L ratios in dwarfs and normals with the effect of adapting to fasting having dissipated between 12 to 16 days. Resistance to Eimeria tenella infection was greater in normals than dwarfs, and for fasted than ad libitum fed chicks. Neither genotype nor feeding regimen had an effect on antibody response to SRBC.

Frustration induced by thwarting of feeding resulted in a dramatic elevation in H/L ratios and conspicuous behavioral responses, suggesting responses to fasting may be
physiologically and psychologically modulated. Provision of sand during feed withdrawal attenuated physiological responses, possibly via alteration of psychogenic components.

An experiment was designed to evaluate the impact of mild feed restriction at several ages on heat tolerance. Normal chicks feed-deprived during the neonatal stage had lower H/L ratios, improved resistance to marble spleen disease and growth during heat treatment than those fed ad libitum or fasted at older ages. A companion experiment examined habituation and concomitantly the role of glucocorticoids in stress response. Neonatal fasting-induced stress responses without concurrent increases in adrenal steroidogenesis did not enhance ability of normal chicks to withstand high ambient temperatures.

The hypothesis regarding modulatory roles of corticosteroids in neonatally-elicited adaptation was affirmed in a subsequent study. Feed withdrawal for 8 or 24 h at 36 days of age did not induce either H/L or plasma corticosterone response of chicks stressed early in life. In contrast, neither criterion provided evidence for adaptation to feed deprivation by neonates treated with an adrenal steroidogenesis blocker during the fast. Corticosterone-mediated inhibitory feedback regulation of the hypothalamic-pituitary-adrenal axis which down-regulates ACTH response was also evident.
Dedicated to those who shared with the author the

STRESS in making the dissertation possible
"Stress like relativity, is a scientific concept which has suffered from the mixed blessings of being too well known and too little understood."

- HANS SELYE (1907-1982)
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INTRODUCTION

According to Selye (1974), the grandmaster of stress research, "The word 'stress', like 'success', 'failure' or 'happiness' means different things to different people." He further stated "...essentially different things as cold, heat, drugs, hormones, sorrow and joy could provoke an identical biochemical reaction in the body." These statements reflect the complexity of understanding the concept of stress. Selye's (1937; 1950) "General Adaptation Syndrome", generally considered a breakthrough in understanding "stress", has been the primary basis for investigations on changes induced by stressors. Generally, stress is considered to have a detrimental influence on livestock and poultry because it is a threat to the animal's homeostasis. However, stress at an optimal level can possess beneficial qualities as demonstrated by disease resistance. For diseases of bacterial origin (e.g., Escherichia coli, Streptococcal fecalis and Staphylococcus aureus), stress elevated host resistance (Gross, 1962; Gross and Siegel, 1965; 1979; 1982; Katanbaf et al., 1987) despite lowering circulating antibody level (Siegel, 1985). In contrast, chickens that were stressed were more susceptible to Newcastle disease, hemorrhagic enteritis, Marek's disease and Mycoplasma gallisepticum infection (Gross, 1972; Gross and Colmano, 1969; Gross and Siegel, 1965). Thus, we can conclude that too much or too little stress is unfavorable for chickens. The
challenge is to assess the appropriate degree of stress and provide an "optimum stress" environment. With intense selection for economic traits, advances in mechanization, and husbandry changes, poultry are exposed to challenges which may be stressful. Feed restriction, a routine management practice in poultry production to control obesity, while perhaps imposing a stressor (e.g., Freeman et al., 1981; Nir et al., 1975) may have long term benefits for livability (Katanbaf et al., 1989a; O'Sullivan et al., 1991). There is, however, a dearth of research on the relationship between various feed restriction programs and resistance to infectious diseases and parasites especially during the period when those under feed restriction have their feed allocations increased.

Ruth Harrison's "Animal Machines" published in 1964, may be considered as a major impetus for the public and scientific awareness of livestock welfare under intensive husbandry system. Although considerable discussions, debates, and investigations pertaining to the subject were conducted for the past three decades, there are gaps in the knowledge of understanding the psychology and emotions of farm animals in response to their environment. Psychoneuroendocrine approaches are needed to reassess the psychogenic constitution of animal well-being.

Habituation is vital in helping an animal to survive in an environment in which it has to live. Chickens have been
reported to adapt quickly to fasting (Gross and Siegel, 1986; Katanbaf et al., 1989a) and to repeated injections of ACTH (Freeman et al., 1979). However, information on the period required for habituation to feed restriction is fragmentary. Earlier studies have indicated that chickens exposed to heat treatment early in life were more tolerant to high ambient temperatures than those not acclimated (Sykes and Fataftah, 1986a,b; May et al., 1987; Arjona et al., 1988).

Acclimation to acute heat stress may also occur by preconditioning to a non-temperature stressor. Bowen and Washburn (1984) acclimated chickens to heat stress by repeated handling. Feed deprivation 24, 48, and 72 h prior to exposure to a high environmental temperature reduced body temperature and mortality during acute heat stress (McCormick et al., 1979; McCormick and Garlish, 1982; Teeter et al., 1987). This association could be partly explained by the close relationship between nutritional status and metabolic heat production. Kohne et al. (1973) reported that feed in the digestive tract during periods of high temperature had a profound influence on body temperature regulation of turkeys. Lacking, however, is information regarding the effect of feed deprivation early in life on acclimation to high ambient temperatures. Gross and Siegel (1982) reported that chickens subjected to water deprivation early in life had higher weight gains than control birds following two days of fasting. Thus, there is a strong possibility that stresses
early in life, while many systems of the chicks are still developing, can have a long lasting impact and could possibly modify the genetic potential of chickens (Gross, 1983).

The nature of adaptation has not been fully explained, particularly the ability to habituate by imposing different stressors. The occurrence of non-specific stress responses which involves the hypothalamic-pituitary-adrenal axis may play a role in acclimatization of feed-deprived birds to heat distress. Munck et al. (1984) indicated that non-specific responses were beneficial to stress resistance at an acute stage. According to Gross (1983), chickens with increased plasma corticosterone levels appeared to be more adaptable to harsher environments than those with "normal" or low levels of this hormone. Thus, involvement of adrenal corticoids in the occurrence of habituation by preconditioning to different stressors needs further investigation. Research is needed to provide insights into mechanisms of habituation and to explore frontiers for further work to combat the adverse effects of thermal stress on performance of poultry.

The sex-linked dwarf gene (dw) in chickens has been studied extensively during the last several decades. In studies of layer-type hens of light or medium weight, dwarf chickens exhibited greater tolerance to heat than nondwarfs (Mather and Ahmad, 1971; Mérat and Bordas, 1974; Horst and Petersen, 1977; Khan et al., 1987). Superiority of dwarfs in hot environments may be related to their small body size.
(Guillaume, 1976) and improved water retention capacity (Singh et al., 1980). Apart from the effect of dw on thermal stress, there is a dearth of work on the impact of dwarfism on heat tolerance in meat-type chickens and ability to habituate to other environmental stressors. Additional information on the response of dwarf chickens to various stressors and their capability for adaptation will become increasingly important if utilization of dw is to have potential in tropical poultry breeding.

Several research groups have studied the effect of the dw allele on response to specific diseases (Hass et al., 1975; Mauldin et al., 1978; Mérat, 1990) and on immunoresponsiveness (Marsteller et al., 1980; Mauldin et al., 1978; Reddy et al., 1975). Information, however, is needed to evaluate relationships of feed restriction, disease resistance, and immunity of dwarf and nondwarf chickens.
The Stress Syndrome

Introduction

Stress is inevitable for an individual or a population and this is clearly emphasized by Selye (1974), who stated "complete freedom from stress is death". Yet, the definition of stress is imprecise and subjective. During the early stages of research in stress, there was a lack of agreement on the definition of "stress" among scientists and authorities in the field were even doubtful that the complications over the terminology would ever be solved (Mason, 1975). Selye (1976) defined stress as "a state manifested by a syndrome which consists of all nonspecifically induced changes in a biologic system".

According to Rice (1987), the term "stress" is commonly used in three different ways. First, it refers to any environmental stimulus that causes tension or arousal, which is more appropriately to be called stressor. Second, it refers to as physiological resistance and tension, associated with the occurrence of interpretive, emotion, defensive and coping processes. The third view of stress is as a physical reaction of the body to demand or damaging intrusions. Whether or not stress is harmful depends upon interactions between (1) individual characteristics and the properties of stressors, stress and the physiological systems, and (2) the nervous system, peripheral organ systems and the
neuroendocrine system (Bohus et al., 1987).

Theories of Stress

Based on extensive research on stress, psychological, social, holistic health and biological theories have been derived. In the context of biological theories, Selye's "General Adaptation Syndrome" (1937; 1950; 1976) has been widely considered as one of the most influential. The syndrome is comprised of three stages which are linked with the hypothalamic-pituitary-adrenal (HPA) axis. First, there is the alarm reaction which is under sympathetic nervous control and occurs at the first appearance of a stressor. If the first reaction fails, the body will call for a full scale mobilization and trigger the second reaction known as stage of resistance or adaptation. An animal enters the final stage of the syndrome, the stage of exhaustion, if the stressor is chronic and severe with eventual death.

Mason (1975) made a major breakthrough in stress research and concomitantly challenged Selye's nonspecificity concept. He demonstrated that the psychological component of physical stressors played a decisive role in triggering the HPA axis. Thus, elimination of emotional arousal associated with physical stimuli may reduce the severity of physiological disruption. The "General Adaptation Syndrome" was also criticized for lacking the consideration of psychosocial factors which are vital to understand stress in
humans and probably in other animals too (Hamilton, cited from Rice, 1987). Freeman (1985) in his review "Stress and the domestic fowl: Physiological fact or fantasy?", questioned the validity of Selye's theory and indicated that it appeared too simple to explain the stress syndrome in domestic fowl. He concluded, however, that there was insufficient information to prove the invalidity of the theory.

There is no doubt that genetic make-up and response to environmental changes are closely related. The genotype of an individual can have a major influence on variation of structural, behavioral, physiological and life strategy characteristics (Gross, 1983). Genetic-constitutional theories address the close relationships between genetics and response to environmental stimuli. They relate genetic background and certain physical characteristics that lower the overall ability to resist stress. Reduced resistance to stress could be induced by altering the genetic influences on structure or function of organs and body systems such as the cardiovascular (risks of hypertension and arteriosclerosis), digestive (peptic ulcers) and nervous (imbalance in autonomic system) systems (Rice, 1987).

**Physiological Responses to Stress**

Responses to stress may be viewed as specific and non-specific (Siegel, 1983). A particular condition which induces
for example, peripheral vasodilation to maximize heat loss when the ambient temperature is high, is considered a specific response. In contrast, non-specific responses occur when regardless of the environmental stimulus, the animal responds in a generalized manner. Both of these responses occur concurrently, could be interrelated (Siegel, 1980) and are beneficial to stress resistance at an acute stage but not at a chronic stage (Munck et al., 1984).

The nervous and endocrine systems play major roles in the response to stressors. External and internal stimuli are channeled via the nervous system to the hypothalamus. Upon receiving stimuli, two distinct pathways but not two separate aspects of stress reaction are activated (Oliverio, 1987). The first pathway, which involves the sympathetic-adrenomedullary (SA) system, is responsible for the "flight or fight" mechanism or the alarm stage of Selye's "General Adaptation Syndrome" (Freeman, 1971). According to Ladewig (1987), at this stage an animal may be capable of altering the situation through some behavioral responses. Manifestations of the "flight or fight" mechanism such as increase in blood pressure, muscle tone, nerve sensitivity, respiration rate and blood sugar level could be attributed to actions of adrenalin and noradrenalin (Siegel, 1983). Adrenalin is the main catecholamine produced in animals where the adrenal cortex and medulla are contiguous. The predominant catecholamine among species with the chromaffin
tissue separated from the adrenal cortex is noradrenalin (Axelrod and Reisine, 1984). When an animal fails to cope with the stressors and behavioral activity is suppressed, the HPA axis is activated. Sensory inputs to the hypothalamus cause liberation of a neurohormone, corticotrophin releasing factor (CRF), which stimulates the anterior pituitary to secrete adrenocorticotrophin (ACTH). Vasopressin and catecholamines stimulate ACTH release by direct action on the anterior pituitary (Axelrod and Reisine, 1984). ACTH is carried by blood to the adrenal cortical tissue and elicits the synthesis and release of adrenal corticoid. Liberation of adrenal corticoids is associated with the adaptive stage of the "General Adaptation Syndrome" (Freeman, 1971).

The main hormone linked with stress in chickens is the steroid, corticosterone (Turner and Bagnara, 1976). Corticosterone travels through the blood stream to all cells in the body and invades the nuclei of the cells (Gross, 1983). According to Munck et al. (1984), glucocorticoid receptors have been found in every nucleated cell in the body. The result is modification of production of proteins and enzymes by the cell which leads to signs associated with long-term stress such as cardiovascular and gastrointestinal diseases, hypercholesteremia, metabolic derangements and modifications of immunological activities (Siegel, 1985). Recent findings, however, indicated that the major effects of adrenal corticoids may be through secondary consequences
influenced by actions of intercellular mediators such as prostanoids and cytokines, which are under the regulation of adrenal corticoids, rather than direct effects on target cells (Munck and Guyre, 1986).

Despite sharing similar neurohumoral pathways, regulation of the avian anterior pituitary and adrenal cortical tissues appears to be more complex than that of mammals. Unlike mammals the avian pituitary is less subject to hypothalamic control (see Siegel, 1980). Miller and Riddle (1942) reported functional adrenal glands in adenohypophysectomized pigeons, suggesting involvement of factors other than ACTH. Frankel (1970) postulated that there were areas of the brain that produced an ACTH or an "ACTH-like substance" responsible for adrenal function in hypophysectomized birds. On the contrary, some researchers believed that the function of avian adrenal was independent of ACTH or "ACTH-like substance" actions (Siegel, 1980). Apart from reactions involving the HPA axis and SA system, there are considerable data indicating that stress response involves many neurochemical (monoaminergic) and neuroendocrinological (peptidergic) reactions at the brain (Oliverio, 1987).

Immune Response

The immune system, once considered an autonomous system, is integrated with other physiological systems and is
sensitive to regulation of the brain (Ader, 1983). A relatively new discipline, psychoneuroimmunology, has broaden knowledge on the relationship between nervous and immune systems. According to Besedovsky et al. (1979), a significant decrease in level of noradrenaline in the spleen during immune response is considered as evidence for a link between the two systems. Although the immune response is influenced by the nervous system via direct innervation and/or involvement of the HPA axis (Ballieux and Heijnen, 1987), the effects of HPA axis and corresponding hormones of the immune system appear to be more important. This thesis was supported by alterations in immunosuppressive effects of stress when chickens were given pre-stress treatment with metyrapone, an inhibitor of the adrenocortical synthesis of steroid hormone (Thaxton and Siegel, 1973). Thus, stress which activates the HPA system has a profound influence on the immune system.

According to Comsa et al. (1982), output from the adrenals (cortisol, aldosterone and deoxycorticosterone) stimulates the immune system and leads to initiation of defensive reactions, increased resistance and rapid recovery. Rice (1987), however, indicated that cortisol may also suppress the immune system. Whether or not this occurs will depends on dosage level.

Stress influences both cell mediated and antibody-dependent immune functions (Ballieux and Heijnen, 1987). Consequences of the direct effect of corticosteroid or
indirect effects of ACTH are reductions in lymphocytic mass
(thymus, spleen and bursa of Fabricius), and reduction and
elevation in the number of circulating lymphocytes and
polymorphs, respectively (Siegel, 1985). There is
considerable information regarding mechanisms involved in the
effect of corticosteroid on immune response. In vitro studies
showed that corticosteroids bind to specific protein
receptors in the cytoplasm of lymphatic cells, and the
steroid-receptor complex is transferred to the nucleus. In
the nucleus, the complex modifies enzymatic activity, nucleic
acid metabolism (Sullivan and Wira, 1979) and production of
interleukin II (lymphoid activator) by the T helper cells
(Gillis et al., 1979). Direct lysis or apoptosis (Munck and
Guyre, 1991), and delay in maturation (Sapolsky, 1992a) of
lymphocytes due to stress steroids have also been reported.

Some studies have shown that immunosuppression is not
always characteristic of stress. Thaxton and Briggs (1972)
reported that neither immobilization nor injection of
formaldehyde (both are stressors) influenced the
immunological responsiveness of chicks. There are also
inconsistencies concerning the effects of thermal stress on
the immune system (Morgan et al., 1976) which may be
attributed to genetics (Gross and Colmano, 1971; Siegel and
Gross, 1980; van der Zijpp, 1983), nutrition (Pardue and
Thaxton, 1984; O'Sullivan et al., 1991; Maatman et al.,
1993), antigen concentration (Ubosi et al., 1985) and
interactions of non-specific and specific environmental effects (Siegel, 1985).

Interest in studying the possibility of a bilateral relationship between stress and immune responses is growing (see Marsh and Scanen, 1994; Siegel, 1994). Besedovsky and his coworkers were among several pioneers to provide evidence for a bidirectional link between the nervous and immune systems. Based on a series of experiments, Besedovsky et al. (1979) summarized the phenomenon of the two-way relationship of the immune and neuroendocrine systems as: (1) complex bidirectional interactions demonstrable between the endocrine and the immune system during early ontogeny, (2) hormonal changes induced by the immune response itself, (3) changes in the firing rates of neurons in discrete zones of the hypothalamus during the immune response, and (4) diminution in norepinephrine content of the spleen during immune response. Thus, we can conclude that the immune system is capable of transmitting signals to the nervous system and eventually activating a neuroendocrine pathway.

Although information on the actual transmission of signals from lymphoid tissue to the hypothalamus is fragmentary, several possibilities have been proposed. Ballieux and Meijnen (1987) indicated that lymphokines which were produced by antigen-stimulated lymphocytes are responsible for the stimulation of the nervous system. Other postulations include immune complexes, pharmacological
mediation liberated during ongoing immune response, or transmission of electrical signals from nerve endings of lymphoid tissue (Besedovsky et al., 1977). In vitro studies suggested that stimulation of interferons induced production of an ACTH-like substance from human lymphocytes (Blalock and Smith, 1980; cited from Smith et al., 1982). An ACTH-like substance acts directly on the adrenal and activates release of adrenal corticoids in chickens (Siegel, 1985). Working with hypophysectomized mice, Smith et al. (1982) reported that infection with Newcastle Disease virus (NDV) showed a time-dependent increase in corticosterone production with peak concentration eight h post-infection. The same workers, based upon the findings that spleen cells from NDV-infected but not from control hypophysectomized mice showed positive immunofluorescence with antibody to ACTH, concluded that lymphocytes produced an ACTH-like substance. Earlier, van Wyck (cited from Smith et al., 1982) reported a marked elevation of plasma corticosteroid levels in seven of eight hypophysectomized human patients treated with a bacterial polysaccharide. According to Smith et al. (1982), production of neuroendocrine hormone by lymphocytes is regulated by the major histocompatibility complex. There is a possibility, however, that the ACTH-like substance is also produced by other areas of the brain. Frankel (1970) suggested that regions of the brain that produce ACTH or ACTH-like substance are capable of activation of adrenal function in
hypophysectomized birds. Siegel et al. (1985, cited from Siegel, 1987) postulated the presence of a direct splenic leukocyte-adrenal activity without the mediation of ACTH or ACTH-like substances.

The above cited literature strongly suggests a two-way connection between immune response and neuroendocrine pathways. If the proposed bidirectional relationship between the two systems exists, how can we relate this theory to vaccination practices as a disease control strategy? It appears contradictory to activate production of antibody by injecting antigen and at the same time, the ongoing immune response itself increases corticosteroid level which leads to suppression of lymphocytic activities. Probably there is a certain threshold where ongoing immune response has no significant effect on the stress response. Thus, antigen concentration and the environment of the host itself could be important factors in determining threshold responses.

Disease Resistance

Disease resistance is complex and involves various factors at the individual, population, and host-pathogen levels. There is considerable evidence indicating that response and adaptation to environmental changes can modify mechanisms of disease resistance. Although response to noxious environmental stimuli or stressors leads to changes in allocation of resources (Gross, 1983), they have both
beneficial and adverse influences on disease resistance, a contradiction studied by Gross and his colleagues. Stressed chickens had increased defense against bacterial diseases (Gross, 1962; Gross and Siegel, 1965; 1979; 1982) with exceptions of *Salmonella worthington* infections (Thaxton et al., 1974) and some parasitic diseases but the reverse was observed for viral diseases and tumors (Gross, 1972; Gross and Colmano, 1969; Gross and Siegel, 1965). These conflicts are due to effects of corticosteroid on either the particular pathology involved or on immunological defense mechanisms (Siegel, 1980). The anti-inflammatory action of corticosteroid is useful against bacterial diseases where the major pathology involves local or generalized inflammation or endotoxin formation. On the other hand, viral infections cause direct invasion of tissue and inflammation is required to localize the infection.

Another possible explanation for these contradictions is associated with the circulating white blood cell components (Siegel, 1980). Stress causes involution of lymphoid tissues (thymus, spleen, and bursa of Fabricius) which reduces the number of circulating lymphocytes and increases the number of heterophilic or neutrophilic granulocytes (Garren and Shaffner, 1954; 1956; Glick, 1967; Siegel 1980; 1983). Defenses against viral and bacterial infections are mainly lymphocytic and heterophilic, respectively (Gross, 1962). Thus, stress which increases the number of circulating
heterophils and reduces the number of circulating lymphocytes may be advantageous for resistance to bacterial diseases, but not viral diseases.

Apart from infectious diseases, considerable evidence suggests that stress may act as a potent carcinogenic agent (see Selye, 1979). This relationship has been observed as early as 1759 by Sir Richard Guy (cited from Selye, 1979), who noted that predominantly, breast cancer patients had history of bereavement or griefs. Decline in natural killer cell activity, which is vital in cancer resistance, due to physiological and psychological disruptions may have accounted for the phenomenon (Bloom, 1980).

Habituation to Stress

As the process of stress itself, habituation or adaptation to stress is a syndrome that has yet to be fully understood. Complexity of the nature of habituation may be attributed to variations in the physiological mechanisms involved with different stressors. Selye (1974) explained adaptation with the concept of heterostasis, defined as "the establishment of a new steady state by treatment with agents which stimulate the physiologic adaptive mechanisms through the development of normally dormant defensive tissue reactions". Habituation, however, may help an animal adapt to the environment in which it has to live and Freeman et al. (1979) considered habituation as a prerequisite for survival.
Because of acclimatization, it is possible for commercial poultry hybrids in various geographical locations to perform with a high degree of uniformity despite major climatic differences (Sykes and Fataftah, 1986a).

According to Gross and Siegel (1993), habituation occurs when the same stressor is continually imposed on an animal and the magnitude of each subsequent response declines. Siegel (1969) in reviewing response to stress stated "the ability to habituate is subjected to mechanisms which regulate the internal state, its ability to "normalize" after resumption of a nonstressful environment, the capability of behavioral adaptive patterns and finally, the ability to survive and continue ontogeny". Extensive investigations have been conducted to study acclimation to heat stress and the accompanying physiological mechanisms. Chickens acclimated to acute heat stress were able to withstand high ambient temperatures better than those that had not been acclimated (Hutchinson and Sykes, 1953; Arieli et al., 1980; Sykes and Fataftah, 1986a;b; May et al., 1987; Arjona et al., 1988;). Hutchinson and Sykes (1953) indicated that factors contributing to acclimation to heat stress included decreases in resting metabolism, activity, panting respiration, and increases in heat convection through the tissues by cardiovascular means, heat transfer through the feathers by control of feather erection, and evaporative cooling. On the other hand, several studies indicated inconsistencies in the
benefits of inducing acclimation by prior exposure to heat treatment. Exposure to high temperatures at five days of age failed to improve livability (McDonald et al., 1990; Smith and McGhee, 1990), growth, or body temperature (Smith and McGhee, 1990) when chickens were exposed to heat distress later in life. Further, Shoop and Birrenkott (1990) reported comparable elevation of plasma corticosterone levels when broilers were exposed to high ambient temperatures, regardless of previous heat treatment.

Chickens habituate quickly to feed deprivation. Freeman et al. (1981) noted that greater relative adrenal weights and plasma corticosterone levels initially observed among chickens under feed restriction decreased with time. Stress responses to feed restriction as measured by heterophil to lymphocyte (H/L) ratios reflected adaptation (Gross and Siegel, 1986; Katanbaf et al., 1989a). Habituation to repeated injections of ACTH has been reported (Bell, 1961; Siegel and Beane, 1961; Freeman et al., 1979). An animal does not always have to be preconditioned to the same stressor for habituation to take place. Bowen and Washburn (1984) acclimated chickens to heat stress by repeated handling.

Despite protection from acute stress responses by adaptation to different stressors, the relationship may not be bidirectional. Sykes and Fataftah (1986a) reported that, although laying hens acclimated when transferred from 5°C to 30°C, they failed to acclimatize from ambient temperatures of
30°C to 5°C. They interpreted the conflicts as the result of changes in metabolic rate and feed intake. The influence of feed intake levels on heat tolerance of acclimated chickens has also been observed by Teeter et al. (1992). In another experiment, while initial water deprivation reduced feed restriction stress (Gross and Siegel, 1980), chickens previously fasted failed to reduce the stressfulness of a period of water restriction (Gross and Siegel, 1986). It is evident that the nature of these findings is still poorly understood.

In practical situations, there are conflicting views regarding the practice of controlled acclimation. Sykes and Fataftah (1986a) implied that preconditioning chickens to controlled high environmental temperatures was not a practical strategy to combat heat prostration. Evidence has shown the possibility of naturally occurring acclimatization. For example, laying hens of strains imported to the Sudan were more heat tolerant than similar strains kept in the United Kingdom. Earlier, Arieli et al. (1980) reported that metabolic rate and heat tolerance of chickens in the Middle East changed according to season. Fluctuating temperatures appeared to be a disadvantage for habituation to heat stress (Sykes and Fataftah, 1986b). In contrast, Mueller (1961) and Smith and Oliver (1971) suggested that cyclic temperatures were better than uniform temperatures in terms of capability to withstand high ambient temperatures as well as
productivity.

Despite the pessimistic view by Sykes and Fataftah (1986a) regarding the practicality of controlled habituation, Arjona et al. (1988) demonstrated the possibility of improving livability and feed efficiency of broilers under high ambient temperatures by preconditioning them to a controlled heat stress for 24 h at five days of age. With laying hens, gradual acclimation to temperatures up to 44°C had no detrimental effect on reproductive performance (Arad et al., 1981).

Stressful experiences during the neonatal stage may have considerable impact on various physiological and behavioral aspects of an animal response to subsequent environmental insults (see Sapolsky, 1992b). One of the earliest studies on the impact of early stress on adult adrenocortical function was by Levine (1962), who reported that infantile stimulation through handling elicited long lasting modifications of the HPA axis in rodents. Handled rats, as adults had lower plasma concentration of corticosterone and enhanced recovery from perturbation of homeostasis. Sapolsky (1992b) attributed the phenomenon to increased concentrations of hippocampal corticosteroid receptors, and of corticosterone binding globulin-like receptors in the pituitary. There is uncertainty, however, whether habituation may occur without the presence of glucocorticoids during an initial stressful event, particularly when different stimuli are imposed. There
is a possibility that the stress-evoked increases in corticosteroids are essential in preparing the body to respond to subsequent physiological disruptions.

Positive Side to Stress

Stress is often viewed as detrimental and affiliated with injury, pain, disease or death. Webster's New Collegiate Dictionary (1981) defined stress as "physical, chemical or emotional factors that cause bodily or mental tension and may be factors in disease causation." There is a plethora of literature indicating the relationship between stress and organ lesions. In animal production, problems such as gastric and pyloric ulcers in pigs (Kowalczyk, 1969) and calves (see Dämmrich, 1987), paralytic myoglobinuria in horses (McLean, 1973) and porcine stress syndrome (Allen et al., 1970) are typical examples of stress-related diseases.

In contrast, Selye (1976), stated "stress is not always the nonspecific result of damage". Several studies on pathological, immunological, and behavioral aspects of stress are in accord with this thesis. For example, stress enhanced protection against bacterial and parasitic infections, and toxicity in poultry (see Gross and Siegel, 1993). Gross and Siegel (1981) reported that understressed chickens became lethargic, anorectic, flabby, appeared less preened and sometimes died from starvation. Behavioral responses, as in disease resistance, may be positively influenced by stress.
ACTH-treated rats and monkeys had superior perception, sensory detection and ability to learn to avoid threatening stimuli (Levine, 1971). Thus, the obvious question is whether stress may possess desirable qualities.

According to Everly and Sobelman (1987), the stress response serves as physiological mechanism of mediation linking the stressor to a target organ, and target end-organ effects can be negative or positive. Selye (1976) used the term "eustress" (from the Greek eu = good) and "distress" (from the Latin dis = bad) to denote stresses that possessed positive and negative qualities, respectively. Eustress heightens awareness, increases mental alertness and often lead to superior cognitive and behavioral performance (Rice, 1987).

In poultry, although stress elevates resistance to bacterial diseases, extremely high levels of stress are detrimental. Gross and Siegel (1993) indicated that too much stress (H/L ratio of 0.8 or more) reduced feed efficiency, body weight and response to genetic selection. Similar adverse consequences were also observed when there was too little stress (H/L ratio of 0.2 to 0.3). This may be explained by the Yerkes-Dodson Law (Rice, 1987) which relates to degree of arousal and performance efficiency. The law states that performance will be enhanced as arousal (or stress) increases but only up to a certain point or optimum level. Exceeding the optimum leads to inefficiency. The
implication of this law to animal production is that the ultimate aim of successful husbandry is not to eliminate stress but to maintain it at an optimal level. As indicated by Selye (1980), "...our goal should be to strike a balance between the equally destructive force of hypo- and hyperstress, to find as much eustress as possible, and to minimize distress."

To assess the degree of stress and provide an appropriate "optimum stress" environment is a difficult task. In their review, Gross and Siegel (1993) suggested H/L ratios of about 0.2, 0.5, and 0.8 characterized low, optimum, and high levels of stress, respectively. Variability at the population (e.g., inter- and intrapopulation genetic variation) and individual (e.g., social status, prior experiences, age, sex, subclinical infections) levels, however, are major factors requiring consideration before quantifying the optimum level of stress by a specific number.

**Feed Restriction**

Feed restriction has been a routine management practice in some phases of poultry production. Shortage of feedstuffs during World War II was partly responsible for the growing interest in studying methods of feed restriction (Lee et al., 1971). In subsequent years various methods have been proposed and studied, and these methods may be categorized into limiting the time of access to feed (Novikoff and Biely,
1945; Schneider et al., 1955), quantitative feed deprivation (Berg et al., 1963; Bullock et al., 1963; Proudfoot and Gowe, 1967) and qualitative feed restriction (Berg and Bearne, 1958; Berg, 1959; Waldroup et al., 1966). Based on these early studies, it was concluded that restriction by limiting the time of access to feed (the earliest method of restriction) was unsuccessful, and that quantitative feed restriction was a satisfactory technique.

Limitation of feed intake is vital in controlling obesity among meat-type chickens which have undergone intense selection for rapid growth. According to Denbow (1989), long-term selection for growth rate has altered the brain and peripheral mechanisms of feed intake, leading to a voracious appetite. Meat-type chickens eat at near gut capacity when feed is available ad libitum (McCarthy and Siegel, 1983). Obesity is closely associated with reproductive complications (Katanbaf et al., 1989b; McDaniel et al., 1981; Wilson and Harms, 1986; O'Sullivan et al., 1991). Katanbaf et al. (1989b) observed inferior hen-day ovulation, lower normal egg yield and reduced duration of fertility when broiler breeders were fed ad libitum. For health and livability, advantages of feed limitation have been clearly demonstrated. Feed-restricted chickens had higher plasma titers to sheep red blood cell antigens, better resistance to Escherichia coli infections (O'Sullivan et al., 1991) and neoplastic diseases (Han and Smyth, 1972). Although earlier findings included
higher mortality among restricted egg-type birds during the pre-laying period (Schneider et al., 1955; Gowe et al., 1960; Strain et al., 1965; Proudfoot and Gowe, 1967), Katanbaf et al. (1989a) reported otherwise for meat-type chickens.

Feed restriction can cause stress response (e.g., Nir et al., 1975; Freeman et al., 1981; Harvey et al., 1983; Gross and Siegel, 1986; Katanbaf et al., 1989a; Maxwell et al., 1992) and it may increase aggressive pecking behavior (Mench, 1988; Mench et al., 1990; Shea et al., 1990). Evidence is accumulating to show that chickens readily habituate to fasts of moderate duration (Freeman et al., 1981; Rees et al., 1985; Gross and Siegel, 1986). According to Lee et al. (1971), chronic feed deprivation induces habituation which increases tolerance to other forms of deleterious environmental stressors. Advantages of feed limitation in meat-type breeders on health and livability have been demonstrated (Robbins et al., 1986; O'Sullivan et al., 1991). Thus, long-term benefits from feed restriction appear to outweigh short-term stress effects.

Effects of High Temperatures on Poultry

Chickens, like reptiles from which they evolved, are heterothermic at hatching, and gradually become homeothermic during the first two weeks post-hatch (Dunnington and Siegel, 1984). As homeotherms, body temperature has to remain stable, and heat production and loss should be in equilibrium. There
is evidence that the thermoregulatory center of birds, as in humans, is located in the hypothalamus. Mills and Heath (1972) reported that heat production of house sparrows decreased when the anterior hypothalamus-preoptic region of the brain was heated locally, whereas cooling the same region showed the opposite effect. In the domestic fowl, lesions of the anterior hypothalamus retarded the shivering response (Lepkovsky et al., 1968).

Normal body temperature of a mature chicken is in the range of 41°C to 42°C (Freeman, 1965; Richards, 1971). Individual body temperature will vary according to size, breed, sex (Kettlewell, 1989) and age (Freeman, 1988). The upper and lower lethal body temperatures of chickens are 47.3°C and 23.4°C, respectively (Moreng and Shaffner, 1951). Heat which is produced continuously from the body core is liberated as sensible (through conduction, convection and radiation) and insensible (through evaporation of water) heat (Smith and Oliver, 1971). Romijn and Lockhorst (1966) reported that as ambient temperatures reached 34°C and 40% relative humidity (RH), 80% of all heat generated was lost as insensible heat. In contrast, when RH was 90% only 39% was lost as insensible heat. This pattern suggests that temperature and/or moisture gradient between the bird and its environment has considerable influence on insensible heat loss.
Respiratory and Circulatory Adjustments

At ambient temperatures above 32°C, thermoregulating mechanisms of a bird fail to control adequately the body temperature within the normal range and produce hyperthermia (Heywang, 1938). Hyperthermia causes alteration of the normal physiological processes. Thermal polypnea or panting, an important source of evaporative cooling, is initiated when the ambient temperature reaches 26.6°C (Wilson, 1948) or body temperatures rises by 0.1°C to 0.4°C above normal (Randall and Hiestad, 1939; cited from Smith and Oliver, 1971). In chickens, at a body temperature of 44°C, rate of respiration rises to 140 to 170 per min (Siegel, 1968). Because panting involves an increase in the respiratory minute volume, a decrease in tidal volume (Whittow, 1976) and eventually loss of carbon dioxide in the blood there is a shift in acid-base balance in the direction of respiratory alkalosis (Mueller, 1966).

Declines in blood pressure, tachycardia (Whittow et al., 1964), and increases in cardiac output (Darre and Harrison, 1987) and peripheral blood flow (De Andrade et al., 1977; Wolfenson et al., 1978) are major cardiovascular changes that occur during heat stress. Elevation in peripheral blood flow, particularly in the head appendages (wattle and comb), is vital for heat dissipation (Richards, 1971). A consequence of higher blood distribution to the periphery is reduced blood flow to the intestines, liver, kidneys (Kettlewell, 1989),
and reproductive organs (De Andrade et al., 1977).

Poultry Meat and Egg Production

Despite "hi-tech" management systems, adequate feeding and stringent disease control measures, the production efficiency of broilers and layers in the equatorial countries is still low. A probable constraint is the prevailing hot climate, which is a disadvantage for optimum production.

Reductions in growth rate with increases in climatic temperature have been reported widely (e.g., Huston and Edwards, 1961; Prince et al., 1965; Ahmad et al., 1974; Cowan and Michie, 1978; Charles et al., 1981a;b). Milligan and Winn (1964) reported that weight gain, feed conversion, pigmentation, and feathering were adversely affected for broilers reared under ambient temperatures of 29.8°C to 31.3°C. Body weights of fast-growing male broilers were more severely affected by high ambient temperature than those of slower growing females (Cahaner and Leenstra, 1992). Depression in body weight could be attributed to declines in voluntary feed intake as temperatures increased (Huston and Edwards, 1961; Davis et al., 1973), with the relationship between feed intake and ambient temperatures within the range of 10°C to 34°C being curvilinear (Marsden and Morris, 1987). Reduced feed intake under heat stress is due, in part, to lower energy requirements because allocation of energy for heat conservation is minimal (Freeman, 1988). Howlider and
Rose (1987) indicated that feed conversion ratios (FCR) had a curvilinear response to ambient temperature which was similar to that for body weight and feed intake. Charles (1986) in his review, "Temperature for broilers", cited that during the finishing period, FCR improved by 0.7% per 5°C rise above 12°C and deteriorated by 5% per 5°C reduction below 12°C. Thus, FCR improved as temperature rose until the point where energy was required to reduce the heat load. Cahaner and Leenstra (1992) reported that, compared to broilers reared under moderate temperatures, feed efficiency of broilers was not affected by high ambient temperatures (32°C to 33°C) to four weeks of age. Thereafter, however, it deteriorated with detrimental effects of high temperatures being more pronounced in males than in females.

Excessive abdominal and clavicular fat is a problem for broiler producers and processors, that can be exacerbated by high environmental temperatures. Kubena et al. (1972) reported a positive relationship between ambient temperatures of 10°C to 30°C and fat deposition with male broilers depositing fat at a faster rate than females as temperatures rise (Howlider and Rose, 1987). Cahaner and Leenstra (1992) suggested that broilers with a higher capacity for energy storage in fat depots were more heat tolerant than those with a greater tendency to deposit protein. The authors, however, indicated that this thesis is subject to genetic variation.
Numerous studies, under both field and controlled situations over a period of years, demonstrate conclusively that thermal stress is one of the major factors responsible for declines in egg production (Benion and Warren, 1933; Hutt, 1938; Fox, 1951; Smith, 1973; De Andrade et al., 1974; 1976; 1977). Bray and Gessel (1961) and Mowbray and Sykes (1971) reported that both rate of lay and egg weight declined at temperatures above 30°C. A reduction of about 15 to 20% in egg size when hens were subjected to heat stress was observed by Benion and Warren (1933). Heat-stressed hens also produced eggs that had inferior shell quality (De Andrade et al., 1974; 1976; 1977) and abnormal shapes (Arad and Marder, 1982). Detrimental effects of high ambient temperatures on egg production traits could be due to decline in feed consumption which give rise to specific nutritional deficiencies such as calcium (De Andrade et al., 1974; 1976; 1977), respiratory alkalosis as a result of hyperventilation (Mueller, 1966), and reduced blood flow to the reproductive organs as a consequence of elevated blood distribution to the peripheral tissues (De Andrade et al., 1977; Wolfenson et al., 1978).

From this brief review it is evident that considerable literature on the response of poultry to high ambient temperatures exists. With an expanding poultry industry in tropical nations, it is essential that research be conducted on procedures to reduce the deleterious effects of heat
stress on the well-being and production performance of poultry.
CHAPTER I

HETEROPHIL TO LYMPHOCYTE RATIOS DURING PERINATAL AND NEONATAL STAGES IN CHICKENS
SUMMARY

Heterophils (H) and lymphocytes (L) were measured in embryos that had pipped into the air cell and through the shell, and in chicks 0 through 8 days posthatch. Numbers of lymphocytes in the perinatal stages were very low with H to L (H/L) ratios greater than 5.0. Posthatching H/L ratios decreased in a quadratic manner from 1.76 at hatch to 0.39 on Day 8.

INTRODUCTION

Degrees of stress experienced by chicks during perinatal and neonatal periods have not been quantified. Embryonic development is complicated, and perinatal stages, particularly at the time of air cell penetration and initiation of pulmonary respiration, may be stressful. Chicken embryos have the capability to synthesize adrenal corticoid, a physiological regulator in stress responses, from the 10th day of incubation onwards (Dawson, 1953). Wise and Frye (1973) reported that corticosterone rose steadily through the 20th day of embryonic development, peaked during hatching, and then exhibited a gradual decline until 7 days after hatching. Analogous patterns, where corticosteroid elevated gradually during gestation and reached its highest concentration at the time of parturition have also been documented in rats (Holt and Oliver, 1968) and sheep (Alexander et al, 1968). Despite the ability of chick embryos
to secrete corticosterone from the 10th day of incubation, Betz (1967) and Wise and Frye (1973) reported that a functional hypothalamic-pituitary-adrenal axis developed at about 15 to 16 days of incubation.

Gross and Siegel (1983) indicated that heterophil to lymphocyte (H/L) ratios may be a more reliable indicator of avian stress than plasma corticosterone concentrations. In his review, Maxwell (1993) discussed advantages and shortcomings of the procedure. Newly hatched chicks have lymphopenia and heterophilia (Lucas and Jamroz, 1961; Burton and Harrison, 1969), however, it is not clearly understood whether the phenomenon is a stress response, the normal leucocytic count during the particular physiological stage, or both. Moreover, there is a paucity of information regarding H/L ratios during perinatal stages. Lucas and Jamroz (1961) indicated that dramatic alterations occurred in circulating blood, with regard to leucocytic number, during the 24 h after hatching. Thus, there may be major differences in H/L ratios during perinatal and neonatal stages. The objective of the present study was to evaluate H/L ratios during perinatal and neonatal stages in a weight-selected line of chickens.

MATERIALS AND METHODS

Embryos and chicks from White Plymouth Rock chickens which had undergone 35 generations of selection for high 8-
week body weight (Dunnington and Siegel, 1985) were used in this experiment. Data were obtained from embryos at two perinatal stages and chicks at various ages up to 8 days after hatching. Perinatal stages were defined as embryos that had pipped into the air cell (AC), and embryos pipped through the shell (TS) and chicks were 0, 1, 2, 3, 4, 5, 7, and 8 days posthatch. All eggs were obtained from the same parental flock which was 60 weeks of age. At hatching, chicks were reared in litter floor pens and were fed ad libitum a mash diet (24% CP; 3,146 kcal ME/kg). Water was always available and continuous lighting was provided throughout the experiment. At each sample time blood from 5 to 7 individuals was obtained via major chorioallantoic vessels (embryos) and decapitation (chicks) in tubes containing EDTA as anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain with H and L counted to a total of 60 cells (Gross and Siegel, 1983).

Regression analysis was conducted for H/L ratios from 0 to 8 days of age with the aid of General Linear Models (GLM) procedure (SAS® Institute, 1982). Statistical significance was considered as $P \leq 0.05$.

**RESULTS AND DISCUSSION**

Number of heterophils were markedly predominant over lymphocytes during the perinatal stages with mean H/L ratios of 5.77 and 5.72 for AC and TS, respectively. The low number
of lymphocytes is consistent with an embryonic differential count of 44% thrombocytes, 19% mature heterophils, 24% heterophil myelocytes, 1% macrophages, 8% monocytes, and 4% lymphocytes (Lucas and Jamroz, 1961). Although, heterophils and lymphocytes were reported to be detectable in hemopoietic organs as early as the 12th day of incubation (Sandreuter, 1951; cited from Romanoff, 1960), occurrence of these cells in circulating blood during the perinatal stage was sporadic. Lucas and Jamroz (1961) indicated that large numbers of granulocytes produced by lymphoid organs were stored until at time of hatching.

There was a dramatic decline in H/L ratios from TS (5.72) to hatching (Day 0) (1.77). During the 15- to 17-h interval between initial pipping through shell and liberation from shell (Dunnington et al., 1992a), the occurring hematological events are poorly understood. The sustained decline in the ratio observed here, however, strongly suggests that major alterations occur in the circulating blood and lymphoid organs with regard to number of heterophils and lymphocytes. Lucas and Jamroz (1961) reported that during the transition from perinatal to neonatal stage, massive production of lymphocytes occurred in hemopoietetic organs, particularly the spleen.

Analysis of neonatal data from 0 to 8 days posthatch revealed a large initial decrease followed by levelling in H/L ratios at about Day 6, resulting in a highly significant
quadratic response (Figure 1). An analogous observation by Burton and Harrison (1969) demonstrated that there was a positive linear regression of lymphocyte to heterophil ratios (L/H) with age during the first 2 weeks of posthatch. The H/L ratios observed at 7 days posthatch in our experiment are consistent with previous results (Lucas and Jamroz, 1961), but somewhat earlier than the normal L/H ratios noted at about 12 days of age by Burton and Harrison (1969). Factors such as variation in rate of growth attributable to genetic differences in stocks may account for such discrepancies.

Defenses against bacterial infections are mainly heterophilic, thus, increases in numbers of circulating heterophils may be advantageous for resistance against bacterial diseases (see Gross, 1983; Siegel, 1985; 1987; Gross and Siegel, 1993). However, despite relatively greater numbers of circulating heterophils than lymphocytes during the neonatal period, newly hatched chicks are susceptible to bacterial infections. This conflict may be attributed to the large number of circulating immature heterophils which are not fully functional against bacterial infections (W. B. Gross, personal communication).

Studies indicate adrenocortical activation in embryos towards time of hatch (Woods et al., 1971; Wise and Frye, 1973; Wentworth and Hussein, 1982) and in neonatal chicks (Webb and Mashaly, 1982) with a steady decline during the first week after hatching (Wise and Frye, 1973; Latour et
al., 1993). Thus, the trend of plasma levels of corticosterone fits with that of H/L ratios observed in the present study. Although direct and indirect effects of corticosteroids on number of circulating heterophils and lymphocytes are well established (Gross, 1983; Siegel, 1985; 1987; Sapolsky, 1992a), it is not clearly understood whether the associated lymphopenia and heterophilia during both perinatal and neonatal stages could be attributed to the hormone. Elevated plasma corticosterone levels during perinatal and neonatal stages may be related to stress responses since embryos from 16 days onwards respond to stressors (Wise and Frye, 1973). Although, the heterophilia and lymphopenia observed may reflect normal hematological events (see Lucas and Jamroz, 1961) which take place during perinatal and neonatal stages, interactions between both normal physiological and stress responses may have accounted for the elevated H/L ratios. Thus, although H/L ratios may be a reliable measure of stress responses in avian species (see Gross and Siegel, 1993; Maxwell, 1993), their value as such a criterion during embryonic stages and the early posthatch is limited.
Figure 1. Regression of heterophil to lymphocyte ratios on age posthatch. $Y = 1.684 - 1.376x + 0.022x^2$ ($r^2 = 0.54$ with $P \leq 0.001$).
CHAPTER II

RESPONSES OF DWARF AND NORMAL CHICKENS TO FEED RESTRICTION, EIMERIA TENELLA INFECTION, AND SHEEP RED BLOOD CELL ANTIGEN
SUMMARY

Relationships among stress responses, habituation to feed restriction, resistance to *Eimeria tenella*, and antibody responses to SRBC were studied in dwarf and normal White Plymouth Rocks. Transfer of chicks at 22 days of age from starter to grower batteries resulted in an increase within 24 h of heterophil to lymphocyte (H/L) ratios of chicks of both genotypes. Restriction of feed intake from *ad libitum* (AL) to 60% of *ad libitum* reduced body weight and increased size of the crop-esophagus. As measured by H/L ratios, the effect of adapting to the 60% feed restriction dissipated between 12 to 16 days after initiation. Release of 60% restricted chicks to 80% of *ad libitum* also elicited a stress response as measured by H/L. These effects were noted in both dwarf and normal chicks. Neither feeding regimen nor genotype had an effect on antibody response to SRBC. Resistance to *E. tenella* was greater in normal than dwarf chicks and greater for restricted than *ad libitum* chicks.

INTRODUCTION

Intense selection for economic traits in concert with increased mechanization and husbandry changes contribute to the dynamics of poultry production. Routine husbandry procedures may result in a stress response as measured by blood corticosterone (e.g., Beuving, 1980). When feed intake is restricted, animals may not fully satisfy their basic
physiological and behavioral needs (Ewbank, 1985). Thus, feed deprivation may cause a stress response (e.g., Nir et al., 1975; Freeman et al., 1980; Harvey et al., 1983), and influence aggressive behavior (Mench 1988; Shea et al., 1990).

Chickens are able to adapt to stress associated with overheating (Sykes and Pataftah, 1986a; b; May et al., 1987), repeated injections of adrenocorticotropic hormone (Freeman et al., 1979; 1980), and feed withdrawal (Gross and Siegel, 1986). Although imposing stress, feed restriction plays a crucial role in controlling obesity among meat-type chickens that have undergone intense selection for rapid growth. Obesity is deleterious to reproduction and viability (McDaniel et al., 1981; Siegel and Dunnington, 1985; Wilson and Harms, 1986; O'Sullivan et al., 1991), and health benefits of mild periods of restriction outweigh the associated stress. Additional studies, however, are needed to provide insights into relationship between various feeding programs and resistance to infectious diseases and parasites particularly during the period when feed restriction is relaxed.

The sex-linked dwarf gene (dw) in chickens has been extensively studied during the last several decades (Guillame, 1976; Mérat, 1984; Decuypere et al., 1991). Apart from the superior effect of dwarfism on thermal stress (Mather and Ahmad, 1971; Mérat and Bordas, 1974), there is a dearth of work on the role of this allele on ability of
habituation to other environmental stressors and its link with disease resistance and immunocompetence.

The present experiment reported here was conducted to evaluate the relationship of stress response and habituation to feed restriction, disease resistance, and immunity of dwarf and nondwarf chickens selected for high body weight.

MATERIALS AND METHODS

Genetic Stocks, Husbandry and Traits Measured

Dwarf and nondwarf (normal) White Plymouth Rock chickens were used in this experiment. Normal chickens were obtained from a line selected 34 generations for high body weight at 8 weeks of age (Dunnington and Siegel, 1985). In the S13 generation the sex-linked allele dw for dwarfism was introduced into a random sample of chickens from that line. Four generations of repeated backcrossing to the selected line resulted a dwarf line with 97% of the background genome of the selected line (Reddy and Siegel, 1977) after which selection was relaxed. The dwarf chicks used in this experiment were from the 17th generation of relaxed selection.

Eggs obtained from age contemporary dwarf and nondwarf parents were incubated in the same machine. At hatching, chicks were wingbanded, vaccinated for Marek’s disease, and five chicks were assigned at random within genotype to each pen (8 pens of dwarf and 15 pens of normals) in starter
batteries with wire floors. A mash diet (without coccidiostat) containing 2,685 kcal of ME/kg of feed and 20% CP was provided for ad libitum consumption. Water was always available and lighting was continuous throughout the experiment.

At 22 days of age, each pen of chicks was transferred from starter to developer batteries with wire floors. Ten chicks from each genotype were chosen randomly and, just prior to moving, their blood was collected in tubes containing EDTA as anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain with heterophil (H) and lymphocytes (L) counted to a total of 60 cells (Gross and Siegel, 1983). This procedure was repeated the following day with a different sample of 10 chicks from each genotype.

On Day 29 (seven days after moving), 6 and 10 pens of dwarf and nondwarf chicks, respectively, were subjected to 60% feed restriction (60R) based on the mean feed intake for the previous day of their respective ad libitum (AL) pens. Time required by each pen of restricted chicks to consume their daily allotment of feed was recorded. At 30, 34, 38, 41, 45, 50, and 55 days of age (i.e., 1, 5, 9, 12, 16, 21, and 26 days after feed restriction commenced), blood was obtained just prior to feeding from 10 chicks per feeding regime-genotype subclass for H and L counts. An interval of at least five days was allowed before a chick was bled again. Prior to feeding, individual body weights (g) were obtained
at 22, 29, 43, 50, and 56 days of age. At 43 days of age, half of the dwarf (three) and normal (five) 60R pens were released to a feed allowance that was 80% (80R) of ad libitum intake on the previous day. The bleeding procedure for H and L was the same as previously described except there were now three groups: AL, 60R, and 80R.

On Day 50, 20 chicks from each feeding regime-genotype subclass were given a 0.1 ml intravenous injection of 0.25% SRBC. On the same day, each of 30 chicks per subclass was dosed orally with 30,000 *Eimeria tenella* oocysts. Different pens of chicks were randomly assigned to receive either SRBC or *E. tenella*. Antibody production to SRBC antigen was measured five days post immunization by the microtiter hemagglutination procedure (Wegmann and Smithies, 1966). At this time blood was also obtained for H and L counts. On Day 56, all chicks were weighed, killed by cervical dislocation, and sex determined by gonadal determination. Weight (to the nearest 0.01 g) and length (to the nearest millimeter) of crop-esophagus and heart weight (to the nearest 0.01 g) were recorded. Intestinal lesions were scored from 0 to 4 according to the criteria described by Johnson and Reid (1970) for coccidiosis.

**Statistical Analyses**

Statistical analyses were by ANOVA unless otherwise specified. Because chicks were fed *ad libitum* to 29 days of
age, body weights to this age were analyzed within sex with genotype as the main effect. At subsequent ages, feeding regime and the interaction between it and genotype were added to the model. This same factorial arrangement was used for analyses of crop-esophagus data at 56 days of age. Prior to analyses, body weights were transformed to common logarithms. Weights and lengths of organs were expressed as weight per 100 g body weight and transformed to arc sine square roots prior to analyses. Daily ad libitum feed intake from 31 to 56 days of age was regressed on days for each genotype. Feed efficiencies from 29 to 50 and from 29 to 56 days of age were analyzed with genotype, feeding regime and the interaction between them as main effects. Regression analyses and ANOVA were conducted for the time it took for a pen of chicks to consume its daily allotment of feed. Genotype, days of restriction, and the interaction between them were the main effects for analyses conducted from 31 to 42 and 31 to 49 days of age. Feeding regime (either 60R or 80R) and challenge effects were included as the main effects in the analyses for 43 to 49 and 50 to 55 days of age, respectively.

Several ANOVA were conducted for H and L data, responses to SRBC and E. tenella. Counts of H and L were converted to a ratio of H/L. Analyses of ratios on Days 22 and 23 were made to measure the effect of moving from starter to developer cages in a factorial arrangement with genotype. To measure the effect of feed restriction on H/L ratios,
analyses included two time periods. One period was the first 26 days of restriction (30 to 55 days of age) for which the main effects were days on restriction (1, 5, 9, 12, 16, and 21), genotype (dwarf versus normal), feeding regime (AL versus 60R), and interactions among them. The other was the period from 45 to 55 days of age, when feeding regime was comprised of AL, 60R, and 80R. For responses to *E. tenella* challenge, and SRBC antigen, measurements included heart weights (g/100 g body weight), lesion scores to *E. tenella*, and SRBC antibody titers. Genotype, feeding regime, and the interaction between them were as main effects. Comparison of number of responders to number of nonresponders to *E. tenella* were made by chi square.

All analyses were carried with aid of General Linear Models (GLM) procedure of SAS® software (SAS Institute, 1982). When significant ($P \leq 0.05$) effects were found, comparisons among multiple means were done by Duncan's multiple range test. When interactions between main effects were significant, comparisons were made within each experimental variable. Statistical significance was considered as $P \leq 0.05$ throughout the paper.

RESULTS

Growth and Feed Efficiency

Normal chicks were heavier than dwarf chicks at 22, 29, 43, 50 and 56 days of age (Table 1). The magnitude of
dimorphism between genotypes for body weight [(mean of normal - mean of dwarf)/mean of normal] increased from 42 to 55% in males and 35 to 47% in females. Thus, although suppressive effects of dwarf were greater for males than females, the increase with age remained proportional. Effects of feed restriction on body weight were large by 43 days of age (14 days of restriction) with 60R chicks weighing less than AL chicks, regardless of genotype and sex. Although males released to 80R on Day 43 were heavier at 50 and 56 days of age than those continued under 60R, body weights of females were similar for these two restricted feeding regimens.

Dwarfs consumed about 50% less feed than normals. There were significant positive linear regression of daily ad libitum feed consumption on days (Figure 1) with regression coefficients different for normal and dwarf chicks. Genotype by feeding regimen interactions were significant for feed efficiency from 29 to 50 and 29 to 56 days of age (Table 2). Regardless of feeding regime, feed efficiency was superior for normal than dwarf chicks. Within both normal and dwarf chicks, efficiencies were superior for the AL regimen; within dwarf chicks the two restricted regimens did not differ, but 60R was least efficient for normal chicks.

There was a significant days of restriction by genotype interaction for time required for the 60R chicks to consume their daily feed allowance. Within each genotype, there was a dramatic initial decrease and then leveling in time needed
to consume the daily feed allowance resulting in a significant quadratic response. The plateau, however, was at a lower level for dwarf than normal chicks (Figure 2). When chicks were released from 60R to 80R, dwarf and normal chicks were taking 121 and 208 min, respectively to consume their daily feed allotments. Comparison between 60R and 80R revealed a significant genotype by feeding regime interaction for time to consume the daily allotments (Table 3). The interactions were found for both time periods because both relatively and absolutely, time required by normal chicks to consume the additional feed was greater than for dwarf chicks.

Relative to body weight, lengths and weights of crop-esophagus and weights of heart were greater for dwarf than normal males and females (Table 4). Organ weights and lengths were influenced by feeding regimen, with relative crop-esophagus lengths shorter for AL than 60R and 80R males and females. Between 60R and 80R regimens, lengths were similar for females but were longer for 60R than 80R males. For crop-esophagus weight the pattern was the same for length except the differences between 60R and 80R were present for females and not males. Growth of heart and body was proportional in both sexes regardless of feeding regimen. This pattern was evident from the lack of differences among feeding regimens in relative heart weight.
Heterophil to Lymphocyte (H/L) Ratios

At the time chicks were moved (22 days of age) from starter to developer battery cages, H/L ratios were 0.52. Twenty-four h later, ratios increased to 0.72. Response was similar for normal and dwarf chicks, suggesting that the transfer was stressful to both genotypes.

Interaction of feeding regimen (AL versus 60R) by age was significant for H/L ratios during the period from 30 to 55 days of age (1 to 26 days of restriction) (Table 5). The interaction resulted from similar ratios for chicks fed AL and elevated ratios during the first 12 days of restriction from 60R chicks. Then there was a rapid decline of H/L ratios to AL levels between Days 12 to 16. When some 60R chicks were released to 80R (at 43 days of age), again there was an increase, with H/L ratios at 45 days of age (2 days later) higher (0.72) than those at 50 (0.61), and 55 (0.57) days of age. There was no differences in H/L ratios between dwarf and normal chicks throughout the experiment.

Responses to E. tenella Challenge and Sheep Red Blood Cell Antigen

The percentage of chicks exhibiting intestinal lesions to E. tenella challenge was 52 with more dwarf (86%) than normal (35%) chicks having lesions. Scores for intestinal lesions among responders were greater for dwarf than nondwarf chicks (2.11 versus 1.38), as well as for the combination of
both responders and nonresponders (1.82 versus 0.49). There was no difference among feeding regimens in proportion of chicks exhibiting lesions for E. tenella. Among those showing lesions, however, scores were higher for AL (2.38) than those under 60R (1.58), and 80R (1.60). Relative heart weights (grams per 100 g body weight) were heavier for dwarf (0.44) than for normal (0.37) chicks at 56 days of age with no effect from E. tenella inoculation. Body weight and feed consumption of dwarf and normal chicks were not affected by E. tenella challenge.

No difference was found between dwarf (1.9) and normal (1.8) chicks for SRBC antibody titers. Means for chicks fed AL (1.3), 60R (2.4), and 80R (1.9) were similar.

DISCUSSION

When discussing results of this experiment, it should be remembered that after introduction of dw in the 13th generation the dwarfs remained unselected for four generations of backcrossing followed by 17 generations of relaxed selection whereas selection continued for the normals. Thus, there are some differences in genetic background as well as for dw. The data presented here confirm previous observations (see Guillaume, 1976; Mérat, 1984; Decuypere et al., 1991) that body weight of dwarf chickens are lower at the ages studied than their normal counterparts with the reduction in weight greater in males than females.
Accelerated growth following release to less severe feed restriction resulted in greater body weight responses in males than females. This observation is inconsistent with previous reports for sexual dimorphism in compensatory growth of broiler chickens following feed restriction (Plavnik et al., 1986; Plavnik and Hurwitz, 1988). Wilson and Osbourn (1960) attributed the manifestation of sexual dimorphism for accelerated growth in chickens to male hormones. Also consistent with previous studies (see Guillaume, 1976), was the inferior feed efficiency of dwarfs compared to normal chicks. Dwarf meat-type chickens waste more protein than their normal siblings and appear rather hyperphagic for their growth performance (Guillaume, 1976).

The negative correlation between time required to consume daily feed allotments and number of days on feed restriction reflects adaptation by chicks to the feeding regime. Also, enlargement of the upper gastrointestinal tract enabled more effective feed storage, thus allowing consumption of more feed in a shorter period. Nir and Nitsan (1979) and Katanbaf et al. (1989c) also reported greater increases in relative weight and length of crop-esophagus in feed restricted chickens than in chickens fed ad libitum. Previous studies suggest that dwarfism results in alteration of feeding behavior, as evidenced by changes in the amount of time spent in feeding activity (Barbato et al., 1980), and in capability of adapting to intermittent feeding regimes.
(Simon, 1972). Dwarf chicks required less time to consume their daily allotments than normal chicks, which was consistent with observations by Huey et al. (1982). There is no clear explanation for the phenomenon, although it reflects variation in appetite control mechanism and regulation of caloric intake (Ahmad et al., 1974). These observations do not imply that feeding rate is the function of body weight or amount of feed consumed. Huey et al. (1982) indicated that dwarf and normal chickens selected for low and high body weight, respectively, even though differing in body weight and amount of feed consumed, had similar rates of feed intake.

Elevation of H/L ratios 24 h following transfer of chicks from starter to developer batteries indicated stimulation of stress response. Handling of chicks during transfer is a stressor (Freeman and Flack, 1980), and exposing an animal to novelty is one of the most potent situations to elicit responses by the pituitary-adrenal axis (Levine, 1985). Chicks probably perceived a new unfamiliar environment with a degree of uncertainty that acts as a psychological stimulus. In pigs, short term exposure to a novel environment induced both emotional reactions such as increased locomotor activity, escape attempts, and vocalization as well as hormonal reactions such as elevated plasma cortisol and ACTH concentrations (Dantzer and Mormède, 1985). The degree of discrepancy between the initial and new
environment is vital in determining the level of stress response. Hennessy et al. (1979) reported that placing rats in cages, different but identical with their home cages, elevated plasma corticosterone levels. The rise, however, was less than that for rats subjected to totally novel living conditions containing none of the elements of its familiar living conditions. Therefore, it appears that an animal prefers familiar over novel environments even though the new living conditions may not threaten their well-being or pose a form of physical stimulus.

Despite the necessity of feed restriction to control obesity in meat-type chickens, the current findings are in agreement with those showing that feed restriction may be a stressor (e.g., Nir et al., 1975; Freeman et al., 1981; Katanbaf et al., 1989a). Initial stress response to feed deprivation and negative associations between the level of stress response and number of days on feed restriction (as indicated by H/L ratios) supported results of Freeman et al. (1981). Following 12 days of restriction, H/L ratios of feed-restricted chicks were similar to those fed ad libitum and suggest adaptation to feed restriction. Habituation occurred when the same stressor was continually imposed on an animal and the magnitude of each subsequent response declined (Gross and Siegel, 1993). Working with Light Sussex chickens, Freeman et al. (1981) observed normal plasmal corticosterone concentrations levels after five weeks of feed restriction
showing habituation. Contradictions among investigations in the time required for adaptation may be attributed to variation in the level of feed restriction and genetic background.

That H/L ratios two days following release from 60R to 80R were higher than at 7 and 12 days following release. This increase suggests that although the chicks were subjected to a less severe feed deprivation, withdrawal from the more severe restriction, which chicks had habituated was also stressful. An analogous observation by Freeman et al. (1980) showed that two weeks after exogenous injections of corticotrophin had ceased, chickens that had adapted to the treatment showed adrenal hypertrophy and cholesterol depletion. This phenomenon could also be linked with exposure to novelty and uncertainty which involves underlying psychological processes (Levine, 1985). Thus, under practical situation, gradual release from feed deprivation should reduce stress response.

Literature regarding the influence of dw on stress response is conflicting. Carsia and Weber (1986) observed lower relative adrenal weights and cellular sensitivity to ACTH in dwarfs than in normal siblings, which reflect lower hypothalamic-pituitary-adrenal activities in the former. On the other hand, Rombaut et al. (1983), as cited by Decuypere et al. (1991), reported higher plasma corticosterone concentrations in dwarf than normal White Leghorns following
46 h of feed deprivation. In the current study, both lines responded similarly (as measured by H/L ratios) to feed deprivation. Age and genetic background may be responsible for such discrepancies (Decuypere et al., 1991).

Although $d_w$ reduced resistance to $E.\ \text{tenella}$ infection in the current experiment, Haas et al. (1975) found no effect of $d_w$ on susceptibility to $Eimeria\ \text{necatrix}$, $Eimeria\ \text{acervulina}$, or $Eimeria\ \text{brunetti}$ infections. The inconsistency between studies may be attributed to the diversity of genetic background and the species of coccidia used. It was noted earlier that effects of $d_w$ on susceptibility to $Escherichia\ \text{coli}$ infection was modified by genetic background (Mauldin et al., 1978; Marsteller et al., 1980).

Feeding regimen can influence resistance to neoplasms (Han and Smyth, 1972), $E.\ \text{coli}$ (O'Sullivan et al., 1991; Maatman et al., 1993) and streptococcal (Katanbaf et al., 1987) infections of chickens. Feed-restricted chickens in the present experiment were less susceptible to $E.\ \text{tenella}$ infection than those that were fed ad libitum. Limitation of feed consumption elicits the pituitary-adrenal axis responses that influence immunocompetence and thus disease resistance. The existence and significance of environment-disease interaction are well documented (Gross, 1983; Kelley, 1985). Gross (1985) reported that defenses against coccidiosis are mainly cell-mediated and sensitization of chickens to cell-mediated immunity may be enhanced under stressful situations.
Because in the present experiment H/L ratios were similar for all chicks at the time of oocysts inoculation, the superior resistance of feed-restricted chicks to coccidiosis may not be attributed to higher stress response that increased sensitization to cell-mediated immunity. A likely explanation for the phenomenon may be associated with adaptation and prior experiences (Gross, 1983; Saplosky, 1992b). An earlier stressful experience due to feed restriction appeared to induce habituation which increased resistance to the deleterious effects of E. tenella infection. Others (e.g., Gross and Siegel, 1980; Bowen and Washburn, 1984) reported that prior responses to previous stressors may reduce the detrimental effects of subsequent perturbations of homeostasis. Another possible explanation for the superior resistance among feed-restricted chicks is the postulated interaction between trypsin activity and susceptibility to coccidiosis. Britton et al. (1964) suggested that excystation of sporozoites from oocysts was positively correlated with trypsin activity. Thus, feed restriction that reduces trypsin activity (Britton et al., 1964; Nir et al., 1987), may enhance resistance to coccidial infection. From a practical point of view, feed restriction, a routine management practice in poultry husbandry, may evoke short-term stress responses, but there can be long-term benefits in improving resistance to other forms of deleterious environmental stressors.
The effect of dw on antibody response to SRBC has been inconsistent. Among meat-type chickens, dwarf chicks had higher antibody titers than their normal siblings (Mauldin et al., 1978; Marsteller et al., 1980; Martin et al., 1988). On the other hand, working with dwarf and normal White Leghorn chickens, Marsh (1983) observed a reversed picture and postulated the inferiority of dwarf chicks to lower triiodothyronine concentration. Martin et al. (1988), however, observed no correlation between plasma triiodothyronine or thyroxine concentrations with antibody response to SRBC in dwarf and normal chicks of the line used in this experiment. They postulated that interactions among plasma thyroid hormone concentrations, age, dose and source of antigen, and prior experience of individuals or populations can be responsible for the inconsistencies. Although a lack of differences between genotypes supports the results of Martin et al. (1988) and Lilburn et al. (1986), inconsistencies in the literature strongly suggest that expression of dwarfism on immunocompetence, as in other traits, is dependent upon background genome. Variations in route of immunization (van der Zijpp et al., 1986) and concentration of SRBC (Ubosi et al., 1985) could yield discrepancies in results obtained.

The lack of feeding regimen effect on antibody titers to SRBC is in disagreement with the findings of O'Sullivan et al. (1991). Although feed restriction could circumvent
obesity and thus improve immunoresponsiveness, acute psychological and physical stresses imposed by restriction may also impair the immune system. There is considerable evidence that stress is detrimental to immunoresponse (Kelley, 1985; Ballieux and Heijnen, 1987; Siegel, 1987). At the time of immunization in the present study, all chicks exhibited similar stress level (as indicated by H/L ratios) regardless of feeding regimen. Thus, it supports the observation that immunoresponse is independent of feeding regimen.
Table 1. **Mean body weights (g) for males (M) and females (F) by genotype and feeding regimen**\(^1\) at various ages

<table>
<thead>
<tr>
<th>Genotype</th>
<th>22 days</th>
<th>29 days</th>
<th>43 days</th>
<th>50 days</th>
<th>56 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dwarf</td>
<td>M 204(^a)</td>
<td>F 211(^a)</td>
<td>M 299(^a)</td>
<td>F 298(^a)</td>
<td>M 403(^a)</td>
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<tr>
<td>Normal</td>
<td>M 353(^b)</td>
<td>F 324(^b)</td>
<td>M 536(^b)</td>
<td>F 484(^b)</td>
<td>M 822(^b)</td>
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</table>

<table>
<thead>
<tr>
<th>Feeding regimen</th>
<th>22 days</th>
<th>29 days</th>
<th>43 days</th>
<th>50 days</th>
<th>56 days</th>
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</thead>
<tbody>
<tr>
<td>Ad libitum (AL)</td>
<td>M 986(^a)</td>
<td>F 734(^a)</td>
<td>M 1233(^a)</td>
<td>F 902(^a)</td>
<td>M 1459(^a)</td>
</tr>
<tr>
<td>60% of AL (60R)</td>
<td>M 606(^b)</td>
<td>F 546(^b)</td>
<td>M 672(^b)</td>
<td>F 633(^b)</td>
<td>M 749(^b)</td>
</tr>
<tr>
<td>80% of AL (80R)</td>
<td>M 853(^c)</td>
<td>F 680(^b)</td>
<td>M 1008(^c)</td>
<td>F 788(^b)</td>
<td></td>
</tr>
</tbody>
</table>

| Pooled SEM     | 5 | 4 | 8 | 5 | 10 | 7 | 13 | 8 | 16 | 9 |

\(^{a,b,c}\) Means within a column-subgroup with no common letters differ significantly \((P \leq 0.05)\).

\(^1\) All chicks consumed feed AL from hatch to Day 28. On Day 29, some chicks were subjected to 60R. Commencing from Day 43, some chicks under 60R were released to 80R and the remainder continued under 60R.
Table 2. Mean feed efficiencies [body weight to feed consumed (kg/kg)] from 29 to 50 and 29 to 56 days of age where genotype by feeding regimen interactions were significant

<table>
<thead>
<tr>
<th>Genotype</th>
<th>29 to 50 days$^2$</th>
<th></th>
<th>29 to 56 days$^3$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL</td>
<td>60R</td>
<td>80R</td>
<td>AL</td>
</tr>
<tr>
<td>Dwarf</td>
<td>0.38$^a$</td>
<td>0.22$^b$</td>
<td>0.26$^b$</td>
<td>0.37$^a$</td>
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<td></td>
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<td>*</td>
<td>*</td>
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<tr>
<td>Normal</td>
<td>0.41$^a$</td>
<td>0.29$^b$</td>
<td>0.34$^c$</td>
<td>0.40$^a$</td>
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$^{a,b,c}$ Means within a row-subgroup with no common letters differ significantly ($P \leq 0.05$).

$^1$ All chicks consumed feed AL from hatch to Day 28. On Day 29, some chicks were subjected to 60R. Commencing from Day 43, some chicks under 60R were released to 80R and the remainder continued under 60R.

$^2$ Pooled SEM = 0.0006.

$^3$ Pooled SEM = 0.0006.

* A significant difference ($P \leq 0.05$) between means within a column.
Table 3. Mean time (min) required to finish daily feed allotment from 43 to 49 and 50 to 55 days of age where genotype by feeding regimen interactions were significant.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>43 to 49 days(^2)</th>
<th>50 to 55 days(^3)</th>
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<td></td>
<td>60R</td>
<td>80R</td>
</tr>
<tr>
<td>Dwarf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>234(^a)</td>
<td>355(^b)</td>
<td>195(^a)</td>
</tr>
<tr>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>372(^a)</td>
<td>580(^b)</td>
<td>336(^a)</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Means within a row-subgroup with no common letters differ significantly (\(P \leq 0.05\)).

\(^7\) All chicks consumed feed AL from hatch to Day 28. On Day 29, some chicks were subjected to 60R. Commencing from Day 43, some chicks under 60R were released to 80R and the remainder continued under 60R.

\(^2\) Pooled SEM = 9.

\(^3\) Pooled SEM = 8.

\(*\) A significant difference (\(P \leq 0.05\)) between means within a column.
<table>
<thead>
<tr>
<th>Genotype</th>
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<th>Crop-esophagus</th>
<th>Heart</th>
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<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Dwarf</td>
<td>1.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Normal</td>
<td>0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.70&lt;sup&gt;b&lt;/sup&gt;</td>
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<table>
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<th>Feeding regimen</th>
<th>Length</th>
<th>Crop-esophagus</th>
<th>Heart</th>
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<tr>
<td>AL</td>
<td>0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>60R</td>
<td>1.63&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.93&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>80R</td>
<td>1.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.86&lt;sup&gt;b&lt;/sup&gt;</td>
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</tbody>
</table>

| Pooled SEM      | 0.051  | 0.043          | 0.013 | 0.010 |

<sup>a,b,c</sup> Means within a column-subgroup with no common letters differ significantly ($P \leq 0.05$).

<sup>1</sup> All chicks consumed feed AL from hatch to Day 28. On Day 29, some chicks were subjected to 60R. Commencing from Day 43, some chicks under 60R were released to 80R and the remainder continued under 60R.
Table 5. Mean heterophil to lymphocyte ratios where age by feeding regimen\textsuperscript{1} interactions were significant

<table>
<thead>
<tr>
<th>Days of restriction</th>
<th>Days of age</th>
<th>Feeding regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AL</td>
</tr>
<tr>
<td>1</td>
<td>30</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>0.52</td>
</tr>
<tr>
<td>9</td>
<td>38</td>
<td>0.47</td>
</tr>
<tr>
<td>12</td>
<td>41</td>
<td>0.53</td>
</tr>
<tr>
<td>16</td>
<td>45</td>
<td>0.60</td>
</tr>
<tr>
<td>21</td>
<td>50</td>
<td>0.57</td>
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<tr>
<td>26</td>
<td>55</td>
<td>0.57</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td></td>
<td>0.013</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} Means within a row-subgroup with no common letters differ significantly ($P \leq 0.05$).
\textsuperscript{1} All chicks consumed feed AL from hatch to Day 28. On Day 29, some chicks were subjected to 60R. Commencing from Day 43, some chicks under 60R were released to 80R and the remainder continued under 60R.

\* A significant difference ($P \leq 0.05$) between means within a row.
Figure 1. Average daily feed intake of dwarf (solid line) and normal (dashed line) chickens from 29 to 55 days of age. $Y_{\text{dwarf}} = 22.0 + 0.46x$, ($r^2 = 0.58$ with $P \leq 0.01$); $Y_{\text{normal}} = 39.8 + 1.30x$, ($r^2 = 0.77$ with $P \leq 0.01$).
Figure 2. Average time for dwarf (solid line) and normal (dashed line) chicks to consume 60% of the previous day's ad libitum intake of feed when restriction started at 29 days of age (first day of restriction). $Y_{\text{dwarf}} = 1195 - 113x + 3.2x^2$, ($r^2 = 0.94$ with $P \leq 0.01$); $Y_{\text{normal}} = 1174 - 107x + 3.5x^2$, ($r^2 = 0.92$ with $P \leq 0.01$).
CHAPTER III

AGE AND PSYCHOCGENIC FACTORS IN RESPONSE TO FEED DEPRIVATION AND REFEEDING IN WHITE LEGHORNS
SUMMARY

Physiology, behavior, and egg production were measured in White Leghorn chickens (21 and 329 days of age) subjected to (1) ad libitum feeding (AL); (2) feed withdrawal for 48 h, with the provision of sand (SAND); (3) feed withdrawal for 48 h (NO FEED); or (4) feed withdrawal for 48 h, imposed by blocking access to feed by a wire mesh which allowed visual and olfactory cues (WIRE). Fasting-elicited elevation in heterophil to lymphocyte (H/L) ratios was evident by 24 h. Following 48 h of fasting, WIRE chickens had the highest H/L ratios followed by SAND, NO FEED, and AL. Within 36 h of refeeding, older chickens had recovered from fasting-induced stress but H/L ratios of younger ones from WIRE and SAND treatments showed erratic fluctuations. When released to ad libitum feeding, feed intake of older chickens varied according to prior treatment and remained the same during both days of replenishment, whereas all younger ones consumed similar amounts of feed with an intake higher on the second than first day of refeeding. Fasted chickens displayed prominent behavioral responses with occasional age-related discrepancies. Chickens subjected to WIRE showed the most frustration. Provision of sand appeared to reduce "emotional" aspects of feed deprivation.
INTRODUCTION

Withdrawal of feed for varying periods of time is a husbandry practice that may have both physiological (e.g., Freeman et al., 1981; Katanbaf et al., 1989a; Maxwell et al., 1992) and psychological (see Duncan, 1970; Mench, 1992) consequences on an animal. While most feed restriction studies emphasize the importance of physiological effects with regard to stress response, there is a dearth of information on the involvement of underlying psychogenic factors. When an animal fails to fulfill its biological and behavioral needs, frustration may be evoked (Levine, 1985). Psychological stimuli contributing to thwarting or frustrating situations may be as potent as physical insults in triggering both autonomic and neuroendocrine responses (Dantzer and Mormède, 1985). Psychogenic factors may activate the hypothalamic-pituitary-adrenal (HPA) axis despite the absence of homeostatic perturbation (Sapolsky, 1992a). In his review, Mason (1975) indicated that plasma glucocorticoid concentration declined in fasted primates provided with a non-nutritive flavored placebo. Thus, satisfaction obtained from oral manipulation of a non-nutritional substance may act as a displacement activity, or frustration outlet among feed-deprived primates. Most research on effects of fasting has emphasized physiological and behavioral consequences that occurred during the fast. Little information is available on the response, particularly leucocytic alterations, following
refeeding after feed deprivation.

Experiments with rats and mice have demonstrated that the HPA axis may be affected by aging (Chiuheh et al., 1980; Sapolsky et al., 1983; Ida et al., 1984). Although, aged rats were capable of secreting glucocorticoid in response to various stressors, they had a marked inability to terminate glucocorticoid elevation at the end of stress as well as delayed adaptation to mild stressors. Sapolsky (1992b) attributed the phenomenon to loss of adrenal corticoid negative feedback receptors, which was accompanied by loss of neurons. The purpose of the present study was to evaluate the importance of age and psychogenic factors in response to fasting and refeeding in White Leghorn chickens.

MATERIALS AND METHODS

Genetic Stock

Chickens used in Trial 1 were from a White Leghorn population developed from lines divergently selected for antibody response to SRBC (Siegel and Gross, 1980). The two selected lines were crossed, then chickens from the F1 generation were backcrossed to each parental line for three generations. Only individuals from the high antibody genetic background with B13B21 genotypes at the major histocompatibility complex (Dunnington et al., 1992b) were utilized in this trial. The chickens used in Trial 2 were offspring of those used in Trial 1.
Trial 1

At hatching (Day 1), chicks were wingbanded, vaccinated for Marek's disease and floor reared with continuous lighting. At 56 days of age, they were transferred to grower pens with wood shavings litter and exposed to natural lighting. Pullets were moved on Day 112 into individual cages (46 cm high, 30 cm wide and 46 cm deep) with vertical rods on the front and horizontal bars on the back and sides. Cages were in a windowless room with a photoperiod from 0600 to 2000 h. Starter (20% CP; 2,865 kcal ME/kg), developer (14% CP; 2,827 kcal ME/kg) and layer (16% CP; 2,761 kcal ME/kg) diets were provided ad libitum in mash form from Days 1 to 63, Days 64 to 139, and Day 140 onwards, respectively. Feed was in a trough attached to the outside of the front of the cages.

On Day 317, 75 hens were assigned at random to one of four feeding regimens as follow: (1) ad libitum feeding (AL); (2) feed withdrawal for 48 h, with the provision of sand (SAND); (3) feed withdrawal for 48 h (NO FEED); or (4) feed withdrawal for 48 h, imposed by blocking access to feed by a wire mesh which allowed visual and olfactory cues (WIRE). Each group was comprised of 20 hens, except for AL, where there were 15 hens. The AL hens were housed at the upper decks to prevent hens in the other feeding regimens from seeing them because allelomimetic behavior (Hughes, 1971) and social facilitation (Franchina et al., 1986) occur during
feeding and drinking. Prior experiments have not shown tier effects for the measurement criteria used in this study. In no case could chickens face each other when eating. Feed withdrawal commenced at 0800 h on Day 329. Hen-day ovulation percentage and total number of normal and defective eggs (van Middlekoop and Siegel, 1976) were recorded for each hen daily from Days 317 to 342. Blood samples were obtained from the wing vein with EDTA as anticoagulant at 12, 24, 36, and 48 h after onset of feed deprivation. Five hens per treatment were bled in each period, except for the AL group, where four and three hens were sampled in the first three and last bleeding period, respectively. A duration of 48 h was allowed before the same hen was bled again. Blood smears were prepared using May-Grunwald-Giemsa stain and heterophils (H) and lymphocytes (L) were counted to a total of 60 cells (Gross and Siegel, 1983). At 0800 h on Day 331, all hens were released to ad libitum feeding and similar bleeding procedures were conducted at 12, 24, 36, and 48 h after refeeding. Water was available at all times.

Behavioral observations were made during the onset of the feeding regimens and prior to each bleeding period. Observations consisted of recording the general behavior of each group of five hens when treatments were imposed and whenever the researchers entered the room where the chickens were maintained. During the period of feed withdrawal, mash and sand consumption of AL and SAND groups, respectively were
measured for groups of five hens daily. Similar measurements were made in all feeding regimens during refeeding.

**Trial 2**

At hatch, 120 chicks (males and females) were wingbanded and assigned at random, in groups of 6, to 20 starter batteries with wire floors and electric heat. Location of the AL pens was the upper levels as described in Trial 1. Chicks were fed a starter diet with the same formulation as for Trial 1. Lighting was continuous and water was available throughout the experiment. At 0800 h on Day 21, chicks were subjected to one of the four feeding regimens (AL, SAND, NO FEED, and WIRE) described in Trial 1. All chicks were refed at 0800 h on Day 23. Similar blood sampling (1 pen per feeding regimen for each bleeding period), feed intake measurements (pen basis), and behavioral observations as described in Trial 1 were carried out.

**Statistical Analyses**

Data from both trials were used in analysis of variance with a factorial arrangement of treatments in a fixed effect model. Heterophil to lymphocyte ratios were analyzed separately for fasting and refeeding with age and feeding regimen as main effects. Feeding regimen and age were the main effects for total feed and sand intake analyses, while stage of refeeding was also included in the model for daily
feed consumption. For Trial 1, egg production prior to and after onset of fasting was examined separately. When interactions were significant, separate analyses were carried out within each main effect. When significant differences among treatments were observed, comparisons among means were made by Duncan's multiple range test. Significant differences were assumed at $P<0.05$. All analyses were conducted with the aid of General Linear Models (GLM) procedure (SAS® Institute, 1982)

RESULTS
Heterophil to Lymphocyte (H/L) Ratios

During feed withdrawal, there was no evidence of feeding regimen interactions with other main variables for H/L ratios. There was no effect on H/L ratios 12 h after subjecting chickens to the various feeding regimens (Figure 1). By 24 h, however, ratios for SAND and NO FEED chickens were elevated. By 36 h, responses were similar for SAND, NO FEED, and WIRE groups with all greater than for AL. All groups differed from each other by 48 h, with the WIRE regimen having the highest ratios followed by NO FEED, SAND, and AL. Chickens fasted on Day 329 (OLD) exhibited higher rises in H/L ratios by 12, and 24 h of feed withdrawal than those that were feed deprived at 21 days of age (YOUNG) (Figure 2). The difference between age groups disappeared by 36 h with a reversal of trend by 48 h.
Although H/L ratios declined within 12 h following refeeding, ratios did not return to the level of AL for any of the other feeding regimens (Figure 3). At this time the SAND regimen had lower ratios than NO FEED and WIRE chickens, which were similar. Age by feeding regimen interactions were observed for the subsequent duration of refeeding. The interactions resulted from delayed recovery among YOUNG chickens during release to ad libitum feeding. After 24 and 36 h of refeeding, except for WIRE where H/L ratios of YOUNG chickens were elevated, there were no age-related differences in response among feeding regimens. Among OLD hens, however, regardless of prior treatment, all had similar H/L ratios following 36 h of refeeding and these levels were maintained thereafter. On the contrary, withdrawal from fasting for 48 h resulted in a marked rise in H/L ratios of YOUNG chickens under SAND and WIRE regimens.

Egg production

Prior to the onset of feed deprivation (Days 317 to 328), percentage hen-day ovulation was similar for all treatment groups (Figure 4) and no defective eggs were produced. During the 48 h fasting period (Days 329 to 331), production patterns of the four groups remained similar. However, commencing from Day 331, there was a dramatic decline in percentage hen-day ovulations among WIRE and SAND hens while those under NO FEED had a moderate drop in
production. There was a rapid rise in ovulations among SAND and WIRE hens following 60 h of refeeding (Day 334) with a lag in the rise in the NO FEED group by a few days. Percentage hen-day ovulations from Days 329 to 342 (post fasting period) were higher for AL and NO FEED regimens than those under SAND and WIRE which were similar. For percentage of defective eggs (relative to total number of ovulations), there was no difference between AL (0.74) and NO FEED (2.92). However, only the former was superior to both SAND (9.87) and WIRE (10.68) while NO FEED and SAND shared similar values.

*Feed and Sand Consumption*

For each age group, consumption of feed and sand was indexed relative to the AL regimen which was considered as 100%. No age effect was found in percentage of sand consumed during feed deprivation when measured daily (YOUNG, 13.3%; OLD, 9.3%) and as a total (YOUNG, 18.6%, OLD, 26.6%). Regardless of age, chickens consumed more sand on the second (14.0%) than the first (8.2%) day of feed withdrawal.

Feeding regimen by age interactions were significant for both daily and total feed intake when chickens were refed. However, the interactions may be inherent because feed intake of AL regimens at both ages were considered as 100%. Upon releasing to ad libitum feeding, all YOUNG chickens consumed similar amount of feed, whereas daily (two days) and total feed consumption among OLD chickens varied according to
feeding regimen (Figure 5).

When released to *ad libitum* feeding, chickens fasted at 21 days of age consumed more feed on the second than the first day of refeeding. The amount of feed consumed by the OLD chickens was similar for both days resulting in a significant age by stage of refeeding interaction (Figure 6).

**Behavior**

Unless stated otherwise, behavioral descriptions were similar for both age groups. AL chickens spent a major proportion of their time eating and resting during morning and evening observations, respectively. When presented with sand during onset of fasting (0 h), chickens initially approached the feeders, pecked at and ate some sand for a min or two. They then either engaged in sand pecking or moved away from the troughs. Unlike their SAND counterparts, WIRE chickens pecked constantly at the wire mesh instead of moving away from feeders. Several OLD hens, showed "exploratory" behavior by lowering their heads to see the undersurface of feed troughs. At the time when feeders were removed, NO FEED chickens did not exhibit any specific reaction. However, a few OLD hens put their heads out from cages and pecked at the wire grills where the feed troughs had been located.

Age-related differences in response were observed following 12 h of feed withdrawal. Even though possible disturbances were minimized by having these chickens isolated
from all others, appearance of the observers initially caused excitement and flightiness among OLD chickens. These reactions were followed by pecking of wire and sand for those groups. On the contrary, at this time YOUNG chicks were either resting or sleeping under the heat source.

After 24 h of fasting, appearance of observers again induced excitement and flightiness with the reaction less intense in AL chickens. While NO FEED chickens remained flighty and excited, several activities seen during the onset of fasting such as eating and pecking of sand, and occasional wire pecking were again observed. Some OLD hens subjected to WIRE were found facing in the opposite direction of feed troughs. This was particularly cogent because the water cups were just adjacent to the feeders.

Observations conducted at 36 and 48 h were analogous to those at 12 and 24 h for OLD hens, respectively. For the YOUNG ones, however, after 36 h of fasting, instead of resting under the heat source, chicks stood around feed troughs and periodically engaged in pecking of wire and sand.

When feed was returned, feeding responses were similar for all OLD chickens. Among YOUNG chicks, however, responses of those from the SAND and WIRE groups were slower than those of their NO FEED counterparts. Observations following 12, 24, 36 and 48 h revealed that, regardless of prior treatment, chickens were either eating or resting.
DISCUSSION

The present findings add to the growing body of evidence that response to fasting may be physiologically and psychologically modulated (see Duncan, 1970; Mench, 1992). After being feed-deprived for 48 h, WIRE and NO FEED chickens had similar durations of physiological disruption but the WIRE chickens, probably due to greater psychological stimulation, were more distressed as measured by H/L ratios. These observations suggest the thwarting nature of the WIRE treatment and concomitantly the potency of underlying psychogenic factors in eliciting the HPA axis. In earlier studies, Duncan and Wood-Gush (1971; 1972) proposed from behavioral observations that presenting feed under a visible cover caused frustration among fasted chickens. Koene (1993) confirmed these observations and found differences in vocalization among white and brown layers stocks.

Suppression of H/L ratios among WIRE chickens following 24 h of feed withdrawal may be mediated by visual stimuli which act as a cue for them to expect the forthcoming event, refeeding. Similarly, Harvey et al. (1983) noted that fasted chickens exhibited a decline in plasma concentration of corticosterone when simply shown feed. However, in the present study, and as noted by Harvey et al. (1983), the suppression of stress response was only transient. They suggested reactivation of the HPA axis if visual stimuli were not followed by metabolic reinforcement and attributed the
phenomenon to "hope-disappointment syndrome".

In discussing the crucial role of psychogenic factors in eliciting stress response, Mason (1975) postulated the possibility of minimizing activation of the HPA axis due to physical stressors by modifying the involved emotional arousal. Studies with pigs (Dantzer and Mormède, 1981), primates (Mason, 1975) and rats (Sapolsky, 1992a) support this thesis and suggest the importance of displacement activities or frustration outlets in ameliorating the psychological threats. The present data provide additional support to these findings, because H/L ratios of SAND were higher than for AL but lower than for NO FEED and WIRE chickens. Apart from the actual consumption of the nutritionally inert sand, the displaced behavior of redirected pecking and "sand playing" may have been important in helping them cope with psychological components of fasting and eventually attenuate physiological responses.

The OLD hens in this experiment did not exhibit impaired recovery from disruption of homeostasis due to age which is inconsistent with earlier studies with rats (Chiueh et al., 1980; Sapolsky et al., 1983; Ida et al., 1984). In terms of "physiological age", the OLD hens may have been different from the rats used in previous work and the reported age-associated hippocampal degeneration (Sapolsky, 1992b) may not have occurred extensively among the aged hens used in this study. The inconsistency may also be attributed to age-
related variation in feeding behavior during refeeding, where the lighting program and reproductive status (Sykes, 1983) could be responsible. Feed intake of refed OLD hens varied according to fasting regimen and remained the same on both days of refeeding, whereas all YOUNG ones consumed similar amounts of feed with a higher intake on the second than first day of release to ad libitum feeding. As reported in Chapter II, chicks exhibited a transient rise in H/L ratios when withdrawn from a more severe fasting. Rapid replenishment following water deprivation may also be stressful (Marsden et al., 1965), suggesting that gradual releases from environmental insults may enhance termination of poststress response. A third possible explanation is related to early experiences and response to stress. Because OLD hens were exposed to various lighting programs and types of housing, they may have had more stressful early experiences than their YOUNG counterparts that were kept in the same pens with a constant photoperiod throughout the experiment. Early stressful experiences may have considerable impact on response to subsequent perturbations of homeostasis (see Gross, 1983; Sapolsky, 1992b).

Mechanisms involved for the erratic and robust fluctuations of H/L ratios during refeeding among YOUNG chickens subjected to WIRE and SAND are unclear. It is possible, however, that the phenomenon could also be linked to feeding behavior during refeeding.
As expected, feed withdrawal suppressed ovulation and increased incidence of defective eggs. Elevation of plasma concentration of adrenaline, due to stress, which caused among other factors, delay in oviposition, cessation of uterine motility and retention of eggs (Sykes, 1955; Hughes et al., 1986) may have accounted for the inferior egg yield of fasted hens. There is a lack of agreement on methods to assess welfare or degree of stress in livestock. Despite skeptical views and inconsistencies among reported findings, productivity is considered as one of the typical indicators of welfare (see Duncan, 1981; Mench, 1992). These data suggest that productivity and physiological indexes do not necessarily correlate well. For example, although NO FEED hens had higher H/L ratios than those under SAND and AL regimens, their hen-day ovulations were greater than and similar to the former and latter, respectively.

Psychological events, such as in thwarting situations, may provide stimuli that can influence aggressive behavior (see Duncan, 1970; Mench, 1992). Frustration-induced aggression has been noted in baboons (Sapolsky, 1990), pigs (Dantzler et al., 1980), and domestic fowl (Duncan and Wood-Gush, 1971). Despite the thwarting nature of the WIRE group, the experiment procedure was such that physical aspects of aggression were precluded. This was because chicks used in Trial 2 were only 21 days of age, prior to establishment of pecking rights (Dawson and Siegel, 1967), and results showed
no aggressive behavior. As for the OLD hens, physical contact among individuals was precluded because they were caged individually.

Excitement and flightiness exhibited by the fasted chickens when observers appeared suggest that presence of humans provide a cue signalling the chickens to expect presentation of feed. Fish (Davis and Bardach, 1965), and finches (Clifton, 1979) were active during anticipation of feed. This reaction, however, may further exacerbate the stressfulness of fasting due to "hope-disappointment syndrome" (Mowrer, 1960; cited from Harvey et al., 1983).

It was unclear whether the tendency of redirected pecking at wire mesh was detrimental to the ongoing frustration, beneficial to the physiology of the chickens as in displacement activities or neither. When layers were denied feed by covering feed troughs with opaque boards, thus attenuating thwarting, pecking of covers was also noted (Preston, 1987). In the present experiment, it is unlikely that wire pecking could be linked to social facilitation or allelomimetic feeding behavior because the AL chickens were out of their sight.

The opposite orientation of several WIRE hens may be considered as a conspicuous display of frustration. The behavior could be interpreted as a "looking for an exit" action or avoidance from thwarting situations (Duncan, 1970). Emotional arousal such as exposure to a novel environment
elicited locomotor activity and escape attempts by pigs (Dantzer and Mormède, 1985).

There is evidence from several sources (see Dantzer and Mormède, 1985; Mench, 1992) suggesting that Mason's (1975) thesis regarding elimination of emotional components and attenuation of detrimental physiological responses could offer some promising solutions for stress-related management procedures. Data in this study suggest the possibility of reducing activation of the HPA axis in feed-deprived chickens by providing a nutritionally inert substance, which modifies the psychological threats of the procedure.
Figure 1. Mean heterophil to lymphocyte ratios at various stages of fasting by feeding regimen. a,b,c,d: Means within a duration of fasting with no common letters differ significantly ($P \leq 0.05$). Pooled SEM = 0.02 (12 h); 0.04 (24 h); 0.05 (36 h); 0.07 (48 h).
Figure 2. Mean heterophil to lymphocyte ratios at various stages of fasting at 21 and 329 days of age. a, b: Means within a duration of fasting with no common letters differ significantly (P<0.05). See Figure 1 for pooled SEM.
Figure 3. Mean heterophil to lymphocyte ratios at various stages of refeeding where age (21 days, solid line; 329 days, dashed line) by feeding regimen interactions were significant (except for 0 and + 12 h). a, b, c: Means within an age with no common letters differ significantly (P≤0.05). Difference between ages P≤0.05. Pooled SEM = 0.07 (0 h); 0.05 (+ 12 h); 0.04 (+ 24 h); 0.04 (+ 36 h); 0.06 (+ 48 h).
Figure 4. Percentage hen-day ovulation by feeding regimen. Feed was withdrawn (↓) on Day 329 and returned (↑) on Day 331.
Figure 5. Mean percentage daily (top), and total (bottom) feed consumption (relative to ad libitum intake) during refeeding where age (21 days, solid line; 329 days, dashed line) by feeding regimen interactions were significant. a,b,c: Means within an age with no common letters differ significantly (P ≤ 0.05). *Difference between ages P ≤ 0.05. Pooled SEM = 1.31 (top); 3.06 (bottom).
Figure 6. Mean percentage daily feed consumption (relative to ad libitum intake) during refeeding where age (21 days, solid line; 329 days dashed line) by stage of refeeding interactions were significant. a, b: Means within an age with no common letters differ significantly ($P \leq 0.05$). *Difference between ages $P \leq 0.05$. Pooled SEM = 1.22.
CHAPTER IV

FEED RESTRICTION EARLY OR LATER IN LIFE AND ITS EFFECT ON ADAPTABILITY, DISEASE RESISTANCE, AND IMMUNOCOMPETENCE OF HEAT-STRESSED DWARF AND NONDWARF CHICKENS
SUMMARY

Dwarf and nondwarf chickens placed under 60% feed restriction from either 4 to 6 (early) or 24 to 26 (late) days of age were exposed to high ambient temperatures (35±2°C) from 36 to 43 days age. As measured by heterophil to lymphocyte (H/L) ratios, stress response to feed restriction was similar at both ages for dwarfs while less at the younger than older age for nondwarfs, resulting in a significant genotype by age of feed restriction interaction. Nondwarf chickens feed restricted at younger age had smaller increase in H/L ratios, improved resistance to marble spleen disease infection and greater growth than those restricted at the older age or fed ad libitum in response to the high ambient temperatures. For dwarf chickens feeding regimen had no influence on response to the environmental insults. Antibody response to sheep erythrocyte antigen was not affected by genotype or feeding regimen.

INTRODUCTION

Ability to habituate can be vital in helping an animal to survive in the environment to which it is exposed. In the context of poultry production, acclimatization has allowed world-wide distribution of commercial stocks with a considerable degree of uniformity in performance despite climatic variations (Sykes and Fataftah, 1986a). Several studies have demonstrated the possibility of improving
livability, growth, and feed efficiency of heat-stressed chickens by prior exposure to controlled thermal stressors (Hutchinson and Sykes, 1953; Reece et al., 1972; May et al., 1987; Arjona et al., 1988) while others reported otherwise (McDonald et al., 1990; Smith and McGhee, 1990; Shoop and Birrenkott, 1990). Several factors, including age when chickens were preconditioned to initial stimuli, could yield discrepancies in the results obtained. Gross (1983) suggested that stresses which occur early in life, while many systems of the chicks are still developing, may have a long-lasting impact and could possibly modify expression of their genetic potential. There is, however, a paucity of information regarding disease resistance, and immunocompetence of acclimated chickens when exposed to high ambient temperatures.

Most approaches for the elicitation of heat-tolerance in chickens involved exposure to mild or acute thermal stressors (Hutchinson and Sykes, 1953; Reece et al., 1972; May et al., 1987; Arjona et al., 1988). Sykes and Fataftah (1986a) considered that to acclimate in advance by exposing chickens to controlled rises in environmental temperatures was difficult under practical situations such as when rearing in open-sided houses, where manipulation of house temperature was a major problem. Bowen and Washburn (1984) indicated that repeated handling increased ability of broilers to withstand acute heat stress the following day. Overheated or water
deprived chicks had lower stress responses when subsequently subjected to fasting (Gross and Siegel, 1980). These findings suggest that an animal does not have to be exposed to the same stressors in order for habituation to occur. Thus, additional studies are required to evaluate the possibility of enhancing heat tolerance by feed restriction early or later in life.

Success in developing distinct lines of quail (Satterlee and Johnson, 1988), turkeys (Brown and Nestor, 1973; 1974) and chickens (Wilson et al., 1966; 1975; Gross et al., 1984) through selection for high and low responsiveness to environmental stimuli indicates genetic variation for responses to stressors. In studies of layer-type hens of light or medium weight, dwarf hens tolerated high environmental temperatures better than nondwarfs (Mather and Ahmad, 1971; Horst and Petersen, 1977; Khan et al., 1987). Carsia and Weber (1986) reported alterations in adrenal stress response and adrenocortical cell function due to the autosomal and the sex-linked dwarfing alleles. Thus, apart from their smaller body size, lower thyroid activity (Guillaume, 1976) and improved water retention capacity (Singh et al., 1980), genetic-dependent alteration of adrenal function may contribute to the superior heat tolerance of dwarf chickens. There is a dearth of information with regard to the effect of dwarfism on heat tolerance and habituation in meat-type chickens. The present study was conducted to
evaluate effects of feed restriction early and later in life on adaptability, disease resistance, and immunocompetence of dwarf and normal White Plymouth Rock chickens when exposed to high environmental temperatures.

MATERIALS AND METHODS

Genetic Stocks, Husbandry, and Traits Measured

Dwarf and nondwarf (normal) chickens were obtained from a line selected for high body weight at 8 weeks of age (Dunnington and Siegel, 1985). The nondwarf chickens were from the $S_{35}$ generation. In the 13th selected generation the sex-linked allele $dw$ was introduced into a random sample of chickens from that line. Four generations of repeated backcrossing to the selected line resulted in a dwarf line with 97% of the background genome of the selected line (Reddy and Siegel, 1977), after which selection was relaxed. The dwarf chickens used in this experiment were from the 17th generation after selection was relaxed.

Eggs obtained from age-contemporary dwarf and normal parents were incubated in the same machine. At hatching (Day 0), chicks were wingbanded, vaccinated for Marek's disease and weighed to the nearest g. Six floor pens with wood shavings as litter were allocated randomly for each genotype with 22 nondwarf or 17 dwarf chicks per pen. Room temperature was maintained by hot air brooding at $35\pm1^\circ$C during the first week of life and then gradually reduced to $20\pm1^\circ$C by Day 35.
Chicks were fed a mash diet (24% CP; 3,146 kcal ME/kg) and water was available at all times. Lighting was continuous.

On Day 0, chicks were assigned to one of the three feeding regimens with two pens per group for each genotype. The feeding regimens were as follows; (1) 60% feed restriction at 4, 5, and 6 days of age (E60) (2) 60% feed restriction at 24, 25, and 26 days of age (L60) (3) ad libitum feeding (AL). Sixty percent feed restriction was based upon the previous day feed consumption of the ad libitum feeding group of each genotype. Prior to feeding, individual body weights (g) were obtained on Days 7, 14, 21, 28, 35, and 43.

The day following each feed restriction period, six chicks from each feeding regimen-genotype subgroup were chosen at random and blood samples were obtained from the wing vein with EDTA as the anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain and heterophils (H) and lymphocytes (L) were counted to a total of 60 cells (Gross and Siegel, 1983).

Commencing from Day 36, all chicks were exposed to ambient temperatures of 35±2°C for 7 days. The increase from 20±1°C to this temperature occurred over a 5-h period. The temperature in each pen during the heat treatment was monitored and daily maximum and minimum temperatures were recorded. Relative humidity was not controlled but measurements showed that it remained below 45%. One day prior
to heat treatment (Day 35), six chicks per feeding regimen-genotype subclass were bled for H and L counts. Similar procedures were repeated two (Day 37) and seven (Day 43) days after the onset of heat treatment. On Day 37, 11 chicks from each feeding regimen-genotype subclass were given 0.1 ml intravenous injection of 0.5% SRBC. On the same day, each of 14 chicks per subclass was inoculated via wing vein with 0.1 ml of a 1% suspension of spleen extract from chickens infected with marble spleen disease (MSD) virus (Domermuth and Gross, 1991). Different pens of chicks were assigned to receive either SRBC or MSD virus. Antibody production in response to SRBC antigen was measured 6 days postinjection by the microtiter hemagglutination procedure of Wegmann and Smithies (1966). On Day 43, all chicks were weighed, sex was determined, and chicks were killed by cervical dislocation. Spleens of all chicks in pens with MSD infected birds were removed and weighed (0.01 g).

Statistical Analyses

Data were subjected to analysis of variance with main effects and their interactions in a factorial arrangement in a fixed effect model. When interactions were significant, separate analysis were conducted within each main effect. When significant, separation of multiple means was by multiple range test. Prior to analysis, body weight data were transformed to common logarithms and relative spleen weights
to arc sine square roots. Untransformed means are presented in the Tables and significance was considered as $P \leq 0.05$. All analyses were conducted with aid of General Linear Models (GLM) procedure (SAS® Institute, 1982).

RESULTS

Growth

There was no sexual dimorphism for body weight at hatch (Table 1). By Day 7 and thereafter, males were heavier than females. Nondwarf and dwarf chicks had similar body weights at hatch. From Day 7, even when genotype by feeding regimen interactions were significant (Days 7 and 21) nondwarf chicks were heavier than dwarfs. The interaction on Day 7 occurred because although, there was lower body weight for the E60 than AL regimen, the difference attributable to suppressed growth was greater for nondwarf (28%) than for dwarf (19%) chicks (Table 2). Upon release of E60 chicks to ad libitum feeding, dwarfs had greater compensatory growth than nondwarfs. By Day 21, although, feeding regimen had no effect on growth in dwarf chicks, nondwarf AL chicks were heavier than those under E60 resulting in the genotype by feeding regimen interactions. Late 60% feed restriction suppressed body weight on Day 28, regardless of genotype and sex, however, the reduction was not evident by Day 35. During the period from Days 36 to 43 when chicks were exposed to heat there were significant genotype by feeding regimen

100
interactions for relative weight gain [(BW Day 43 - BW Day 35)/BW Day 35]. The interactions were caused by different effects of feeding regimen on relative weight gain of dwarf and nondwarf chicks (Table 3). Within nondwarf chicks, E60 improved relative weight gain, whereas feeding regimen had no effect on the trait in dwarf chicks. Except for those subjected to L60, nondwarf chicks exhibited greater relative weight gains than dwarfs. There was no sexual dimorphism for relative weight gain.

**Heterophil to Lymphocyte (H/L) Ratios**

Regardless of age, imposition of 3 days of 60% feed restriction increased H/L ratios for chicks of both genotypes (0.48 for AL, and 0.79 for 60% restriction). There was a significant genotype by age at feed restriction interaction for H/L ratios. The interaction resulted from similar ratios between ages for dwarfs (0.54 for E60, and 0.64 for L60) and elevated ratios for nondwarfs during the later feed restriction (0.55 for E60, and 0.81 for L60).

Genotype by feeding regimen interactions were significant for H/L ratios when chicks were exposed to high ambient temperatures for 7 days (from Days 36 to 43). Among nondwarf but not dwarf chicks, the E60 regimen greatly reduced H/L ratios due to heat treatment (Figure 1). Differences between genotypes for H/L ratios attributable to heat treatment were observed only among chicks fed E60. There
was a significant feeding regimen by number of days under heat treatment interaction (Figure 2). One day prior to heat treatment (Day 35), H/L ratios were not affected by feeding regimen. On Day 38, however, two days after exposure to heat, there was a dramatic elevation in H/L ratios among AL and L60 but not E60 chicks. Whereas H/L ratios of E60 and AL chicks were consistent from Days 38 to 43, ratios of L60 chicks declined after 7 days.

Responses to Marble Spleen Disease Challenge and Sheep Red Blood Cell Antigen

There was no difference among feeding regimens in response of dwarf chicks to MSD infection as measured by relative spleen weights (g/100 g body weight). In contrast, relative spleen weights of nondwarf chicks were greater for AL and L60 than the E60 feeding regimen, resulting in significant genotype by feeding regimen interactions (Figure 3). Relative spleen weights were greater for females (0.18) than males (0.15), regardless of feeding regimen and genotype. Neither genotype, sex, or feeding regimen had significant effect on relative spleen weights of chicks that were not challenged with MSD virus. Body weights of dwarf and nondwarf chicks were not affected by MSD virus challenge. There was, however, significant genotype by challenge interactions for relative weight gain, because gains among unchallenged (0.25) dwarf chicks were greater than for those
inoculated (0.22) with MSD virus. In contrast, the converse was observed among nondwarf chicks (0.27 compared with 0.30).

Antibody titers to SRBC were similar for dwarf (7.41) and nondwarf (7.79) chicks. Mean titers for chicks fed E60 (7.57), AL (7.75), and L60 (7.50) were similar.

**DISCUSSION**

Genetic bases for compensatory growth were reflected in the greater weight gains of dwarf than nondwarf chicks upon release from E60. There is delayed sexual maturity of dwarfs (Nutt, 1959; Bernier and Arscott, 1960; Polkinghorne and Lowe, 1973) and in their review, Wilson and Osbourn (1960) indicated that slower maturing animals had more rapid compensatory growth from undernutrition than those that matured earlier. An alternative explanation for the phenomenon may be associated with discrepancies in relative length and weight of gastrointestinal tract between dwarfs and nondwarfs. Because of the relatively heavier and longer crop-esophagus of dwarf than nondwarf chicks (Chapter II), dwarfs were able to store feed more effectively, hence allowing consumption of feed in a shorter period. In addition to genetic dimorphism in compensatory growth, an effect of stage of development when feed restriction commenced on accelerated growth was also observed. Imposition of fasting early in life or near the point of inflection of the sigmoid growth curve of an animal, where there is rapid development...
of "supply organs" such as gastrointestinal tract and liver (Katanbaf et al., 1988), may be more detrimental for compensatory growth (Wilson and Osbourn, 1960).

The present study, confirmed earlier evidence that feed restriction is a stressor. Feed restriction elicited alteration in both leucocytic count (e.g., Cross and Siegel, 1986; Katanbaf et al., 1989a; Maxwell et al., 1992) and steroidal hormone status (e.g., Nir et al., 1975; Freeman et al., 1981; Harvey et al., 1983). Fasting-induced increases in corticosterone may be essential for stimulating gluconeogenesis. O'Neil and Langslow (1978) reported that adrenalectomy may result in fatal hypoglycemia during starvation. Increased plasma of corticosterone during fasting may also be important in stimulating hyperphagia during refeeding (Bouillon and Berdanier, 1981). The greater H/L ratio response among nondwarf chicks, when fed L60 than E60, contrasts with the findings of Constantin et al. (1977) who reported that feed withdrawal during the neonatal period was more stressful than at later ages. However, because different genetic stocks were used in the cited study, and there were genotype by feeding interactions in this study, inferences should be made with caution.

The results of this study confirm that stressful experiences during the neonatal stage may have a profound impact on various physiological and behavioral aspects of an animal's response to subsequent environmental stimuli. One of
the earliest studies on effect of early stressors on adult adrenocortical function was by Levine (see Sapolsky, 1992b), who reported that infantile stimulation through handling elicited long lasting alteration of the hypothalamic-pituitary-adrenal (HPA) axis in rodents. Handled rats, as adults, had lower circulating corticosterone concentration both basally and during recovery period after withdrawal of stressors than those that were not handled. Enhanced negative feedback sensitivity due to increased concentrations of hippocampal corticosteroid receptor, and decreased numbers of corticosterone binding globulin-like receptors in the pituitary may have accounted for the phenomenon (Sapolsky, 1992b). Similar findings, however were not observed when rats were stimulated at later ages. Data presented here indicated that nondwarf chicks fasted early in life (E60) had lower stress response, enhanced disease resistance and improved growth when exposed subsequently to high ambient temperatures. Ben Nathan et al. (1976) and McFarlane and Curtis (1989) noted heat-elicited alteration of leucocytic counts in chickens. Persisting modification of the HPA axis attributable to neonatal feed restriction was reflected in this experiment by lower H/L ratios of nondwarf E60 than AL and L60 chicks during the imposition of heat treatment. Despite the lack of nonheated control in the present study, the H/L ratios (0.49) of nondwarf E60 chicks during heat exposure may be their "normal" range. As reported in Chapter
II, chicks of similar genotype reared under ambient temperatures of 21±1°C had H/L ratios of 0.47 and 0.53 at 38 and 41 days of age, respectively.

Stressors may reduce ability to produce antibodies and effective cell-mediated immunity and thereby increase susceptibility to viral infections (Gross, 1983; Gross and Siegel, 1993). Based on relative spleen weights, nondwarf E60 chicks were more resistant to MSD infection when exposed to high ambient temperatures than those under AL and L60. This result may be attributed to a lower stress response, based on H/L ratios, when they were exposed to heat, which is consistent with the association between level of stress and resistance to viral infection. The improved relative weight gain among nondwarf E60 chicks during the heat treatment also suggested better ability of these chicks to tolerate heat.

Significant genotype by feeding regimen interactions for several traits measured during the heat treatment suggested that dwarf and nondwarf chicks responded differently to stressors. Despite the ability to acclimate nondwarf chicks to high environmental temperatures by neonatal feed restriction, neither feeding regimen improved heat tolerance among the dwarf chicks. There is evidence that the \( dw \) allele alters adrenocortical cell function (Carsia and Weber, 1986). Genetic differences in habituation have also been reported (Bowen and Washburn, 1984). While repeated handling did not
increase heat resistance in White Leghorns, the converse was observed in broilers. The lack of heat tolerance superiority attributable to the dw allele observed here for White Rocks is in contrast with that reported in layer-type dwarf hens (Mather and Ahmad, 1971; Horst and Petersen, 1977; Khan et al., 1987). These inconsistencies suggest that responses to environmental factors associated with the dw allele may be influenced by genetic background. Also the typical correlation between body weight and heat tolerance (see Smith and Oliver, 1971) may not be consistent.

Detrimental effects of extreme temperatures on immunocompetence in chickens have been well established (see Thaxton, 1978). The inhibitory and suppressive actions of glucocorticoids on the immune system may be the most important mediators for heat-induced immunosuppression. These findings indicate that although the 7-day heat treatment was relatively less stressful to nondwarf E60 than AL and L60 chicks, all chicks, regardless of feeding regimen and genotype, responded similarly to SRBC antigen. The conflict may be attributed to the duration of heat treatment. Thaxton et al. (1968) noted that circulating antibody levels were not affected by high temperatures if chickens were exposed to chronic heat treatment. In conclusion, these data suggest the possibility of acclimating chickens to high ambient temperatures by mild feed restriction early in life, which may be more realistic under practical situations than prior
exposure to thermal stressors.
Table 1. Mean body weights (g) by sex, genotype, and feeding regimen\(^1\) at various ages

<table>
<thead>
<tr>
<th></th>
<th>Day</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>14</td>
<td>21</td>
<td>28</td>
<td>35</td>
<td>43</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td>40(^a)</td>
<td>81(^a)</td>
<td>189(^a)</td>
<td>338(^a)</td>
<td>470(^a)</td>
<td>704(^a)</td>
<td>896(^a)</td>
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<tr>
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<td>77(^b)</td>
<td>170(^b)</td>
<td>290(^b)</td>
<td>388(^b)</td>
<td>560(^b)</td>
<td>709(^b)</td>
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<td></td>
<td></td>
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<tr>
<td>Dwarf</td>
<td>40(^a)</td>
<td>128(^a)</td>
<td>313(^a)</td>
<td>459(^a)</td>
<td>567(^a)</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>206(^b)</td>
<td>534(^b)</td>
<td>793(^b)</td>
<td>1016(^b)</td>
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<td></td>
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<td>Ad libitum (AL)</td>
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<td>185(^a)</td>
<td>457(^a)</td>
<td>647(^a)</td>
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<tr>
<td>Early 60% of AL (E60)</td>
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<td></td>
<td></td>
<td></td>
<td>162(^b)</td>
<td>440(^b)</td>
<td>629(^a)</td>
</tr>
<tr>
<td>Late 60% of AL (L60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>393(^c)</td>
<td></td>
<td>625(^a)</td>
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<tr>
<td>Pooled SEM</td>
<td>0.15</td>
<td>0.55</td>
<td>1.16</td>
<td>2.03</td>
<td>2.64</td>
<td>3.88</td>
<td>4.45</td>
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</tbody>
</table>

\(^{a,b,c}\) Means within a column-subgroup with no common letters differ significantly (\(P \leq 0.05\)).

\(\square\) Significant (\(P \leq 0.05\)) genotype by feeding regimen interactions, see Table 2.

\(^1\) All chicks were fed \textit{ad libitum} from hatch to Day 3. On Days 4, 5, and 6 some chicks were subjected to E60. On Days 24, 25, and 26 different pens of chicks were fed L60.
Table 2. Mean body weights (g) on Days 7 and 21 where genotype by feeding regimen\(^1\) interactions were significant

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Day 7</th>
<th></th>
<th>Day 21</th>
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<tr>
<td></td>
<td>AL</td>
<td>E60</td>
<td>AL</td>
<td>E60</td>
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<tr>
<td>Dwarf</td>
<td>64</td>
<td>*</td>
<td>51</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>Nondwarf</td>
<td>96</td>
<td>*</td>
<td>70</td>
<td>373</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

* A significant difference ($P \leq 0.05$) between pairs of means.

NS No significant difference ($P \leq 0.05$).

\(^1\) All chicks were fed *ad libitum* from hatch to Day 3. On Days 4, 5, and 6 some chicks were subjected to E60. See Table 1 for Pooled SEM.
Table 3. Mean relative weight gain [(BW 43 - BW 35)/BW35] x 100 where genotype by feeding regimen interaction were significant

<table>
<thead>
<tr>
<th>Feeding regimen</th>
<th>AL</th>
<th>E60</th>
<th>L60</th>
</tr>
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<tbody>
<tr>
<td>Dwarf</td>
<td>24(^a)</td>
<td>22(^a)</td>
<td>25(^a)</td>
</tr>
<tr>
<td>*</td>
<td>*</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Nondwarf</td>
<td>27(^a)</td>
<td>30(^b)</td>
<td>28(^a)</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Mean within a row-subgroup with no common letters differ significantly \((P \leq 0.05)\).

\(^*\) A significant difference \((P \leq 0.05)\) between means within a column.

\(\text{NS}\) No significant difference \((P \leq 0.05)\).

\(^1\) All chicks were fed *ad libitum* from hatch to Day 3. On Days 4, 5, and 6 some chicks were subjected to E60. On Days 24, 25, and 26 different pens of chicks were fed L60.

Pooled SEM = 0.38.
Figure 1. Mean heterophil to lymphocyte ratios at various stages of heat treatment where genotype by feeding regimen interactions were significant. a,b: Means within a genotype with no common letters differ significantly ($P \leq 0.05$). *Difference between genotypes $P \leq 0.05$. Pooled SEM = 0.04.
Figure 2. Mean heterophil to lymphocyte ratios at various stages of heat treatment where feeding regimen by stage of heat treatment interactions were significant. a,b: Means within a feeding regimen with no common letters differ significantly (P<0.05). *E60 < (AL = L60). See Figure 1 for pooled SEM.
Figure 3. Mean relative spleen weights (g/100 g body weight) of marble spleen disease infected chicks on Day 43 (6 days postinoculation) where genotype by feeding regimen interactions were significant. a, b: Means within a genotype with no common letters differ significantly (P<0.05). *Difference between genotypes P<0.05. Pooled SEM = 0.003
CHAPTER V

INHIBITION OF ADRENAL STEROIDOGENESIS, FEED RESTRICTION AND ACCLIMATION TO HIGH AMBIENT TEMPERATURES IN CHICKENS
SUMMARY

White Plymouth Rock chickens placed under 60% feed restriction or ad libitum feeding, with or without metypapone treatment, from either 4 to 6 (early) or 24 to 26 (late) days of age were exposed to high ambient temperatures (35±2°C) from 36 to 43 days of age. Stress due to fasting was not manifested through leucocytic alteration when feed-restricted chicks were supplemented with an adrenal blocking chemical, metypapone. Provision of metypapone during the fasting period resulted inferior compensatory growth during refeeding. Exposure to high temperatures from 36 to 43 days of age did not cause an elevation in heterophil to lymphocyte (H/L) ratios of chicks that had eaten metypapone-treated feed ad libitum during the neonatal stage. During heat exposure, chicks that had been subjected to early 60% restriction with non-metypapone-treated feed had lower H/L ratios and improved resistance to marble spleen disease infection.

INTRODUCTION

When an animal is faced with a stressor there is activation of the hypothalamic-pituitary-adrenal (HPA) axis, which increases synthesis and liberation of adrenal corticoids. Although increased levels of glucocorticoids are crucial for survival during severe disruption of homeostasis (Sapolsky, 1992a), chronic exposure to high levels of glucocorticoids may be detrimental to the health and well-
being of an animal. Whereas stress physiology is closely related to the functions of adrenal corticoid, the mechanisms involved in glucocorticoid-modulation of the stress syndrome are not well understood.

In view of the complex physiological functions of glucocorticoid during stress, questions remain about their involvement in adaptation. There is substantial evidence to indicate that early stressful experiences affect various aspects of an animal's response to subsequent stressors (see Sapolsky, 1992b). There is uncertainty, however, whether habituation may occur without the presence of glucocorticoids during initial stressful experiences, particularly when different stressors are imposed. Complexity in understanding mechanisms of adaptation may be attributed to variation in the physiological actions involved with responses to different stimuli. There is a possibility that the stress-induced increases in glucocorticoid during disruption of homeostasis are essential in preparing the body to respond to subsequent stressors. Several studies suggest the existence of glucocorticoid negative feedback regulation of the HPA axis (Stark et al., 1981; Kamstra et al., 1983; Sapolsky et al., 1984). De Souza and van Loon (1982) noted that corticosterone may reduce adrenal cortex sensitivity to adrenocorticotrophin hormone stimulation in rodents. Inhibitory feedback mechanisms, however, occur only when there is high level of plasma glucocorticoid. Thus, neonatal
or infantile stimulation which helps in response to subsequent stressors later in life may not be attributed to negative feedback regulation.

Acclimation through prior exposure to controlled increases in environmental temperatures (Reece et al., 1972; May et al., 1987; Arjona et al., 1988) has been associated with metabolic alterations (Hutchinson and Sykes, 1953). In Chapter IV, the possibility of acclimating chickens to high ambient temperatures by imposing mild feed restrictions during the neonatal stage was noted. This procedure may be a more practical husbandry procedure than exposure to thermal stressors. Because nonthermal initial stimulation was involved in the elicitation of heat tolerance in that experiment, there was the possibility that different physiological mechanism may have occurred. The purpose of the experiment reported here was to obtain insights into the mechanics of acclimation through neonatal fasting and concomitantly the role of glucocorticoids in the physiology of stress and habituation.

**MATERIALS AND METHODS**

*Genetic Stock, Husbandry, and Traits Measured*

White Plymouth Rock chickens from a line that had undergone long term selection (35 generations) for high 56-day body weight (Dunnittington and Siegel, 1985) were used in this experiment. At hatch (Day 0), chicks were wingbanded,
vaccinated for Marek's disease, and weighed to the nearest g. Chicks were randomly assigned to 14 floor pens with wood shavings as litter, with 22 individuals per pen. Room temperature was maintained by hot air brooding at $35\pm1^\circ C$ during the first week of life and then gradually reduced to $20\pm1^\circ C$ by Day 35. Chicks were fed a mash diet (24% CP; 3,146 kcal ME/kg) and water was available at all times. Lighting was continuous.

On Day 0, chicks were assigned to 1 of 7 feeding regimens with 2 pens per group. The feeding regimens were: (1) ad libitum feeding with untreated feed (AL), (2) 60% feed restriction on Days 4, 5, and 6 with untreated feed (E60), (3) ad libitum provision of metyrapone-treated feed on Day 3 and 60% feed restriction on Days 4, 5, and 6 with the provision of metyrapone-treated feed during the restriction period (E60M), (4) ad libitum provision of metyrapone-treated feed on Days 3, 4, 5, and 6 (EALM), (5) 60% feed restriction at 24, 25, and 26 days of age with untreated feed (L60), (6) ad libitum provision of metyrapone-treated feed on Day 23 and 60% feed restriction on Days 24, 25, and 26 with the provision of metyrapone-treated feed during the restriction period (L60M), and (7) ad libitum provision of metyrapone-treated feed on Days 23, 24, 25, and 26 (LALM). Feed restriction was 60% feed consumption of the ad libitum fed group on the previous day. Feed was treated with metyrapone (2-methyl-1, 2-di-3-pyridil-1-propanone; Lot # 121H2504,
Sigma Chemical Co., St Louis, MO), which inhibits the conversion of deoxycorticosterone to corticosterone (Dominguez and Samuels, 1963) at the rate of 1200 mg/kg. Prior to feeding, individual body weights (g) were obtained on Days 7, 14, 21, 28, 35, and 43.

The day following each feed restriction period, 6 chicks from each feeding regimen were chosen randomly and blood samples were obtained via the wing vein with EDTA as the anticoagulant. Blood smears were prepared using May-Grunwald-Giemsa stain and heterophils (H) and lymphocytes (L) were counted to a total of 60 cells (Gross and Siegel, 1983).

Commencing from Day 36, all chicks were exposed to ambient temperatures of 35±2°C for 7 days. The increase from 20±1°C to this temperature occurred over a 5-h period. The temperature in each pen during the heat exposure was monitored and maximum and minimum temperatures were recorded daily. Relative humidity was not controlled but measurements of it showed that it remained below 45%.

One day prior to heat exposure (Day 35), 6 chicks per feeding regimen were bled for H and L counts. Similar procedures were repeated 2 and 7 days after the onset of heat treatment (i.e., Days 38 and 43). On Day 37, 11 chicks from each feeding regimen were given 0.1 ml intravenous injection of 0.5% SRBC. On the same day, 14 chicks from each feeding regimen were inoculated intravenously with 0.1 ml of a 1% suspension of spleen extract from chickens infected with
marble spleen disease (MSD) virus (Domermuth and Gross, 1991). Different pens of chicks were assigned to receive either SRBC or MSD. Antibody production in response to SRBC antigen was measured 6 days postinjection and antibody titers were expressed as log₂ of reciprocal of the last dilution in which there was agglutination (Wegmann and Smithies, 1966). On Day 43, all chicks were weighed, killed by cervical dislocation and their sex was determined. Spleens of chicks that comprised MSD infected birds were removed and weighed (0.01 g).

Statistical Analyses

Data were subjected to analysis of variance with main effects and their interactions in a factorial arrangement in a fixed effect model. When interactions were significant, separate analysis were conducted within each main effect. When significant, separation of multiple means was by Duncan's multiple range test. Prior to analysis, body weight data were transformed to common logarithm and relative spleen weights to arc sine square roots. Untransformed means are presented in the tables and significance was considered as $P<0.05$. All analyses were conducted with aid of General Linear Models (GLM) procedure (SAS® Institute, 1982). Data for the E50, L60, and AL groups were also presented in Chapter IV where the experiment was conducted concurrently with this study.
RESULTS

Growth

There was no sexual dimorphism for body weight at hatch. From Day 7 onwards males were heavier than females (Table 1). Effect of early 60% feed restriction on body weight was evident by Day 7 with AL and EALM chicks being heavier than E60 and E60M chicks. Due to greater compensatory growth among E60 chicks following refeeding, they were heavier than E60M chicks on Days 14, 21, and 28, but not thereafter. Among E60 chicks compensation for weight gain lost during fasting was completed by Day 28, when they and AL chicks had similar body weights. In contrast, although body weight of E60M and EALM chicks were similar by Day 35, at that age AL chicks continued to be heavier than E60M chicks. Similar to the results observed during early fasting, the late 60% feed restriction (L60 and L60M) suppressed body weights at 28 days of age. Also the depressive effect of metyrapone on accelerated growth was again observed in comparisons between L60 and L60M chicks. One week following refeeding (Day 35), there was no difference in body weight among L60, AL, and LALM feeding regimens, whereas L60M chicks were lighter than AL chicks. By Day 43, body weights were similar for all feeding regimens.

Neonatal feed restriction, regardless the provision of metyrapone-treated feed, improved relative weight gain \{([BW_{43} - BW_{35}] / BW_{35}) \times 100\} from Days 35 to 43, the period
when chicks were exposed to high ambient temperatures (Figure 1). Similar findings were not observed when chicks were fasted at older ages. Relative weight gain during the heat treatment did not differ between the sexes.

**Heterophil to Lymphocyte (H/L) Ratios**

There were no interactions between age and feeding regimen for H/L ratios. The pooled H/L ratio the day after early imposition of various feeding regimes (Day 7) was lower (0.45) than that (0.69) on the day after chicks were subjected to similar treatment at older ages. The H/L ratio of 0.86 for chicks imposed with early and late 60% feed restriction, which was higher than that for fasted chicks provided metyrapone-treated feed (0.53) and those fed AL (0.50) (Figure 2). Regardless of age, the day following imposition of various feeding regimens (Days 7 and 27), the H/L ratio of chicks fed metyrapone-treated feed ad libitum was lower (0.35) than those from the other feeding regimens.

There was a significant feeding regimen by stage of heat treatment interaction. The interaction was caused by an effect of feeding regimen on H/L ratios during the second day of heat treatment (Day 38) but not during other stages (i.e., a day before, and 7 days after heat treatment) (Table 2). Except for E60 and EALM chicks, there was a dramatic elevation in H/L ratios after 2 days of exposure to high ambient temperatures.
Responses to Marble Spleen Disease Challenge and Sheep Red Blood Cell Antigen

There was sexual dimorphism in response to MSD inoculation, where females (0.21) had heavier relative spleen weights (g/100 g body weight) than males (0.17). Relative spleen weights of E60 chicks were less than those under other feeding regimens which did not differ (Figure 3, top). Although body weight on Day 43 was not affected by the challenge, chicks that were inoculated with MSD had greater % weight gains during the period of heat exposure than chicks that were not challenged (31 versus 27). Antibody response to SRBC antigen was lower for LALM than for AL, E60, L60, and L60M groups which were similar. Chicks from feeding regimens E60M and EALM did not differ in SRBC antibody levels from any of the other regimes (Figure 3, bottom).

DISCUSSION

The effect of early and late feed restriction on heat tolerance has been discussed elsewhere (Chapter IV), therefore findings regarding feeding of methyrapone and occurrence of acclimation will be emphasized here. Stimulatory effects of fasting chicken on the HPA axis has been well documented (e.g., Nir et al., 1975; Freeman et al. 1981). There is a question, however, about the physiological significance of elevated plasma corticosterone concentrations during starvation. The results of this study where E60 chicks had greater compensatory growth than those fed E60M, suggest
the importance of corticosterone in promoting accelerated growth during refeeding. Reasons for this assumption may be attributable to modifications in metabolic and feeding behavior. Bouillon and Berdanier (1981) reported that elicitation of gluconeogenesis by corticosterone during starvation may enhance gluconeogenic rate during refeeding. Corticosterone-induced hyperphagia may also facilitate accelerated growth during refeeding (Bouillon and Berdanier, 1981).

Stress due to fasting may not be manifested physiologically when feed restricted chicks are provided with metyrapone. Wilson and Cunningham (1980) reported that metyrapone did not reduce plasma corticosterone level of unstressed chickens. In contrast, we noted that regardless of age, chicks that ate metyrapone-treated feed ad libitum had lower H/L ratios (on Days 7 and 27) than chicks under feeding regimens without metyrapone. Sensitivity of the index utilized to measure the degree of stress response may be responsible for the discrepancies in results. Heterophil to lymphocyte ratio is considered a more reliable indicator of the perceived magnitude of stressors than plasma corticosterone values in avian species (Gross and Siegel, 1983). A lack of heat-induced increase in H/L ratios among EALM chicks was indeed surprising. Gross (1990) indicated that the inhibitory effect of metyrapone on adrenal steroidogenesis may be short-term, which is in accord with
the observation that LALM chicks had a marked elevation in H/L ratios 2 days after heat treatment. It appears, however, that feeding of metyrapone-treated feed ad libitum during the neonatal stage may have caused persistent modifications in the adrenocortical function.

Effects of extreme temperatures on the immune system have been well documented (see Thaxton, 1978; Siegel, 1987). In the present study, however, the significant impact of feeding regimen on antibody response during heat exposure was equivocal and inconclusive.

Since Levine's (see Sapolsky, 1992b) revolutionary finding concerning the effect of infantile experiences on adult HPA axis function in rats, the phenomenon has received considerable attention from physiologists and psychobiologists. In the context of livestock production, these findings provide insights into efforts for counteracting problems of heat prostration. Prior exposure to acute thermal stressors appears to improve heat tolerance in chickens (Reece et al., 1972; May et al., 1987; Arjona et al., 1988). Also in Chapter IV, it was noted that chicks fasted early in life were able to withstand high ambient temperatures during the juvenile stage.

Despite these promising findings, mechanics of acclimation or adaptation remain an enigma. The data presented here indicate that, although E60 and E60M chicks were feed restricted on similar basis and during the same
age, inhibition of adrenal steroidogenesis due to metyrapone feeding in the latter, decreased ability to tolerate high ambient temperatures among E60M chicks. Although stress may increase H/L ratios (see Maxwell, 1993) and susceptibility to viral infections (see Gross, 1983; Gross and Siegel, 1993), E60 chicks had negligible leukocytic alteration and superior resistance to MSD challenge during heat exposure. As for relative weight gain during heat exposure, it seems unlikely that the similar values for E60M and E60 chicks were due to acclimation in the former. Because compensation for weight gain lost during fasting was not completed prior to heat treatment, E60M chicks were still experiencing accelerated growth at that time, thus relative weight gain of E60 and E60M chicks did not differ.

These findings suggest that transient perturbations of homeostasis during the neonatal stage, without concurrent increases in the synthesis and liberation of corticosterone, may not help an animal in responding to subsequent stressors. What corticosterone-elicited physiological modifications have occurred following neonatal stimulation? In discussing Levine's pioneering work, Sapolsky (1992b) postulated that stresses early in life may increase the concentration of hippocampal corticosteroid receptors which enhanced negative feed back sensitivity, and decreased numbers of corticosterone binding globulin-like receptors in the pituitary. He, however, hypothesized that these alterations
were not glucocorticoid related. In contrast, the results of this experiment show markedly elevated H/L ratios by feed restriction early in life, indicating stimulation of the HPA axis. Thus, the data presented here suggest that corticosterone plays a role in inducing persistent alterations of the adrenocortical function and habituation simultaneously. It is uncertain whether Sapolsky's (1992b) thesis on physiological changes due to neonatal stimulation are applicable here.

Munck et al. (1984) and Munck and Guyre (1986), in their review, rejected the thesis that increased levels of glucocorticoid during stress have been associated with enhanced defense against the source of the stress itself. They postulated that rise in glucocorticoid levels during a stressful situation is to curtail the body's normal reaction to stressors from overshooting, which themselves may be detrimental to health and well-being. In reviewing both views, Sapolsky (1992b) indicated that each may have validity. In addition to these earlier theories, findings reported here suggest that stress-induced elevation in adrenal corticoid concentration when an animal is faced with a stressor is primarily to prepare the body for responding to subsequent disruptions of homeostasis.
Table 1. Mean body weight (g) by sex and feeding regimen\(^1\) at various ages

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
<th>35</th>
<th>43</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>88(^a)</td>
<td>210(^a)</td>
<td>381(^a)</td>
<td>569(^a)</td>
<td>851(^a)</td>
<td>1094(^a)</td>
</tr>
<tr>
<td>Female</td>
<td>85(^b)</td>
<td>195(^b)</td>
<td>336(^b)</td>
<td>494(^b)</td>
<td>707(^b)</td>
<td>905(^b)</td>
</tr>
<tr>
<td>Feeding regimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum (AL)</td>
<td>85(^a)</td>
<td>210(^a)</td>
<td>370(^a)</td>
<td>568(^a)</td>
<td>812(^a)</td>
<td>1031(^a)</td>
</tr>
<tr>
<td>Early 60% of AL (E60)</td>
<td>69(^b)</td>
<td>193(^b)</td>
<td>344(^b)</td>
<td>542(^a)</td>
<td>775(^{abc})</td>
<td>1010(^a)</td>
</tr>
<tr>
<td>Early 60% of AL + metyrapone (E60M)</td>
<td>70(^b)</td>
<td>184(^c)</td>
<td>322(^c)</td>
<td>516(^b)</td>
<td>745(^c)</td>
<td>972(^a)</td>
</tr>
<tr>
<td>Early AL + metyrapone (EALM)</td>
<td>92(^a)</td>
<td>205(^a)</td>
<td>367(^a)</td>
<td>546(^a)</td>
<td>776(^{abc})</td>
<td>991(^a)</td>
</tr>
<tr>
<td>Late 60% of AL (L60)</td>
<td>494(^b)</td>
<td>792(^{ab})</td>
<td>1009(^p)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late 60% of AL + metyrapone (L60M)</td>
<td>498(^b)</td>
<td>769(^{bc})</td>
<td>987(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late AL + metyrapone (LALM)</td>
<td>562(^a)</td>
<td>783(^{ab})</td>
<td>992(^a)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pooled SEM

0.6 | 1.2 | 2.4 | 2.9 | 4.2 | 4.9

\(^{a,b,c}\) Means within a column-subgroup with no common letters differ significantly (P ≤0.05).

\(^1\) All chicks were fed *ad libitum* from hatch to Day 3. On Days 4, 5, and 6 some pens of chicks were subjected to E60, E60M, and EALM. On Days 24, 25, and 26 different pens of chicks were subjected to L60, L60M, and LALM.
Table 2. Mean heterophil to lymphocyte ratios where feeding regimen\(^1\) by stage of heat treatment interactions were significant.

<table>
<thead>
<tr>
<th>Feeding regimen</th>
<th>Prior to heat</th>
<th>2 days heat</th>
<th>7 days heat</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ad libitum (AL)</em></td>
<td>0.39(^a)</td>
<td>1.04(^a)</td>
<td>0.95(^a)</td>
</tr>
<tr>
<td>Early 60% of AL (E60)</td>
<td>0.42(^b)</td>
<td>0.44(^b)</td>
<td>0.59(^b)</td>
</tr>
<tr>
<td>Early 60% of AL + metyrapone (E60M)</td>
<td>0.45(^a)</td>
<td>1.04(^a)</td>
<td>1.06(^a)</td>
</tr>
<tr>
<td>Early AL + metyrapone (EALM)</td>
<td>0.39(^a)</td>
<td>0.44(^b)</td>
<td>0.60(^b)</td>
</tr>
<tr>
<td>Late 60% of AL (L60)</td>
<td>0.51(^a)</td>
<td>1.24(^a)</td>
<td>0.90(^a)</td>
</tr>
<tr>
<td>Late 60% of AL + metyrapone (L60M)</td>
<td>0.34(^a)</td>
<td>1.60(^a)</td>
<td>0.90(^a)</td>
</tr>
<tr>
<td>Late AL + metyrapone (LALM)</td>
<td>0.42(^a)</td>
<td>1.08(^a)</td>
<td>0.68(^a)</td>
</tr>
</tbody>
</table>

\(^a,b\) Means within a column with no common letters differ significantly \((P \leq 0.05)\).

\(^x,y\) Means within a row with no common letters differ significantly \((P \leq 0.05)\).

\(^1\) All chicks were fed *ad libitum* from hatch to Day 3. On Days 4, 5, and 6 some pens of chicks were subjected to E60, E60M, and EALM. On Days 24, 25, and 26 different pens of chicks were subjected to L60, L60M, and LALM.

Pooled SEM = 0.029.
Figure 1. Mean relative weight gain \( \text{[(BW43 - BW35)/BW35] x 100} \) during the heat treatment. a, b: Means with no common letters differ significantly \((P \leq 0.05)\). Pooled SEM = 0.003.
Figure 2. Pooled mean heterophil to lymphocyte ratios where early and late imposition of feeding regimens were combined (Days 7 and 27). a, b, c: Means with no common letters differ significantly ($P < 0.05$). Pooled SEM = 0.018.
Figure 3. Mean relative spleen weights (g/100 g body weight) of marble spleen disease infected chicks (top) and mean antibody responses to SRBC antigen (bottom) on Day 43 (6 days postinoculation). a,b: Means with no common letters differ significantly ($P \leq 0.05$). Pooled SEM = 0.007 (spleen); 0.160 (SRBC).
CHAPTER VI

INHIBITION OF ADRENAL STEROIDOGENESIS, NEONATAL FEED RESTRICTION AND PITUITARY-ADRENAL AXIS RESPONSE TO SUBSEQUENT FASTING IN CHICKENS
SUMMARY

White Plymouth Rock chickens placed under 60% feed restriction or *ad libitum* feeding, with or without metyrapone treatment, from 4 to 6 days of age were subjected to either 8 or 24 h feed deprivation at 36 days of age. Chicks subjected to the neonatal 60% feed restriction (60R) but not those provided metyrapone during the procedure (60M) had elevated heterophil to lymphocyte (H/L) ratios. However, there was no difference in plasma corticosterone and ACTH responses between 60R and 60M chicks. Except for increases in H/L and plasma corticosterone concentrations among *ad libitum* fed (AL) and 60M chickens, respectively, there was no indication of stress response attributable to the 8 h fast. Feed withdrawal for 24 h did not cause rises in H/L ratios and plasma levels of corticosterone of chicks that had been subjected to early 60% feed restriction with non-metyrapone-treated feed. In contrast, chicks of other regimens had elevated H/L and plasma corticosterone responses when exposed to a similar procedures. Except for those fed *ad libitum* during the neonatal stage, circulating levels of ACTH declined following the 24 h fast.

INTRODUCTION

Stressful experiences early in life may have long-term benefits in improving resistance to other forms of environmental insults (see Gross, 1983; Sapolsky, 1992b). One
of the earliest studies on effects of prior experiences on adrenocortical functions was by Levine (1962). He demonstrated that infantile stimulation through handling, which was stressful, evoked long lasting alterations of the hypothalamic-pituitary-adrenal (HPA) axis in rodents. Handled rats, as adults, had lower circulating corticosterone concentrations than those that were not handled both basally and during recovery after withdrawal of stressors.

Additional support for the phenomenon reported by Levine (1962) was obtained in the study conducted for this dissertation. Chicks fasted early in life were better able to withstand high ambient temperatures as juveniles than those that were not fasted or were fed restricted at a later age as indicated by lower heterophil to lymphocyte (H/L) ratios, improved resistance to adenoviral infection and weight gain (Chapters IV and V). Neonatal feed limitation, however, did not enhance heat tolerance when adrenal steroidogenesis was chemically blocked during the restriction period (Chapter V). Hence, these findings suggest that transient perturbations of homeostasis during the neonatal stage, without concurrent rises in the synthesis and liberation of glucocorticoids, may not aid an animal in responding to subsequent stressors. The mechanism responsible for the phenomenon, however, is unknown. The purpose of the research reported in this chapter was to evaluate and define further the effect of neonatal feed restriction and inhibition of adrenal steroidogenesis on
anterior pituitary and adrenocortical responses to subsequent fasting.

**MATERIALS AND METHODS**

**Genetic Stock, Husbandry, and Traits Measured**

One hundred and sixty-eight newly hatched straight-run chicks (Day 0) from a line selected 37 generations for high juvenile body weight (Dunnington and Siegel, 1985) were wingbanded, vaccinated for Marek’s disease, and randomly assigned in groups of seven to 24 pens in a starter battery with wire floors. Chicks were reared under constant light with feed (24% CP; 3146 kcal ME/kg) in mash form and water available ad libitum. There were four feeding regimens with six replicate pens per regimen. Pen assignments were at random within the battery. Feeding regimens were (1) ad libitum feeding with untreated feed (AL), (2) ad libitum provision of metyrapone-treated feed on Days 3, 4, 5, and 6, (ALM), (3) 60% feed restriction on Days 4, 5, and 6 with untreated feed (60R), (4) ad libitum provision of metyrapone-treated feed on Day 3, and 60% feed restriction on Days 4, 5, and 6 with the provision of metyrapone-treated feed during the restriction period (60M). The 60% restriction was based upon feed intake of the ad libitum feeding regimen from previous studies (Chapters IV and V). Feed was treated by adding metyrapone (2-methyl-1, 2-di-3-pyridil-1-propanone; Lot # 121h2504, Sigma Chemical Co., St Louis, Missouri),
which inhibits the conversion of deoxycorticosterone to corticosterone (Domínguez and Samuels, 1963), to untreated feed at the rate of 1200 mg/kg. The day following the imposition of various feeding regimens, two chicks from each pen were chosen at random and blood samples (3 ml) were obtained via decapitation for differential leucocyte counts (EDTA, anticoagulant), and plasma ACTH and corticosterone concentrations assays (heparin, anticoagulant). Each chick was caught and sampled, one immediately after another, in a separate room. Time elapsed from catching to obtain the blood sample was less than 50 s. All sampling was between 0800 to 0900 h. This procedure should not influence circulating corticosterone levels (Craig and Craig, 1985; Lagadic et al., 1990). Blood smears were prepared using May–Grunwald–Giemsa stain, and H and L were counted to a total of 60 cells (Gross and Siegel, 1983). Blood samples for hormone assays were centrifuged and plasma was separated, frozen and maintained at -70°C until shipped (packed in dry ice) to Pennsylvania State University for assays. All H and L counts, and hormone assays were done blind.

On Day 20, each pen of chicks was transferred as a group from starter to developer batteries with wire floors. On Day 36, chicks were assigned to one of four groups as follows; (1) 8 h of feed deprivation; (2) ad libitum feeding for a control to the 8 h fast (3) 24 h of feed deprivation; (4) ad libitum feeding for a control to the 24 h fast. Each control
and fasted group consisted of one pen and two pens of chicks, respectively, from each of the earlier feeding regimens (AL, ALM, 60R, and 60M). Following each duration of feed withdrawal, blood was obtained from each chick for H and L counts, and plasma ACTH and corticosterone assays. The bleeding procedure was the same as described previously except blood sampling was from the wing vein, and conducted between 1200 and 1400 h.

Plasma hormones were assayed in duplicate by radioimmune assay (RIA) using commercial kits. Corticosterone was determined by a $^{125}$I RIA (ICN Biochemicals, Inc., Costa Mesa, CA 92626). ACTH was determined by a $^{125}$I heterologous assay for mammalian ACTH (Diagnostic Products Corp., Los Angeles, CA 90045) as modified by Carsia et al. (1988) for avian plasma. Both assays were performed in single runs, thus no interassay coefficients of variability (CV) were calculated; intra-assay CV's were 2.38% and 2.87% for corticosterone and ACTH, respectively. Neither assay required extraction of plasma.

Statistical Analyses

Data were subjected to analysis of variance. For the earlier feeding regimens (AL, ALM, 60R, and 60M), the statistical model was:

$$Y_{ij} = \mu + FR_i + e_{ij}$$
where \( i = 1, 2, 3, 4 \) feeding regimens, and \( j = 1, 2, \ldots, n \) individuals. Data for traits measured at 36 days of age were analyzed separately for 8 and 24 h feed withdrawal using the model:

\[
Y_{ijk} = \mu + FR_i + FW_j + (FRFW)_{ij} + e_{ijk}
\]

where \( i = 1, 2, 3, 4 \) feeding regimens, \( j = 1, 2 \) feed withdrawal (fasted or non-fasted), and \( k = 1, 2, \ldots, n \) individuals. When interactions were significant, separate analyses were conducted within each main effect. Prior to analyses, plasma ACTH and corticosterone levels were transformed to square roots. Untransformed means are presented in Figures. When effects were significant \((P<0.05)\), comparisons were made among multiple means by Duncan's multiple range test. All analyses were conducted with the aid of General Linear Models (GLM) procedure (SAS® Institute, 1982).

**RESULTS**

The effects of various feeding regimens on H/L ratios, and plasma corticosterone and ACTH concentrations during the neonatal stage are shown in Figure 1. Feed restriction of 60% of ad libitum elevated H/L ratios above those of AL, ALM, and 60M regimens. In contrast, there was no difference in plasma corticosterone response between 60R and the other regimes.
Chicks fed ALM had lower plasma levels of corticosterone than 60M chicks. There was no influence of feeding regimen on plasma concentrations of ACTH.

Presented in Figure 2 are H/L ratios, and plasma corticosterone and ACTH concentrations for controls and chicks that underwent 8 h of feed withdrawal at 36 days of age. There were significant earlier feeding regimen by feed withdrawal interactions for these three criteria. The interaction for H/L ratios was caused by feed deprivation having an effect on AL but not the other early regimens. Except for 60M chickens, the 8 h fast did not affect plasma corticosterone levels, thus resulting in the interaction for this trait. The interaction for plasma concentrations of ACTH, was attributable to greater ACTH response among 60M chicks when deprived of feed for 8 h, and no differences between fasted and control groups in other feeding groups.

Interactions of 24 h fast and no feed deprivation and earlier feeding regimen interactions were also present for these traits (Figure 3). Following feed deprivation, the H/L ratios and plasma corticosterone levels of AL, ALM, and 60M chicks were elevated. However, there was no evidence of H/L or plasma corticosterone response among 60R chicks when fasted. This pattern reflected the significant interactions for these measurements. The interaction for ACTH levels in plasma was significant because among AL but not other chickens, the 24 h fast had no influence.
DISCUSSION

Results of this experiment are consistent with previous findings (Chapters IV and V) concerning neonatal stimulation and adrenocortical functions. Significant feeding regimen by feed deprivation interactions for the three measurement criteria suggest that prior experiences influence adrenocortical response to subsequent feed withdrawal. Following withdrawal of feed for 8 and 24 h, H/L ratios and circulating corticosterone levels of 60R chicks were similar to those fed ad libitum and suggest adaptation to fasting. In contrast, as measured by H/L ratios, the 8 h fast was stressful to those fed AL. Within 24 h of feed deprivation, neither H/L nor plasma corticosterone measurements revealed any evidence of habituation to the procedure among AL chickens. Thus, it is evident that establishment of the HPA axis may be manipulated and permanently modified by a mild feed restriction early in life.

Data presented here also affirmed the hypothesis that elevated circulating corticosterone levels during stress may play a modulatory role in neonatally-induced habituation. Although 60R and 60M chicks were subjected to the same limitation of feed during the neonatal stage, suppression of adrenal steroids synthesis and release by metyrapone feeding of 60M chicks reduced their ability to cope with subsequent fasting. Thus, glucocorticoids appeared essential in simultaneously triggering persistent alterations of the HPA
axis and habituation. Based on these results and earlier ones (Chapter V), it can be proposed that increases in corticosteroid synthesis and liberation when an animal is faced with a threatening stimulus may have considerable importance for preparing the biological system in responding to subsequent disruption of homeostasis.

Corticosteroid-mediated inhibitory feedback regulation of the HPA axis which down-regulates ACTH responses is well documented in mammalian species (e.g., Jones et al., 1972; Buckingham and Hodges, 1974; Stark et al., 1981; Kamstra et al., 1983; Vale et al., 1983; Sapolsky et al., 1984). Consistent with other avian studies (see Frankel, 1970), a similar feedback mechanism was observed in the present study. Regardless of feeding regimen, higher ACTH responses to fasting were evident at 8 compared to 24 h of feed withdrawal. Preceding endogenous ACTH stimulation, plasma corticosterone concentrations continued to rise with progressively longer durations of fasting, and may have blunted subsequent ACTH response during the 24 h feed withdrawal. The higher circulating ACTH levels attained by controls may indicate a corticosterone-modulated negative feedback mechanism.

It is generally accepted that long lasting changes in the response to a stimulus can be evoked by stresses early in life. Lacking, however, is information concerning the mechanism(s) underlying this phenomenon in avian species.
Evidence from several sources (see Sapolsky, 1992b) suggests infantile stimulation of rats increases corticosteroid receptor number in the hippocampus and decreases corticosteroid binding globulin-like receptor numbers in the pituitary that are crucial for the inhibitory feedback loop of the HPA axis. The question is whether these alterations are relevant to avian species. If the answer is yes, then 60R chickens should have reduced circulating ACTH concentrations when subjected to subsequent fasting. Data in this study, however, do not support this notion because ACTH response of 60R chicks to subsequent feed deprivation was similar to those fed other regimens. Hence, neonatally-induced habituation in this study did not appear to be secondary to superior inhibitory feedback regulation. Perhaps stress-elicited rises in circulating corticosteroids following feed restriction early in life blunted adrenocortical cell responses to endogenous ACTH. The potent adrenocortical desensitization effect of corticosteroid has been reported in mammals (De Souza and van Loon, 1982).

Studies on neurohumoral control of the avian adrenal raise a caveat to the prior hypothetical physiological paradigm. Although, the typical HPA axis pathway has generally been accepted within the realm of mammalian physiology, regulation of the avian pituitary and adrenocortical tissues appears to be more intricate. Miller and Riddle (1942) and Frankel et al. (1967) reported
functional adrenal glands in adenohypophysectomized pigeons and cockerels, respectively. In his review, Frankel (1970) presented a body of evidence suggesting that there were areas of the brain that produced ACTH or an ACTH-like substance. More recent findings (see Marsh and Scanes, 1994; Siegel, 1994) suggest that avian leucocytes (lymphocytes and macrophages) can synthesize and liberate ACTH or ACTH-like products. It might be argued that although neonatal stimulation of chicks increased hippocampal concentrations of corticosteroid receptors that heightened a negative feedback mechanism, thus reducing ACTH output from the anterior pituitary, these regulations may have been obscured by ACTH of extra-hypophyseal origin.

While the contention concerning the physiological alteration involved in the phenomenon remains unresolved, the persistent impact of stress early in life on the HPA axis establishment is similar for avian and mammalian species. Earlier (Chapter V) and findings in this experiment suggest that mechanism(s) underlying the phenomenon appear to be potentiated by corticosteroids.
Figure 1. Mean heterophil to lymphocyte ratios (top), plasma corticosterone (ng/ml) (middle) and ACTH (pg/ml) (bottom) on Day 7 posthatch. a,b: Means with no common letters differ significantly ($P \leq 0.05$). Pooled SEM = 0.04 (H/L ratio); 0.66 (corticosterone); 22.85 (ACTH).
Figure 2. Mean heterophil to lymphocyte ratios (top), plasma corticosterone (ng/ml) (middle) and ACTH (pg/ml) (bottom) when interactions were significant for fasting [8 h fast (solid line) and control (dashed line)] and early feeding regimen (AL, ALM, 60R, and 60M). a,b: Means within a single line with no common letters differ significantly (P<0.05).

*Difference between fasted and control groups P<0.05. Pooled SEM = 0.02 (H/L ratio); 0.17 (corticosterone); 13.55 (ACTH).
Figure 3. Mean heterophil to lymphocyte ratios (top), plasma corticosterone (ng/ml) (middle) and ACTH (pg/ml) (bottom) when interactions were significant for fasting (24 h fast (solid line) and control (dashed line)) and early feeding regimen (AL, ALM, 60R, and 60M). a, b: Means within a single line with no common letters differ significantly (P≤0.05). *Difference between fasted and control groups P≤0.05. Pooled SEM = 0.03 (H/L ratio); 0.17 (corticosterone); 8.01 (ACTH).
GENERAL SYNTHESIS

The "General Adaptation Syndrome" (Selye, 1937), an influential theory in biology and medical science, continues to draw considerable attention from physiologists and psychobiologists. Although the theory opened promising frontiers in human medicine, and animals in their natural state, its implications in the context of veterinary science and animal agriculture have been a major interest. These implications are particularly true for modern production systems where intense selection for economic traits in concert with increased mechanization and changes in husbandry may result in a stress response. Growing concern regarding animal welfare is partly responsible for the resurging interest to reassess human-animal relationships.

Although the literature on stress is voluminous, understanding of the stress syndrome remains elusive and suffers from confusion and controversy. Complexity in understanding "stress" may be partly attributed to variation in the physiological and psychological reactions involved with different stimuli. The experimental paradigm in this dissertation explored physiological, pathological and immunological aspects of stress, and the nature of adaptation in dwarf and nondwarf chickens.

Evaluation of heterophil to lymphocyte (H/L) ratios during perinatal and neonatal stages in Chapter I is of interest for two reasons. First, embryonic development is
intricate and perinatal stages, particularly at the time of air cell penetration and initiation of pulmonary respiration may be stressful. Second, studies in mammals (Holt and Oliver, 1968; Alexander et al., 1968) indicated that adrenal steroids rose gradually during gestation and reached highest concentration at the time of parturition. Thus, considerable leucocytic alteration might be expected to occur at the time of hatching and first week post-hatch. Despite noting heterophilia and lymphopenia in both stages, it was unclear whether these findings could be attributed to stress response, normal hematological event, or interaction of both factors. Thus, the findings of this study suggest that even though H/L ratios may be a reliable physiological indicator of stress response in avian species (see Gross and Siegel, 1993; Maxwell, 1993), their values as such a criterion during perinatal and neonatal stages need further evaluation. Design for future studies should include comparison of H/L ratios in chicks that were either exposed to controlled stressors, or were inhibited to synthesize glucocorticoids during perinatal and early neonatal stages.

Feed restriction is crucial in controlling obesity among meat-type chickens that have undergone intense selection for rapid growth. Results in Chapter II confirm previous studies (e.g., Freeman et al., 1981; Harvey et al., 1983; Gross and Siegel, 1986) that the procedure elicited the HPA axis. In the present study, as measured by H/L ratios, habituation to
a 60% feed restriction was evident by 12 to 16 days after initiation. Genetic variation and prior experience are but a few of the important factors in determining the period required for adaptation to occur. Relaxing the severity of fasting resulted in transient increases in H/L ratios because the chicks may have successfully adjusted to that procedure. Thus, under practical situation, particularly during severe feed deprivation as during flock recycling, gradual replenishment should reduce the stress response. Resistance to *E. tenella* was greater in normal than dwarf chicks and greater in those that were fasted than those fed *ad libitum*. These findings suggest that although feed withdrawal deprived physiological and behavioral needs and concomitant disruption of homeostasis, there can be long term benefits in enhancing resistance to other forms of environmental insults. This research may also have some implication on the reliability of "preference test", as assessment of animal well-being (see Duncan, 1981; Curtis, 1985). Given the choice to gain *ad libitum* access to feed, meat-type chickens will engorge themselves to near gut capacity (McCarthy and Siegel, 1983) which leads to obesity and eventually their livability will be negatively affected. Hence, it can be concluded that an animal has the tendency to make short-term choices which may be costly to its long-term well-being.

While physiological responses to feed withdrawal have received considerable interest from researchers, there is a
dearth of information regarding psychogenic factors in fasting-induced stress responses. Frustration evoked by thwarting of feeding resulted dramatic elevation in H/L ratios (Chapter III). In the poultry industry, where feed restriction has become a routine husbandry procedure during forced molting in laying hens, and to attenuate obesity in broiler breeders, frustration is likely to occur. To avoid overcrowding of chickens at the feeders during refeeding, availability of feeding space must be assured. Emotional arousal could be induced if during presentation of feed, a chicken failed to eat simultaneously with other members of the flock due to insufficient feeding space. This situation will be further exacerbated by social facilitation and allelomimetic feeding behavior.

Mason (1975) revolutionized Selye's theory on "stress" by postulating that nonspecific aspects of the stress response are produced by a common reaction to emotional arousal, which accompanies all physical stressors. Therefore, minimizing the psychological aspects of physical stimuli may attenuate the deleterious physiological responses. The impact of Mason's thesis on well-being was reflected in Chapter III, where fasting-induced stress response was diminished by providing nutritionally inert sand, which may have altered the accompanying emotional arousal. Thus, it is a challenge to agriculturists and scientists to develop practical strategies for combating the undesirable psychogenic
components of physical stressors and ultimately to improve the well-being of domestic animals.

There is substantial evidence (Sykes and Fataftah, 1986a;b; May et al., 1987; Arjona et al., 1988) to indicate that controlled thermal stimulation in advance of subsequent exposure to heat improved tolerance. This procedure, however, may be considered as unlikely under practical situations, particularly in open-sided houses where manipulation of ambient temperatures is difficult. As indicated by H/L ratios, relative growth rate, and resistance to marble spleen disease, mild feed restriction during the neonatal stage but not at three weeks of age enhanced ability of nondwarf chicks to withstand high ambient temperatures (Chapter IV). Although the \textit{dw} allele improved heat-tolerance in White Leghorns (Mather and Ahmad, 1971; Horst and Petersen, 1977; Khan et al., 1987), such superiority in growth-selected White Plymouth Rocks was not observed in the present study. This inconsistency suggests that response to extreme temperatures associated with the \textit{dw} gene, as in disease resistance and immunocompetence (see Decuypere et al., 1991) may be affected by background genome.

Though the impact of infantile experiences on adult HPA axis function was established more than two decades ago (see Sapolsky, 1992b), the physiological actions involved remain uncertain. Findings presented in Chapters V and VI provided some elucidation to the phenomenon and concomitantly
suggested an additional theory regarding the role of glucocorticoids in stress response. Feed restriction early in life did not induce adaptation to either thermal or nutritional insults in nondwarf chicks when adrenal steroidogenesis was blocked during the procedure. Thus, glucocorticoids play a role in evoking alterations of the HPA axis and adaptation. From the results obtained in these experiments, the thesis can be proposed that elevation of glucocorticoid concentrations during stress response may be crucial in preparing the biological system for responding to subsequent perturbation of homeostasis. This experiment was, to my knowledge, the first to show that stress early in life without concurrent rises in circulating corticosteroid levels, may not aid an animal in coping with subsequent stressors.

The findings presented in Chapters V and VI have implications not only to avian biology but probably to human medicine. It is possible that these experiments, which suggest the importance of stress-elicited rises in glucocorticoid synthesis in preparing the biological system to respond to subsequent stressors, may have opened up new avenues in the pathophysiology of adrenocortical insufficiency or Addison's disease in humans (Frawley, 1967; Vinson et al., 1992)

The nature of adaptation, however, is far from clear. What are the psychoneuroendocrine mechanisms triggered by
glucocorticoids in eliciting habituation? Can adaptation be induced by exogenous administration of adrenal steroids early in life without actual activation of the HPA axis? Why do stresses that occur later in life have no persistent impact on adult adrenocortical function? What role, if any, does the sympathetic-adrenomedullary system play in eliciting habituation? These are but only a small fraction of the many intriguing questions need to be answered before we can understand, and learn to cope with and manage stress efficiently.
LITERATURE CITED


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