ACUTE STRESS EFFECTS ON PIG PERFORMANCE, IMMUNITY, AND BEHAVIOR

by

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ABSTRACT

Stress activates the hypothalamic-pituitary-adrenal axis (HPA). Corticotropin-releasing hormone (CRH) is the primary initiator of the stress response. The hypothalamus releases CRH which in turn stimulates the anterior pituitary to secrete adrenocorticotropin hormone (ACTH) and other peptides. Increased ACTH levels stimulate the adrenal cortex to release glucocorticoids. Stress not only effects the neuroendocrine and endocrine systems, but also alters behavior, immunity and overall physiology. The objectives of these studies were to (1) determine if physiological concentrations of various hormones had an effect on *in vitro* NK cytotoxicity in pigs, and (2) to determine the effects of three common stressors (heat, cold and shipping) on pig performance, physiology and behavior. In experiment 1, peripheral blood was collected from 59 pigs to determine the effects of physiological concentrations of stress hormones on porcine natural killer cell (NK) activity. A standard cytotoxicity assay was utilized with peripheral blood mononuclear cells and ^{51}Cr-labeled K-562 target cells. Hormones and concentrations were: porcine corticotropin releasing hormone (pCRH) at 0, 0.47, 4.7, 47 and 467 ng/mL; β-endorphin at 0, 0.001, 0.01, 0.1, 1, 10 and 100 ng/mL, cortisol at 0, 3.6, 36 and 360 ng/mL, adrenocorticotrophic hormone (ACTH) at 0, 0.045, 0.45, 4.5 and 45 ng/mL, epinephrine at 0, 0.022, 0.22, 2.2 and 21.9 ng/mL and norepinephrine at 0, 0.021, 0.21, 2.1, 20.6 ng/mL. Over the range of doses evaluated, pCRH, ACTH, cortisol and norepinephrine had no significant effect on porcine NK activity. β-endorphin and epinephrine had significant ($P < .01$) effects on porcine NK activity. All doses of β-

endorphin suppressed ($P < .05$) porcine NK activity. Porcine NK activity was greater ($P < .05$) when incubated with 0.22 ng/mL epinephrine than with 0.022 or 21.9 ng/mL epinephrine. These results suggest that physiological concentrations of CRH, ACTH, cortisol, and norepinephrine alone do not modulate pig NK activity. β-endorphin seems to be a potent suppressor of porcine NK activity. Epinephrine influenced pig NK activity in a dose-sensitive manner. In the second study, 132 weanling pigs were used to investigate the effects of three common stressors (and a control) and differing social status on behavior, immunity, plasma cortisol and blood chemical measures. Eleven blocks of 12 pigs each were evaluated. Each block contained four pens, established at weaning, and examined for outcome of agonistic interactions. Pigs were labeled either socially Dominant (DOM), Intermediate (INT) or Submissive (SUB). Two weeks later, random pens of pigs experienced either a control treatment (CON) or for 4 hours they were either shipped (SHIP), heat stressed (HEAT) with overhead heat lamps in their home pens, or cold stressed (COLD) by direct application of water and an air current. A battery of physiological and behavioral measures were collected. Treatments did not influence body weights, however, percentage weight change during SHIP was greater ($P < .001$) than for other treatments (.23, -2.9, .72, .91% ± .96 for CON, SHIP, COLD, HEAT, respectively). Serum glucose was elevated ($P = .02$) for SHIP pig compared with other treatments. Serum cortisol was decreased ($P = .03$) for HEAT compared with COLD and SHIP pigs. Social status influenced ($P < .01$) body weights at weaning and throughout the study with body weights heaviest for DOM pigs than INT and SUB pigs. Social status also influenced serum globulin, albumin/globulin (A/G) ratio, and cortisol concentrations. Globulin

decreased, while A/G increased in DOM pigs. SUB pigs showed an increase in cortisol compared to DOM and INT pigs. An interaction between social status and treatment occurred for NK cytotoxicity at 50:1 E:T (P = .06). Socially DOM and INT pigs had higher NK cytotoxicity than SUB, COLD pigs. An interaction was also observed between social status and stress treatment for lymphocyte blastogenesis under Phytohemagglutinin mitogen. Among HEAT pens, the INT pigs had lower lymphocyte proliferation than SUB pigs, but were not different from the DOM pigs. Among SHIP pens, the INT pigs had higher lymphocyte proliferation than either the DOM or SUB pigs. In conclusion, social status had large effects on plasma cortisol, globulin, A/G ratio, body weight and weight changes, although only acute shipping stress resulted in weight loss. Many immune and blood measures were not changed among acutely stressed pigs, however, the relationship between social status and LTA and NK cytotoxicity was disrupted during acute stress. Pig behavior was largely influenced across all stress treatments. COLD pigs had greater overall activity, including, feeding, standing and attacking and they had less lying behavior. The HEAT and SHIP pigs had depressed activity levels and spent more time lying. During acute stress, behavioral symptoms appears to be the most accurate and reliable indicator of stress.

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ABBREVIATIONS AND ACRONYMS

CRH	= Corticotropin releasing hormone
ACTH	= Adrenocorticotropin hormone
POMC	=Proopiomelanocortin
PVN	=Paraventricular nucleus
GC	=Glucocorticoids
PSS	=Porcine stress syndrome
APR	=Acute phase response
APP	=Acute phase proteins
AGP	=Alpha$_1$-acid glycoprotein
CBG	= Corticosteroid binding globulin
LTA	= Lymphocyte transformation assay
WBC	= White blood cell
IgG	= Immunoglobulin G
A/G	= Albumin/Globulin ratio
SRBC	= Sheep red blood cells

CHAPTER I

INTRODUCTION

The swine industry in the United States generates over $11 billion of farm sales per year. Domestic pigs (sus scrofa) are raised in various environmental situations all across the country and the world. The industry has seen many changes over the years to increase production efficiency to produce a better product. Some of these changes have attracted the attention of various groups, including animal activists, who argue that producers are not concerned about their animals' well being. Admittedly, there are some production practices which appear cruel and painful to the animal, however, these are quite justifiable. During the production and marketing of these animals, situations do arise which may prove stressful.

Khansari described stress (physical, chemical, psychosocial) as representing the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects (Khansari et al, 1990). When an animal or human experiences stress, activation of the hypothalamic-pituitary-adrenal axis is initiated (Baxter and Tyrell, 1981). The primary mediator of the stress response is corticotropin releasing hormone (CRH), which is secreted from the hypothalamus (Britton et al., 1984; Dunn and Berridge, 1990; Chrousos, 1992). The release of CRH stimulates the anterior pituitary to release adrenocorticotropin hormone (ACTH) and β-endorphin, proopiomelanocortin (POMC) derived molecules (Yates et al., 1974). ACTH, then stimulates the synthesis and release of glucocorticoids, primarily cortisol, from the adrenal

cortex (Axelrod and Reisine, 1984; Hillhouse et al., 1991). During the initial stress response, the sympathetic nervous system and adrenal medulla are also activated to release catecholamines (norepinephrine and epinephrine) into the blood stream (Axelrod and Reisine, 1984; Kopin et al., 1988).

Stress affects both humans and animals by promoting the pathogenesis of infectious diseases and tumor development (Peterson, 1991). Disease and sickness leads to production losses which are extremely important to producers. Therefore, producers must try to reduce the amount of stressors their animals experience to prevent disease. Research has shown that stress may alter neuroendocrine responses that disrupt immune system homeostasis, and lead to immunosuppression (McEwen, 1987; Freier, 1990; Dohms, 1991). Immunosuppression is a state of temporary or permanent dysfunction resulting from damage to the immune system (Dohms and Saif, 1984). An animal that is immunosuppressed may have decreased resistance to disease, that could lead to infections (Dohms and Saif, 1984; Kelley, 1985, 1988). The relationship between stress, the immune system and the neuroendocrine system is still not well defined.

Pigs injected intracerebroventricularly with pCRH demonstrated increased overall activity, particularly walking, standing, licking, rooting, and increased overall activity-related sequences (e.g., sit, walk and stand, walk), but showed reduced complex oral/nasal sequences (e.g., root, lick) (Salak-Johnson et al., 1996). It has been reported that cold stress results in increased feeding (Cabanac, 1974) and increased behavioral problems, such as tail-biting, flank-biting and ear-biting (Scheepens et al., 1991). Pigs which were

heat stressed did not experience an increase in aggressive behavior (Morrow-Tesch et al., 1994).

The objectives of this thesis were to (1) assess neuroendocrine effects on pig natural killer cell activity and (2) to evaluate specific endocrine, immune and behavioral responses to common stressors in young pigs.

CHAPTER II

LITERATURE REVIEW

General Concept of Stress

Claude Bernard, a 19th century physiologist, proposed the concept of internal homeostasis. He described the internal physiological reactions of animals to environmental stimuli as the *"milieu interieur,"* or the principle of a dynamic internal physiological equilibrium. Following Bernard, Walter Cannon in 1914 defined homeostasis as the complex biological responses necessary to maintain a steady state in the body.

In a series of landmark experiments in 1911, Cannon and de la Paz were among the first to examine the response of animals to stressful situations. They recognized the importance of the adrenal medulla and sympathetic-adrenomedullary system in reacting to stressful or emergency events evoked by acute physical or psychobiological stressors. They observed that cats frightened by barking dogs had a humoral agent in their tissues which he termed "sympathin." This was later identified as adrenaline (epinephrine). Hitchings prevented this response by adrenalectomy or by section of the splanchnic nerve which innervates the adrenal medulla (Hitchings, 1913). In 1929, Cannon described the autonomic nervous system's (ANS) response to various stimuli and labeled the response the "flight-fight" syndrome (Griffin, 1989).

In 1936, Hans Selye reported that one consequence of the "stress syndrome" is an enlargement of the adrenal cortex. He observed that a variety of noxious stimuli

(stressors) such as cold, heat, pain, toxins and starvation caused physiological changes resulting in the development of pathological states in animals (Selye, 1950; Griffin, 1989). During several stress studies, Selye observed similar responses to a variety of stressful stimuli. He stated, in fact, "I could find no noxious agent that did not elicit the syndrome" (Selye, 1956). Therefore, he concluded that stress responses were "nonspecific." He classified this nonspecific stress response, the General Adaptation Syndrome (GAS). The syndrome was divided into three distinct phases, the alarm reaction; a resistance stage and the stage of exhaustion (Selye, 1956; Griffin, 1989; Mason, 1975).

In 1968, John Mason disputed Selye's concept of the nonspecific response of the pituitary-adrenal cortical system. He explained the role of the SNS in response to stressful events. He postulated that there is a specific response to a single type of stimulus (psychological) which various unpleasant situations share in common (Mason, 1968). Mason suggested that the entire neuroendocrine system was involved in the stress response. He reported that the pattern of hormonal response to stress differed from one stressor to another.

Neuroendocrine Mediators

Corticotropin Releasing Hormone

In 1948, G.W. Harris proposed the existence of hypothalamic hormones that were released from the median eminence of the hypothalamus and caused the release of hormones from the adenohypophysis (Harris, 1948). A few years later, two independent sets of scientists postulated that there was a hypothalamic releasing factor which

stimulated the anterior pituitary to release ACTH, a hormone which stimulates the adrenal cortex to synthesize glucocorticoids (Saffran and Schally, 1955; Guillemin and Rosenberg, 1955; Guillemin and Hearn, 1955; Vale et al., 1981). Saffran and Schally (1955) purified the substance responsible for ACTH release from posterior pituitary extracts and called it corticotropin-releasing factor (CRH or CRF). At the same time, Guillemin and Rosenberg discovered that CRH released ACTH *in vitro*. Guillemin and Hearn found that posterior pituitary extracts released ACTH *in vitro*, yet synthetic vasopressin did not yield the release of ACTH. It was not until 1981 that scientists were able to identify the sequence of CRH.

In 1981, Vale et al. isolated and purified a 41-residue ovine hypothalamic peptide that stimulated the secretion of ACTH and β-endorphin (Vale et al., 1981). Rivier and Plotsky reported that the primary structure of CRH is similar across several species, including sheep, humans, rats, pigs, goats and cows (Rivier and Plotsky, 1986). CRH is mainly synthesized in the paraventricular nucleus (PVN) of the hypothalamus (Lundblad and Roberts, 1988). CRH is released into the hypophysial portal circulation by median eminence nerve terminals originating from PVN perikarya and is the primary and obligatory hypothalamic ACTH secretagogue (Plotsky et al., 1993). Additional CRH immunoreactivity has been detected in the human placenta, gut, thymus and reproductive tract (Heijnen et al., 1991). Considering that CRH is produced in various sites in the body suggests that the action of the hormone is not restricted to the pituitary (Heijnen et al., 1991). It was also demonstrated that CRH could stimulate human peripheral blood lymphocytes to produce POMC *in vitro* (Smith et al., 1986).

ACTH and β-endorphin

CRH induces the release of POMC in the anterior pituitary. POMC is cleaved enzymatically into various peptides such as ACTH (39 amino acids) and the endogenous opioid, β-endorphin (31 amino acids) (Heijnen et al., 1991). Stimuli responsible for the release of ACTH are also capable of eliciting release of β-endorphin (Guillemin et al., 1977). There are several ACTH secretogogues, including CRH, vasopressin, catecholamines, angiotensin II and oxytocin (Vale et al., 1983; Plotsky, 1985; Rivier and Plotsky, 1986). The most potent of the secretogogues is CRH. The others are weak and usually have an additive or synergistic effect with CRH (Rivier and Plotsky, 1986; Chrousos, 1992). The primary role of ACTH is to stimulate the production and release of glucocorticoids from the adrenal cortex, particularly cortisol (Sapolsky, 1992). ACTH *in vitro* has been shown to suppress antibody production (Johnson et al., 1982), to modulate B-cell function (Alvarez-Mon et al., 1985) and to suppress interferon-γ production (Johnson et al., 1984). Shavit et al. showed that the administration of exogenous ACTH *in vivo* suppressed antibody formation, produced atrophy of lymphoid tissue, suppressed inflammation and prolonged the survival of skin grafts (Shavit et al., 1990).

β-endorphin is released concomitantly with ACTH from the pars intermedia during acute stress (Guillemin et al., 1977). This hormone is part of a class of opiate compounds that regulate pain perception and reproductive physiology during stress (Sapolsky, 1992). β-endorphin can be detected in the low picomolar range in the peripheral circulation (Heijnen, 1991). Endogenous opiate peptides (EOP), such as β-endorphin, also have

effects on lymphocyte function, considering lymphocytes exhibit opiate receptors (McEwen, 1987).

Glucocorticoids

Stress actively increases the adrenocortical system (Jacobson and Sapolsky, 1991). Glucocorticoids are secreted by the adrenal cortex in response to ACTH released from the anterior pituitary. Glucocorticoids (GC) exhibit a negative feedback on the pituitary and hypothalamus to shut off ACTH and CRH secretion (McEwen, 1987). GC have an effect on most of the homeostatic systems in the body, but are thought to have their dominant effect on gluconeogenesis, and on the inflammatory and immunological systems (Griffin, 1989). The level of circulating GC is considered the primary indicator of stress and animal well-being. This steroid hormone occurs in a number of different forms. In humans and primates, the dominant form released is cortisol (hydrocortisone), whereas in rodents it is corticosterone (Sapolsky, 1992).

In 1949, a firm link between GC and immune system function was discovered. It's role took on an unexpected form of an anti-inflammatory to suppress defense mechanisms, rather than to stimulate (Hench et al., 1949). McEwen explained that GC suppressed the activity of thymus-dependent immune responses, including production of lymphokines by T cells and antibodies by B-cells. However, stimulation of immunoglobulin production by GC have also been reported (McEwen, 1987). Because of GC immunosuppressive effects, prolonged elevations can cause deleterious tumor growth (Sapolsky and Donnelly, 1985). In aging rats, glucocorticoid elevation is associated with enhanced tumor growth.

Even in younger rats, stress elevates GC levels and may potentiate tumor growth (McEwen, 1987).

Catecholamines

Knowledge of the adrenal medulla developed during the early part of the century in parallel with the concept of homeostasis and the flight-fight responses (Malven, 1993). In the adrenal medulla, chromaffin cells are innervated neuroendocrine cells that secrete catechoaminergic hormones into the blood (Malven, 1993). The primary secretory product of adrenal medullary cells is the catecholamine, epinephrine (adrenaline). In 1899, Abel isolated and characterized epinephrine. Almost half a century later, von Euler discovered norepinephrine, an epinephrine-like chemical mediator released from peripheral sympathetic nerve endings. Both norepinephrine and epinephrine are recognized as activators of physiological responses to emergency situations. The biological actions of these catecholamines appear to be antagonistic in the arterioles of skeletal muscle and skin where blood-borne epinephrine caused vasodilation while norepinephrine (locally released) causes vasoconstriction (Malven, 1993). Epinephrine also promotes glycogenolysis, the induced breakdown of glycogen stored in the liver and in skeletal muscle to generate glucose needed as a metabolic substrate. Various psychological and physical stimuli produce varying levels of epinephrine and norepinephrine, therefore it is important to understand the interaction between these factors and their impact on host function (Griffin, 1989). As a result of the activation of sympathetic nerves or an acute stress response, norepinephrine levels have been shown to increase. Activation of the

adrenomedullary tissues results in epinephrine elevation (Griffin, 1989). Catecholamines also tend to affect the production of ACTH. Increased levels of brain norepinephrine inhibits the pituitary to release ACTH, while plasma epinephrine stimulates ACTH production (Axelrod and Reisine, 1984). Stress results in an immediate increase of both catecholamines and corticosteroid levels, yet catecholamine levels return to normal immediately following termination of stress, whereas corticosteroid levels remain high for 30 minutes. Chronic stress causes persistent elevations in catecholamines, while plasma corticosteroids return to basal levels much earlier (Griffin, 1989).

Stressors, such as cold, thermal stress, exercise, surgery, hemorrhage, myocardial infarction, hypoglycemia and hypoxia cause a marked elevation of plasma catecholamines (Axelrod and Reisine, 1984).

The Stress Response

Stress defies easy definition. Walter Cannon would define stress in terms of stimuli or activation of the sympathoadrenomedullary system, while Hans Selye would emphasize the pituitary-adrenocortical system. Webster's Medical Dictionary claims stress to be " a state of bodily or mental tension resulting from factors that tend to alter an existent equilibrium" (Webster, 1986). In the veterinary context, stress is defined as an abnormal or extreme adjustment in the physiology of an animal to cope with adverse effects of its environment and management (Fraser et al., 1975). The factors which adversely affect the animal are termed stressors. In a broad sense, stress has been used to explain the effects of stimuli (external and internal) on the body's biological system. Thus,

stress would depend on the animal's perception of a stressor, its physiological response and the physiological consequences (Moberg, 1985).

Stressors activate the hypothalamic-pituitary-adrenal (HPA) cortical system (Harris, 1955). Mason suggested that the primary mediator in the activation of the HPA axis is psychological stressors (Mason, 1971). The "flight-fight" reaction is a result of the physiological changes in the sympathetic-adrenal medullary system. The HPA axis is activated under many conditions, including stress. Persistent HPA activation causes biological changes which may produce a pre-pathological state where the animal becomes susceptible to disease (Griffin, 1989). The hypothalamus appears to play a central role in coordinating the endocrine, autonomic, and behavioral responses to stress. Neurons of the PVN are responsible for the release of CRH into the hypophysial portal system, thus initiating the stress cascade (Antoni, 1986; Sawchenko and Swanson, 1989). Within the hypothalamus, specific cells exert neural control over the pituitary-adrenocortical and sympathetic-adrenomedullary axes (Hillhouse et al., 1991). These two axes are responsible for the GC- and POMC-derived peptides and epinephrine and norepinephrine release into circulation.

The stress-response is dominated by the adrenal gland. Epinephrine is released from the adrenal medulla and glucocorticoids are released from the adrenal cortex (Sapolsky, 1992). The synthesis and release of glucocorticoids represents the final step in the neuroendocrine cascade (Swanson and Sawchenko, 1980; Gann et al., 1985). Glucocorticoids appear to play a vital role in maintaining the body's homeostasis under stress conditions.

Stressors (psychological, physical, environmental manipulations, exogenous and infectious agents), initiate the stress-response (Molitor and Schwandt, 1993) by the immediate secretion of CRH from the PVN region of the hypothalamus (Swanson et al., 1980; Lundblad and Roberts, 1988). As CRH concentrations increase, within perhaps 15 seconds, ACTH is released from the anterior pituitary. ACTH, in turn, stimulates the adrenal cortex to release glucocorticoids within minutes (Sapolsky, 1992).

Models of Stress

Cold Stress

During development, animals may experience fluctuations within their environment, particularly varying temperatures. There is both a lower and upper critical temperature of the thermoneutral zone. When lower critical temperature exceeds the amount of heat that an animal would produce as a consequence of metabolism, the animal is forced to elevate metabolism by the processes of cold thermogenesis or to allow body temperature to fall below normal (Webster, 1974). Animals adapt to such stressors by behavioral, physical or physiological changes. Practically all the systems in the body are involved in the metabolic response to cold. The striated muscles shiver, cardiac muscle beats faster, respiration becomes deeper, urine flow is increased and the sympathetic and pituitary controlled endocrine systems are activated to elevate biological oxidations in all tissues. Cold thermogenesis can be divided into two categories: shivering and non-shivering thermogenesis (NST). Shivering is a clearly defined response common to all homeotherms and can be described as a tremor or a rhythmic involuntary movement

consisting of an oscillation about a mid point in one or several muscles (Webster, 1974). The result of such an action would be an elevation in heat production. Mechanisms to produce heat other than shivering would refer to NST. More specifically, catecholamine-induced regulatory non-shivering thermogenesis would be an example of such a mechanism (Webster, 1974). Cold stress activates the sympathoadrenal system, particularly catecholamine synthesis. The effects of catecholamines include lypolysis of white adipose tissue, hyperglycemia, potentiates shivering in striated muscles and increases tone and rhythm in cardiac and smooth muscle (Webster, 1974). In addition to affecting the adrenal medulla, cold also affects the thyroid. The sympathetic system and the thyroid have synergistic effects to facilitate thermogenesis. Acute cold stress results in an elevation of glucocorticoids, particularly cortisol and corticosterone (Webster, 1974).

Cold temperatures, especially wind or draft, may directly influence the animal's economic efficiency by reducing its resistance to infection (Kreukniet et al., 1990). During long-term cold stress exposure, feed intake in all species has been found to be enhanced proportionally to the degrees of cold (Cabanac, 1974). Draft has been found to have negative influences on physiological activity, growth, performance, causes clinical symptoms, such as diarrhea, coughing and sneezing and pathological lesions. Decreasing temperature has been associated with decreased growth (Le Dividich, 1981) and increased susceptibility to disease (Shimizu et al., 1978). Long-term fluctuating temperature did not affect serum glucose, plasma cortisol, monocyte phagocytic function or antibody response (Minton et al., 1988). Growth, feed intake and feed-conversion efficiency was also similar between chronically cold stressed and control nursery pigs (Minton et al., 1988). Newly

weaned pigs exposed to a reduced nocturnal temperature regimen grew faster and ate more feed than pigs subjected to the recommended constant temperature regimen of 30°C (Brumm et al., 1985.) Behavioral problems, such as tail-biting, flank-biting and ear-biting, have also been reported in response to long-term, unpredictable climatic changes (Scheepens et al., 1991). Lymphocyte numbers increased and neutrophil numbers decreased in dairy cattle after acute cold stress (Kelley, 1983). However, chronic cold stress increased both lymphocyte and neutrophil numbers (Kelley, 1983). Growing pigs challenged with *Haemophilus pleuropneumoniae* (Hpp) and then exposed to increased air velocity and lower temperatures had increased mortality rate as compared to non-stressed controls (Osborne et al., 1984).

Pig producers on the average, lose more than 25% of the live pigs farrowed before they are weaned. During the first postnatal day, chilling is directly responsible for many of the deaths and also increases susceptibility to disease (Kelley et al., 1982). Therefore, to decrease death loss and disease, proper thermal environment should be provided for baby pigs.

Heat Stress

During high ambient temperatures, animals may experience unnecessary suffering, reduced performance or even death. Animals have developed heat-loss mechanisms to cope with such environments. Evaporative heat-loss mechanisms, such as sweating, panting and salivating, are essential for homeothermy (Hales, 1974). The catecholamines, 5-hydroxytryptamine (5-HT) and noradrenaline (NA), act as transmitters in the

hypothalamus, to mediate thermoregulatory responses (Hales, 1974). These neurotransmitters have different effects in different species. For example, NA results in a fall and 5-HT in a rise of body temperature in the monkey, cat and dog, while the reverse occurs for the rabbit, sheep, goat and rat (Hales, 1974). Thermoreceptors appear to lie within the walls of the rumen of sheep and intestine and maybe the mesenteric veins, with an afferent pathway in the splanchnic nerves (Rawson, 1972).

Heat stress ultimately affects the work and mental ability in humans and meat and milk productivity in animals. In the ram, 1 to 3 hours of heat exposure induced infertility (Waites, 1970). Weanling pigs exposed to temperatures above 30°C experienced depressed weight gain (Seymour, 1964). The National Research Council reported that pigs which are heat stressed eat less feed and consequently gain less weight (NRC, 1981). Social stress, or regrouping, in addition to long-term thermal stress (32.2°C) depressed gain:feed ratio (McGlone et al., 1987).

Performance was compared between pigs raised in a constant environment verses a tropical environment. Animals in the tropical environment had increased rectal temperature and respiratory rates, but depressed growth rate and efficiency of feed utilization. In addition, the heaviest weight group had reduced feed intake and increased body fat (Christon, 1988). The tropical environment significantly affected the metabolism of the pigs. There was an increased concentration of plasma free fatty acids, triglycerides, cholesterol and adipose tissue lipoprotein lipase activity, while there was a decreased concentration of thyroid hormones (Christon, 1988).

Morrow-Tesch et al. (1994) found that chronic heat and social status had interactive effects on the pigs' immune system. In contrast to cold stress, heat stress had no effect on aggression in pigs, yet heat stress increased neutrophil numbers and decreased antibody production in socially dominant and submissive pigs (Morrow-Tesch, 1994).

Shipping Stress

Animal transportation is not a new concept. Transport of horses by sea was first recorded in 490 B.C. (Hartmann, 1962). In the past, droving was the more common method used to transport animals. Droving is moving livestock on foot, thus allowing them to feed and graze as they move. This method of transport is the least expensive way to move animals (Hails, 1978). However, this method is not feasible in today's fast-paced society. Today, animals can be moved by rail, road, sea or air. Air transport is the most expensive method and is more widely used for zoo animals, wild animals for laboratories, valuable domestic animals, such as racehorses, and breeding animals, rather than for routine transport of livestock (Hails, 1978). Livestock are generally transported long distances to packing plants for slaughter. The feeder pig industry moves pigs at various stages of production, usually by truck. Feeder calves are transported long distances from cow-calf producing areas to feedyards (Cole et al., 1988). It is common to move breeding animals and horses both nationally and internationally. The transport of wild animals has increased because of the increase in both zoos and scientific research facilities.

It has been widely accepted that transportation causes animals to experience stress. It disrupts the animal's ability to maintain homeostasis, both physically and socially.

Besides the actual movement of the vehicle, the animal may come into contact with a multitude of stressors, including separation from familiar environments and social groups; loading and unloading; overcrowding; unfamiliar and loud noises; vibrations, jolting; extremes of temperature and humidity; lack of ventilation; gases (from feces, urine, diesel and exhaust fumes); changes in bacterial environment and deprivation of food and water (Hails, 1978). The stressor which causes the greatest stress reaction is loading and unloading (Augustini, 1976). In mice, shipping caused an increase in corticosterone levels and immune function was significantly lessened (Landi et al., 1982).

The U.S. ships all or most beef cattle long distances either to feedyards or slaughterhouses, depending on stage of production. Cattle are susceptible to shipping stress, particularly for calves in the first few days of life or during long distance transport. Transport mortality by rail has been reported as twice as high as road transport (Rodenhoff and Schonherr, 1971). A study was conducted to evaluate shipping's impact on calves' resistance to experimentally induced respiratory disease. Post-shipping, control and shipped calves were given aerosol inoculations of infectious bovine rhinotracheitis (IBR) virus and *Pasteurella hemolytica* bacteria. Results concluded that corticosteroid concentration was high in transported calves during the journey and immediately after unloading, but returned to baseline by the following day. In addition, the transported calves showed more severe clinical signs to the induced respiratory diseases than the control calves (Brunner, 1989). Another study was designed to determine the effects of duration of transport on weight loss, health, performance and hematologic and serum biochemical constituents of feeder calves. Blood samples were collected prior to shipping

and immediately upon return. Numbers of RBC, WBC, segmented neutrophils, lymphocytes and eosinophils were linearly ($P < .05$) affected by duration of transport. Serum enzymes, iron, urea nitrogen, β-globulin, glucose, and urea nitrogen-to creatinine ratio determinations were also significantly affected linearly by transport duration. Transport duration did not affect average daily gain; however, calves of the short-duration (12 hour) group had significantly ($P < .05$) higher morbidity and mortality than did the control and long-haul (24 hour) groups (Cole et al., 1988).

Some pigs are generally more susceptible to stress than other farm animal species and death during transit may be attributed to the pig's inability to overcome and adapt to the numbers of stressors involved (Hails, 1978). This susceptibility is also known as porcine stress syndrome (PSS). Early signs include, muscle and tail tremors, irregular breathing, blanched or reddened areas of the skin, rapid increase in body temperature, cyanosis and development of extreme acidosis. Continued exposure to stress may result in collapse, muscle rigidity, hyperthermia and ultimately death (Hails, 1978). The syndrome may be seen in other ways including, sudden death; post-mortem deterioration in meat quality; and acute necrosis of back muscles (Hails, 1978). Stress susceptibility or PSS is thought to be inherited by a single recessive gene (n) and homozygous animals (nn) can be identified by exposure to halothane gas (Nyberg, 1988). Animals which inherit this stress gene are prone to higher mortality and lower meat quality (Ville et al., 1993). One important factor involved in porcine stress syndrome is the breed of the animal. Transport deaths are more common in the European breeds and in heavily muscled breeds, such as the Pietrain (Hails, 1978).

Porcine stress syndrome is a definite concern among producers, but may induce an even greater situation among packing plants and thus the pork industry. PSS may result in reduced meat quality, or pale, soft and exudative (PSE) meat. It has been shown that pigs held 2 hours before slaughter resulted in PSE meat, while hogs held for 24 hours had a greater incidence of dark, firm and dry (DFD) meat (Moss and Robb, 1978). Carcass muscle pH is important for meat quality. In cattle, muscle pH ranges from 5.2 to 6.2, while muscle pH in pigs range from 5.5 to 5.8 (Cervenka, 1969). The pH of muscle is affected by the breakdown of glycogen to lactic acid and the rate of glycogen metabolism is affected by short-term or long-term stress and by an individual animals' ability to adapt (Grandin, 1980). Physical stressors cause a depletion of glycogen, while physiological stress results in rapid glycogen catabolism provoked by epinephrine production. Prolonged stress depletes glycogen stores and lactic acid can no longer be produced, thus resulting in dark, firm and dry meat. In 1978, Hendrick reported that in order to produce dark cutting cattle, the animals had to withstand stress for more than 8 hours. Glycogen breakdown just prior to slaughter results in lighter meat. Determination of final pH is dependent on lactic acid produced from glycogen breakdown. PSS seems to negatively affect the rate at which lactic acid is removed from the muscle (Grandin, 1980).

Biology of NK Cells

Natural Killer (NK) cells are a subpopulation of lymphoid cells morphologically known as large granular lymphocytes (LGL), which make up approximately 5% of circulating or splenic leukocytes (Herberman and Ortaldo, 1981). These effector cells are

involved in spontaneous natural cytotoxicity against tumor and virally-infected cells, thus killing rapidly and without prior antigenic sensitization (Trinchieri, 1984; Lotzova, 1991; Herberman and Ortaldo, 1981). In addition to tumor surveillance and resistance and lysis of virus-infected cells, NK cells participate in bone marrow transplant rejection, control of hemopoiesis and cytokine production (Pinto and Ferguson, 1988). Although NK cells are thought to originate and differentiate in the bone marrow, concentrations are low, as well as in the lymph nodes and peritoneal cavity and undetectable in the tonsil or thymus. However, studies using mouse, rat and human cells demonstrated that NK levels are high in the peripheral blood and spleen (Ortaldo and Herberman, 1984). The distinction between T-lymphocytes and NK cells has been controversial since both subsets exhibit similar functional and phenotypic characteristics. For example, both cells when activated by IL-2, are capable of killing a variety of tumor cells. Despite similarities, NK cells differ distinctly from T-cells. T-cells differentiate and function via the thymus, while NK cells are thymus-independent and are functional in athymic mice (Lotzova, 1991, Ortaldo and Herberman, 1984). NK cells lack the cell surface markers, CD3 and CD4, thus they can not be considered T-cells. However, they do express Thy-1 which may indicate T-cell lineage (Ortaldo and Herberman, 1984; Blalock, 1989). The surface markers, CD16 and CD56, have been found to be present on essentially all resting and activated NK cells in human peripheral blood. In addition, human NK cells have surface receptors for immunoglobulin G (IgG) (Lotzova, 1991; West, 1977).

These cytotoxic cells have been described as large lymphocytes with a high cytoplasm to nucleus ratio and a few azurophilic granules arising from the Golgi body

(Welsh, 1986; Trinchieri, 1984). NK cells recognize and bind target cells nonspecifically to ill-defined receptors, then reorient so that the nucleus is distal and its cytoplasm proximal to the target cell. The effector cell is then triggered by target cell determinants to release cytotoxic factors, such as perforin. Perforin polymerizes into a complement-like protein ring deposited on the membrane of the target cell. The NK cell can then dissociate from the target cell leaving it to eventually lyse (Welsh, 1986). There are certain chemical agents which can interfere with or enhance this killing process. In virus infections, IFN can either enhance the cytotoxic activity of NK cells or protect the target cells from any damage. One study demonstrated that IFN (α, β and γ) can increase the proportion of human LGL binding to target cells, especially carcinoma lines (Ortaldo and Herberman, 1984; Trinchieri, 1984). Although all three known types of IFN increase human NK cell activity, IFNγ is considered the most effective. Non-virally, IL-2 is another agent which enhances NK cell activity. There is a complex interplay between IFN and IL-2. IL-2 induces NK-like cells and macrophage production of IFN. IFN can induce IL-2 receptors on NK cells and IL-2 can influence growth and maturation of NK cells (Trinchieri, 1984). Inhibitory agents may act at the level of recognition and binding to the target cell or at the dissociation or lytic stage. Some of these agents (cAMP, cholera toxin, PGE, and some phosphorylated sugars) do not appear to block binding, however, EDTA, phorbol esters and proteases do act at the level of binding to the target cell (Ortaldo and Herberman, 1984).

While most studies concerning NK cells have focused primarily on humans and rodents, information is becoming increasingly available on the role these cells may play in

domestic animals, such as swine. The presence of cells with spontaneous cytotoxic ability has been reported in miniature swine (Kim et al., 1980). Large White/Landrace crosses (Martin and Wardley, 1984), Yorkshire breeds (Pinto and Ferguson, 1988) and other porcine species have been found to have similar cytotoxic cells (Norley and Wardley, 1983; Cepica and Derbyshire, 1986). These porcine studies describe both similarities and differences between the NK cell of the domestic pig and NK cells of other species. In Yorkshire swine, the NK effector cell was reported to be a small, non-granular lymphocyte (Pinto and Ferguson, 1988), quite different from the LGL reported in humans and rodents. Pig NK cells are similar to the human counterpart in that, small lymphocytes exhibit little killing, while large lymphocytes exhibit substantial cytotoxicity. Microscopic examination of porcine NK cells reveal relatively less cytoplasm than human LGL. Porcine NK cells exhibit the surface markers CD2 and CD8 (Pescovitz et al., 1988).

Studies of porcine NK activity indicate that the cells are highly reactive against the YAC-1 lymphoma, the K-562 myeloid leukemia, the P-815 mastocytoma and the TU-5 virally transformed fibroblast. Nevertheless, the MOLT-4 and SB leukemias are NK resistant. The target cell, K-562 myeloid leukemia has been reported to be the most reliable target cell to measure peripheral blood NK activity in pigs (Pinto and Ferguson, 1988; Lumpkin and McGlone, 1992).

Stress Effects on NK Cytotoxicity

Stressors such as surgical stress (Tonnesen et al., 1984; Pollock and Lotzova, 1987), bereavement in humans (Irwin et al., 1988b), foot shock in rats (Shavit et al., 1984;

1987), inescapable stress (Cunnick et al., 1988; Weiss et al., 1989; Irwin et al., 1990; Strausbaugh and Irwin, 1992), isolation stress (Ghoneum et al., 1987), general anesthesia (Markovic and Murasko, 1990), noise stress (Irwin et al., 1989; Segal et al., 1989), cold stress (Aarstad et al., 1983; Jiang et al., 1990) and transportation in mice (Aguila et al., 1988) and pigs (McGlone et al., 1993) have been shown to reduce NK cell cytotoxicity.

Following surgical stress, rats exhibited a 14 day reduction in NK activity (Toge et al., 1981). Aarstad et al. (1983) immersed mice into cold water (cold stress) twice a day for 5 minutes and demonstrated a suppression of splenic NK activity. Restraint stress in both mice and rats reduced NK cytotoxicity (Okimura et al., 1986). In pigs, social status and shipping had an interactive effect on NK activity. Shipped, dominant pigs experienced higher NK cytotoxicity than either the intermediate or submissive pigs (McGlone et al., 1993).

Neuroendocrine Effects on Natural Killer Cell Cytotoxicity

CRH Effects on NK Cytotoxicity

Several studies indicate that corticotropin-releasing hormone (CRH) may play a role in the central modulation of natural killer (NK) cell cytotoxicity (Irwin, 1987, 1988a, 1990a, 1990b; Jain et al., 1991; Strausbaugh and Irwin, 1992) and peripheral blood NK activity (Strausbaugh and Irwin, 1992) independent of its role in activating of the hypothalamic-pituitary-adrenal axis (McGlone, 1991). Synthetic CRH administered intracerebroventricularly (ICV) suppressed splenic natural killer (NK) cell activity in a dose-sensitive manner. Rats pretreated with chronic α-helical CRH, a CRH antagonist,

showed no suppression of splenic NK activity. However, responses of the pituitary-adrenal system were not altered by the antagonist (Irwin, 1987). In another study by Irwin, CRH injected into the lateral ventricles increased noradrenergic function and reduced NK activity in the rat spleen (Irwin, 1990a). The autonomic nervous system is considered to be the link between the central nervous system and the immune system. For example, noradrenergic fibers of the sympathetic nervous system innervate the spleen in which lymphocytes reside (Irwin, 1988, 1990a).

The *in vitro* effects of CRH on NK activity have had conflicting reports. *In vitro* exposure of human lymphocytes to 10^{-6} to 10^{-10} M concentrations CRH inhibited NK activity (Pawlikowski, 1988), where as Irwin et al. (1987) reported that *in vitro* CRH at concentrations 10^{-6} to 10^{-12} M showed no effect on NK activity. Concentrations of 0.1 to 1 nM of CRH have been shown to stimulate NK activity, while 1 to 100 nM had no effect on NK activity (Leu and Singh, 1991).

ACTH and Cortisol Effects
on NK Cytotoxicity

Evidence suggests that ACTH does play a role in the modulation of the immune response. *In vitro* ACTH was ineffective in changing spontaneous NK activity at all concentrations in humans (Gatti et al., 1993), murine (Carr and Blalock, 1990) or pigs (McGlone et al., 1991). However, ACTH was able to significantly reduce the cortisol-dependent inhibition of human NK activity (Gatti et al., 1993). An iv bolus of ACTH (1

IU/kg BW) administration dramatically elevated NK and IL-2-stimulated NK cytotoxicity (McGlone, 1991).

Stress activates the HPA axis, thus increasing glucocorticoid concentrations. Glucocorticoids suppress several aspects of the immune system (Munck and Guyre, 1991). Lymphocytes are known to possess cortisol receptors on their surface, hence the suppression of cortisol may be due to direct effects (Lippman and Barr, 1977). Glucocorticoids interfere profoundly with NK activity. In both animals and humans, Glucocorticoids suppress NK cytotoxicity either *in vivo or in vitro* (Parrillo and Fauci, 1978; Oshimi et al., 1980; Onsrud et al., 1981; Holbrook et al., 1983; Gatti et al., 1987; Pedersen and Beyer, 1986; Masera et al., 1989). Administration of hydrocortisone acetate to mice reduced splenic NK cell activity for up to 120 hours (Hochman and Cudkowicz, 1979).

β-endorphin Effects on NK Cytotoxicity

The role of β-endorphin in the alteration of the immune response is generally unknown. Several studies have demonstrated that NK activity is significantly enhanced by *in vitro* β-endorphin, yet is concentration-dependent (Mathews et al., 1983; Williamson et al., 1987). Endorphins enhanced cytotoxicity in human NK cells and murine splenic cells (Mandler et al., 1986; Williamson et al., 1987; Carr and Blalock, 1990; Gatti et al., 1993). When NK cell were incubated with concentrations of β-endorphin between 10^{-7} and 10^{-10} M, activity was increased by 63% (Mandler, 1986). In contrast, lower concentrations ranging from 10^{-13} to 10^{-18} M indicated a significant reduction in NK activity (Williamson

et al., 1988). In addition to ACTH, *in vitro* β-endorphin also significantly reduced cortisol-dependent inhibition of human NK activity (Gatti et al., 1993).

Catecholamine Effects on NK Cytotoxicity

Studies show that the catecholamines, norepinephrine and epinephrine, do tend to affect NK cell activity and numbers. Direct addition of norepinephrine to lymphocyte/target cell mixtures inhibited NK cell cytotoxicity in a dose-dependent manner. This effect was blocked by propranolol, (β-blocker), but not affected by atenolol (α-blocker) (Takamoto et al., 1991).. When lymphocytes were incubated with epinephrine at concentrations ranging from 10^{-7} to 10^{-9} M, NK activity increased by 30% (Hellstrand et al., 1985). Knudsen et al. (1994) suggests that adrenaline has a dual effect on NK cells: an acute effect where NK cells are mobilized and a chronic effect by which NK cell numbers decrease in peripheral blood. After administration of epinephrine to humans, NK cell activity increased within 15 to 30 min and returned to normal values within two hours (Tonnesen et al., 1984). Another *in vivo* study involving humans, demonstrated that NK cell numbers increased significantly 5 min after administration of adrenaline and noradrenaline. Values peaked 15-30 min post-injection and declined to baseline values 60 min (noradrenaline) and 120 min (adrenaline) after injection (Schedlowski, 1993).

Acute Phase Response

In response to bodily injury, trauma or infection, an immediate series of complex physiological and biochemical reactions occur. This induced reaction is known as the acute phase response (APR). The cell responsible for eliciting this response is normally the macrophage or blood monocyte. A local reaction activates macrophages to release various chemicals, such as interleukin cytokines (IL-1; IL-6), tumor necrosis factor (TNF) and interferons, which mediate the systemic reaction (Baumann and Gauldie, 1994; Heinrich et al., 1990; Le et al., 1989; Kushner et al., 1990). These initial cytokines have a dual purpose. They act proximally on stromal cells and induce a secondary release of cytokines. This secondary reaction initiates and mediates the cellular and cytokine cascades involved in the APR (Baumann and Gauldie, 1994).

During the critical stages of stress, the liver regulates the amount of essential metabolites provided to the organism. This organ also supplies the necessary components involved in protecting the site of tissue damage, limiting tissue destruction, clearing harmful agents and aiding tissue repair (Baumann and Gauldie, 1994). The APR cascade stimulates hepatocytes, via cell-surface receptors, to release acute phase proteins (APP). The concentrations of these proteins normally maintain a steady state which reflects a balance between synthesis and catabolism. As stress occurs and homeostasis is disrupted, the plasma concentrations of various acute phase reactants may change in magnitude, duration and direction (Dowton and Colten, 1988). These proteins are categorized as either positive or negative acute phase proteins, depending on whether they decrease

(negative) or increase (positive) in concentration during the acute phase response (Gruys et al., 1993). The positive APP are stimulated by cytokines and are usually glycoproteins released from hepatocytes. The positive APP function as hemoglobin, free radical and cell nest scavengers, they bind bacterial components, activate complement, play a role in cholesterol distribution and some may promote immunoglobulin production (Gruys et al., 1994). There are several known APP with distinct species differences. C-reactive protein (CRP) is a major acute phase reactant in humans, dogs (Connor et al., 1988), pigs (Kushner and Feldman, 1978) and rabbits (Eckersall, 1989). Ceruloplasmin increases about 50% during inflammation or infection. Serum amyloid A (SAA) is a high density lipoprotein and the precursor for amyloid protein A (Eckersall, 1992). α_1-Anti protease and α_2-macroglobulin are anti-proteases. This thesis will focus on three positive acute phase proteins: Fibrinogen, Haptoglobin and α-1-acid glycoprotein.

Fibrinogen

Fibrinogen has been determined in several species and is regarded as a moderate to low acute phase protein. Studies have shown that fibrinogen concentration increases in response to stress. In beef calves which were weaned and transported, fibrinogen concentrations increased. Yet, genotypes were highly variable in that *Bos indicus* calves had higher fibrinogen concentrations during weaning, assembly and transit than did *Bos taurus* calves (Phillips et al., 1989). In horses, plasma fibrinogen concentrations were shown to increase in response to inflammation (Auer et al., 1989). Following surgery for treatment of subchondral bone cyst and osteochondrosis dissecans lesions, fibrinogen was

elevated two fold (Allen and Kold, 1988). Following turpentine injection of calves, five acute phase proteins increased, including a fibrinogen increase of 100% (Conner et al., 1988a). Administration of a short-acting or long-acting form of ACTH failed to induce an increase of fibrinogen in calves, yet both forms caused a fibrinogen increase in rabbits (Gentry et al., 1992).

Haptoglobin

Haptoglobin is considered to be a major acute phase reactant in ruminants, yet in humans and other species there is only a 2 to 4% increase in circulating concentration, thus making it only a moderate reactant (Eckersall, 1992).

Haptoglobin functions to bind and transport free circulating hemoglobin to the liver, thus recycling heme iron. In humans, haptoglobin is recognized as an immunosuppressive factor in serum (Morimatsu et al., 1992). In cattle, haptoglobin has been used as a diagnostic test in which concentrations were elevated in cases of mastitis, metritis and retained placenta while haptoglobin remained low in cases of metabolic disease (Skinner et al., 1991; Eckersall, 1992). Physical stress did not affect haptoglobin concentrations in calves (Alsemgeest et al., 1995). However, calves which were transported for 2 days experienced elevated haptoglobin concentrations. In addition, a correlation was observed between haptoglobin concentrations and lymphocyte suppression in the sera. These data suggested that bovine haptoglobin may be involved as an immunomodulator in the suppression of lymphocyte blastogenesis (Murata and Miyamoto, 1993). At parturition, haptoglobin concentrations were elevated in 74% of the cows

observed (Uchida et al., 1993). In calves, experimental injections of *Pasteurella hemolytica*, *Ostertagia ostertagi* and endotoxin raised levels of haptoglobin and other APP (Conner et al., 1989). In sheep, haptoglobin has been shown to be useful as a diagnostic tool in the prognosis of dystocia (Scott et al., 1992) and a reliable indicator of infection (Skinner and Roberts et al., 1994). Grass sickness (Milne et al., 1991) and laminitis (Edinger et al., 1992) in horses resulted in a rise of haptoglobin. The highest concentration of serum haptoglobin was found in newborn foals (5.25 ± 2.36 mg/mL) and this value was maintained until about 12 months of age, then decreased with age (2.19 ± 1.54 mg/mL). Inflammation elevated haptoglobin 1.5 to 9 times higher than normal horses (Taira et al., 1992). In the dog, haptoglobin reached peak levels 4 to 6 days following surgical trauma (Conner and Eckersall, 1988b). Pigs naturally or experimentally infected with *Actinobacillus pleuropneumoniae* showed an elevation in haptoglobin (Hall et al., 1992). Eurell et al. (1990) documented a haptoglobin increase following experimentally inducing atrophic rhinitis in swine.

Alpha$_1$-acid Glycoprotein (AGP)

Recently, alpha$_1$-acid glycoprotein has become one of the most extensively studied acute phase proteins because of its distinct differences from other glycoproteins. AGP has several unique properties including acidic isoelectric points and a high solubility in water and other organic solvents. This protein has been isolated from cattle, pigs, dogs, cats and horses. AGP was characterized in fetal and newborn calves. During fetal development and neonatal stages, AGP continued to increase and reached a peak (1368 ± 207 μg/mL)

immediately after birth. Thereafter, concentrations gradually decreased to 249 ± 100 μg/mL, similar to adult bovine concentrations (Itoh et al., 1993a). Within one day of birth, the AGP levels in pigs was 14,263 ± 2,393 μg/mL (40 times the normal adult value). By four weeks of age, this value had rapidly decreased to 699 ± 186 μg/mL. After four weeks of age in conventional pigs, AGP began to increase, averaged 1,428 μg/mL by eight weeks then gradually decreased to adult levels by 20 weeks of age. In contrast, the AGP level in specific pathogen-free (SPF) pigs was 800 μg/mL at 8 weeks of age then decreased more rapidly to normal by 16 weeks of age (Itoh, 1993b). At eight weeks of age, the incidence of clinical pneumonia and antibodies to *Actinobacillus pleuropneumoniae* and *Mycoplasma hyopneumoniae* and consequently AGP concentrations was high in the conventional pigs. As the pneumonia disappeared, AGP levels gradually returned to normal. In comparison, the SPF pigs had very little clinical illness and thus low AGP levels (Itoh, 1993b).

Corticosteroid Binding Globulin (CBG)

Corticosteroid binding globulin belongs to the serpin superfamily of serine protease inhibitors. Through evolutionary processes, CBG has developed its role as the principle serum transport protein for most glucocorticoids (approximately 90%) , delivering them to sites of inflammation. Corticosteroids then modify the inflammatory response by inhibiting the efflux of neutrophils from the circulation (Edgar and Stein, 1995). Free cortisol possesses greater biological activity than does the protein bound cortisol (Bamberg-Thalen et al., 1992; Berdusco et al., 1995). In addition to CBG, glucocorticoids also bind to

albumin, which has a large binding capacity, but a much lower affinity than CBG. The binding of CBG to glucocorticoids plays an important role in protecting the steroids from being metabolized and in ensuring them to reach target cells (Bamberg-Thalen et al., 1992). Although the liver is considered the major site of CBG synthesis (Hammond, 1990; Hammond et al., 1991), it has also been discovered in the kidney of the fetal mouse (Scrocchi et al., 1993), rabbit (Seralini et al., 1990) and rat (Kraujelis et al., 1991). A study was conducted comparing CBG binding capacity and plasma cortisol concentration in normal dogs verses dogs with hyperadrenocorticism. The dogs with hyperadrenocorticism showed a higher cortisol concentration than control dogs. There was no difference in CBG binding capacity between the two groups, thus suggesting that increased cortisol secretion does not induce an increase in CBG binding capacity (Bamberg-Thalen et al., 1992). During late pregnancy, there is an elevated level of cortisol concentration due to maturation of fetal pituitary-adrenal function, consequently signaling the onset of parturition and promoting the maturation of several fetal organ systems (Jacobs et al., 1991; Ali et al., 1992). In conjunction with the rise of fetal cortisol, corticosteroid-binding capacity (CBC) of fetal plasma is also increased. Therefore, at birth, concentrations of fetal CBG exceeds that of the mother (Jacobs et al., 1991). Administration of ACTH also stimulates a rise in fetal plasma CBC and may be mediated by cortisol (Jacobs et al., 1991). In the rat fetus, CBG concentrations are high, decrease at birth, remain low during the suckling period and reach adult values by 4 weeks of age (Haourigui et al., 1994). CBG reduced the ability of cortisol to exert negative feedback on ACTH output by fetal ovine pituitary cells *in vitro*. It was thus suggested that CBG

interacts with cortisol in a manner that maintains a low negative feedback on the pituitary, and perhaps hypothalamus (Challis et al., 1995).

CHAPTER III

EFFECTS OF *IN VITRO* PCRH, ACTH, β-ENDORPHIN, CORTISOL, EPINEPHRINE AND NOREPINEPHRINE ON PORCINE NATURAL KILLER CELL ACTIVITY

Abstract

Peripheral blood was collected from 59 pigs to determine the effects of physiological concentrations of stress hormones on porcine natural killer cell (NK) activity. A standard cytotoxicity assay was utilized with peripheral blood mononuclear cells and ^{51}Cr-labeled K-562 target cells. Hormones and concentrations were: porcine corticotropin releasing hormone (pCRH) at 0, 0.47, 4.7, 47 and 467 ng/mL; β-endorphin at 0, 1, 10, 100 pg/mL and 1 ng/mL, cortisol at 0, 3.6, 36 and 360 ng/mL, adrenocorticotrophic hormone (ACTH) at 0, 0.045, 0.45, 4.5 and 45 ng/mL, epinephrine at 0, 0.022, 0.22, 2.2 and 21.9 ng/mL and norepinephrine at 0, 0.021, 0.21, 2.1, 20.6 ng/mL. Over the range of doses evaluated, pCRH, ACTH, cortisol and norepinephrine had no significant effect on porcine NK activity. β-endorphin and epinephrine had significant ($P < .01$) effects on porcine NK activity. All doses of β-endorphin suppressed ($P < .05$) porcine NK activity. Porcine NK activity was greater ($P < .05$) when incubated with 0.22 ng/mL epinephrine than with 0.022 or 21.9 ng/mL epinephrine. These results suggest that physiological concentrations of CRH, ACTH, cortisol, and norepinephrine alone do not modulate pig NK activity. β-Endorphin seems to be a potent suppressor of porcine NK activity. Epinephrine influenced pig NK activity in a dose-sensitive manner.

Introduction

Stress (physical, chemical, psychosocial) represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects (Khansari et al., 1990). An important physiological response to stressors is the activation of the hypothalamic-pituitary-adrenal axis (Moberg, 1985). Hypothalamic corticotropin releasing (CRH) hormone is a primary mediator of the stress response (Britton et al., 1984; Dunn and Berridge, 1990; Chrousos, 1992). CRH stimulates the anterior pituitary to release adrenocorticotropin hormone (ACTH) and other peptides, including β-endorphin, derived from the proopiomelanocortin molecule (Vale et al., 1981). ACTH in turn stimulates the adrenal cortex to secrete glucocorticoids (Axelrod and Reisine, 1984; Hillhouse et al., 1991), of which cortisol is the most prevalent in the pig. Release of catecholamines (norepinephrine and epinephrine) also constitute an initial response to stress. Catecholamines are also controlled via activation of sympathetic neurons by CRH and discharged from the adrenal medulla (Axelrod and Reisine, 1984; Kopin et al., 1988).

Natural killer (NK) cells are large granular lymphocytes, which are capable of "natural" cytotoxicity. That is, they can kill tumor and virus-infected cells without prior antigenic stimulation. Animals, including pigs, that are stressed normally experience reduced NK activity (Keller et al., 1991; McGlone et al., 1993) which may result in infectious disease. Thus, in the present studies we sought to determine if physiological concentrations of various hormones had an effect on *in vitro* NK cytotoxicity in pigs.

Materials and Methods

Separate experiments were performed in this study using blood samples from 59 prepubertal gilts (one sample per pig). Assays were performed within a few hours of blood collection. Gilts were bled via venipuncture into heparinized syringes. Pigs were commercial-style Camborough-15 crossbreds from lines of Pig Improvement Company, Inc. (Franklin, KY). All subjects were sired by PIC 405 boars. At the time of bleeding, the pigs were 7 to 9 weeks of age.

Natural Killer Cell Assay

Ten mL of heparinized whole pig blood (10 U of sodium heparin ml^{-1}) was collected from each animal and assayed for NK cell activity. The NK cell assay was performed according to standard techniques for porcine NK assay (Lumpkin and McGlone, 1992). Briefly, ten milliliters of blood were centrifuged for 20 min at 850 *g*. The buffy coat, containing the peripheral blood mononuclear cells (PBMC), was removed and mixed with Roswell Park Memorial Institute (RPMI) 1640 (Sigma Chemical, St. Louis, MO, USA; with 2.0 g L^{-1} of NaHCO3 and 100 U mL^{-1} of gentamicin sulfate). The buffy-coat-RPMI mixture was layered onto 4 ml of histopaque 1077 (Sigma Chemical) and centrifuged at 400 *g* for 30 min at 25°C. The PMBC were collected and washed once in RPMI at 850 *g* for 15 min. Adherent monocytes were removed by adherence to sterile plastic Petri dishes for 2 h in a 5% CO_2 humidified chamber. Non-adherent PBMC were collected by gently rinsing Petri dishes with RPMI then centrifuging recovered cells for 15 min at 850 *g*. The recovered PBMC were resuspended in 1 mL of RPMI and were

counted on a Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The samples were diluted in RPMI supplemented with 10% fetal bovine serum (FBS) (Sigma Chemical) and adjusted to a cell concentration of 1 X 10^7 cells mL^{-1}.

The targets for the assays were K-562 cells from a human chronic myelogenous leukemia cell line (American Type culture Collection, Rockville Pike, MD, USA). The targets were maintained in log growth in RPMI-10% FBS. Target cells were labeled with ^{51}Cr by incubating 5 X 10^6 cells in 1 mL of RPMI-10% FBS with 100 μCi of ^{51}Cr for 1 h in 5% CO_2. After 1 h, 10 mL RPMI-10% FBS was added and targets were incubated an additional 1 h, The target cells were pelleted and washed twice and resuspended in RPMI-10% FBS to a final concentration of 1 X 10^5 cells mL^{-1}.

Samples were run in triplicates at effector:target ratios (E:T) of 12.5:1, 25:1, 50:1 and 100:1; 10^4 target cells were added to each well. Total volume per well was 200 mL. Maximum ^{51}Cr release was determined by adding 100 mL of 7.5% Triton-X detergent (Sigma Chemical) to lyse all targets. Spontaneous ^{51}Cr release was determined by adding 150 mL of RPMI-10% FBS to target cells and counting radioactive label in the supernatant. Plates were incubated in a 5% CO_2 humidified chamber for 18 h. Plates were centrifuged for 10 min at 125 *g*. One hundred microliters of supernatants were collected and transferred to a plastic tube. Each tube was counted for 1 min on a gamma counter.

Percentage of cytotoxicity was calculated using the following formula: [(experimental release cpm - spontaneous release cpm) / (maximum release cpm - spontaneous release cpm)] X 100.

Hormone Sources and Concentrations

Hormones were incubated with the effector/target cell mixtures for 18 hours. Porcine CRH (pCRH) was obtained from American Peptide Company (Sunnyville, CA). Porcine CRH was used at 0, 0.467, 4.67, 46.7, and 467 ng/mL (10^{-9} to 10^{-6} moles). ACTH was obtained from Sigma (St. Louis, MO). ACTH was used at 0, 0.045, 0.45, 4.5, and 45 ng/mL (10^{-9} to 10^{-6} moles). β-endorphin was obtained from Sigma (St. Louis, MO). Concentrations ranged from 0, 1, 10, 100 pg/mL and 1 ng/mL (10^{-12} to 10^{-7} moles). Sigma (St. Louis, MO) supplied cortisol and it was used at 0, 3.6, 36, and 360 ng/mL (10^{-8} to 10^{-5} moles). Epinephrine and norepinephrine were obtained from Sigma (St. Louis, MO). Their concentrations ranged from 0, 0.022, 0.22, 2.2 and 21.9 ng/mL (10^{-10} to 10^{-7} moles) and 0, 0.021, 0.21, 2.1 and 20.6 (10^{-10} to 10^{-7} moles), respectively.

Statistical Analysis

Analysis of variance (ANOVA) was calculated using general linear model procedure (SAS, 1990) with NK cytotoxicity as the dependent variable and dose as the independent factor. Effector:Target (E:T) ratios were considered sub-plots in the spilt plot experimental design. The statistical model examined effects of pig, dose, dose*pig, E:T ratio and dose*E:T ratio. The split-plot design used dose*pig to test dose and pig effects while the residual error was used to test the remaining effects. Dose*E:T effects were not significant ($P > .10$) for any of the hormones; therefore, main plot means are only presented. Means were separated by predicted difference test within the SAS (1990) General Linear Models procedures.

Results

Over the range of doses evaluated, pCRH, cortisol ACTH and norepinephrine had no significant effect on porcine NK activity (Table 3.1). However, β-endorphin and epinephrine had significant effects on porcine NK activity. Presented in Table 3.1 are the results of the statistical analysis for each hormone evaluated. Of particular interest are those hormones that had a significant ($P < .05$) effect of dose. For each hormone evaluated, the dose*E:T effect was not significant, indicating that each hormone uniformly influenced (or did not influence) NK activity over E:T ratios.

Hormone dose influenced ($P < .01$) NK activity for β-endorphin and epinephrine. All concentrations of β-endorphin suppressed NK activity (Fig. 3.1). Porcine NK activity was significantly greater when exposed to 0.22 ng/mL (10^{-9} M) epinephrine than 0.022 ng/mL (10^{-10} M) 21.9 ng/mL epinephrine (Fig. 3.2). Physiological concentrations of pCRH , cortisol ACTH and norepinephrine did not modulate pig NK activity.

Individual pig effects were significant for every hormone evaluated. However, only β-endorphin and cortisol significantly influenced NK activity for the E:T effect.

Discussion

Stress is known to suppress the immune system in both animals and humans. Neuropeptides, neurotransmitters and stress hormones, such as CRH, ACTH, cortisol, β-endorphins and catecholamines, have been proposed as mediators of stress-induced immunosuppression because they interact with immune cells of certain species *in vitro* at

certain concentrations (Golub and Gershwin, 1985; Griffin, 1989). The present study attempted to determine the factors that mediate porcine natural killer (NK) activity.

In previous studies, icv administration of CRH in the lateral ventricles of rats resulted in a dose-dependent splenic suppression of NK cytotoxicity (Irwin et al., 1987; 1988; 1990; Jain et al., 1991; Strausbaugh and Irwin, 1992). CRH administered intravenously to rats produced a 25% suppression of NK activity (Jain et al., 1991). *In vitro* concentrations of CRH (10^{-6} to 10^{-10}M) were shown to inhibit NK activity of human NK cells (Pawlikowski et al., 1988). But, Irwin et al. (1987) showed no effect of CRH *in vitro* on rat NK activity. Our research found that CRH also had no significant effect on porcine NK activity. Thus, while *in vitro* CRH may suppress human NK activity, this neuropeptide did not influence rat or pig NK activity.

In pigs, iv administration of ACTH increased NK cytotoxicity dramatically (McGlone et al., 1991). However, *in vitro* administration of ACTH did not affect NK cytotoxicity in humans (Gatti et al., 1993) mice (Carr and Blalock, 1990) or pigs (McGlone et al., 1991 and this study). Thus, any effects of ACTH on *in vivo* porcine NK activity would be mediated by compounds other than ACTH.

Direct addition of norepinephrine *in vitro* caused reduced NK activity in humans (Takamoto et al., 1991). Our data shows that norepinephrine did not significantly affect pig NK activity.

Epinephrine appeared to enhance NK cytotoxicity. *In vivo* administration of epinephrine to humans increased NK cytotoxicity (Tonnesen et al., 1984). In the pig, the effect of epinephrine on NK activity is dose-dependent. Depending on epinephrine

concentration, NK cytotoxicity was either not influenced or enhanced. Physiological concentrations of epinephrine in the pig are approximately 0.09 ng/mL (Baetz et al, 1973). At physiological concentrations (0.22 ng/mL), epinephrine seemed to increase NK activity compared with baseline NK activity.

Human NK cells, treated *in vitro* for 18 to 24 h with increased physiological concentrations of cortisol, resulted in a decrease in NK activity (Holbrook et al., 1983). In the present study, under similar conditions, cortisol did not affect NK activity.

Several studies have reported that in vitro β-endorphin enhances NK cytotoxicity. Both human and mouse NK cells have been shown to be enhanced by β-endorphins (Mandler et al., 1986; Williamson et al., 1988; Carr and Blalock, 1990; Gatti et al., 1993). In sharp contrast, in the pig, *in vitro* β-endorphin suppressed NK activity. If the pig NK cell is opioid sensitive, rather that glucocorticoid sensitive (at physiological concentrations), then treatment regimes could be directed towards blocking endogenous opioids to enhance NK activity among stressed pigs. Normal physiological concentrations of β-endorphin are approximately 22-27 pg/mL (Tsuma et al., 1995). In this range of β-endorphin, *in vitro* NK activity is significantly reduced.

All hormones evaluated were significant for individual pig effects. This should be investigated further to determine what factors influence these effects. For example, genotype (beyond genetic line) may play a role in these differences. Individual sires and dams may exert very large effects on NK activity of offspring. The wide and significant pig to pig variation indicates genetic family should be controlled within *in vivo* stress models and *in vitro* investigations.

Table 3.1. Results of statistical analysis for stress hormone effects on porcine NK activity (Mean is % cytotoxicity)

Hormone	P-values for Effects					N	Mean	SE
	Dose	Pig	Dose*Pig	E:T	Dose*ET			
pCRH	.5407	.0001	.4919	.1578	.9729	8	15.4	1.62
ACTH	.7067	.0001	.0114	.1692	.7346	10	6.7	1.32
β-Endorphin	.0054	.0001	.0054	.0001	.5791	10	12.8	1.63
Cortisol	.172	.0001	.4899	.0001	.7499	11	16.7	1.54
Epinephrine	.0046	.0001	.9396	.1805	.3918	10	8.6	1.65
Norepinephrine	.8931	.0001	.5222	.8554	.4895	10	15.2	2.35

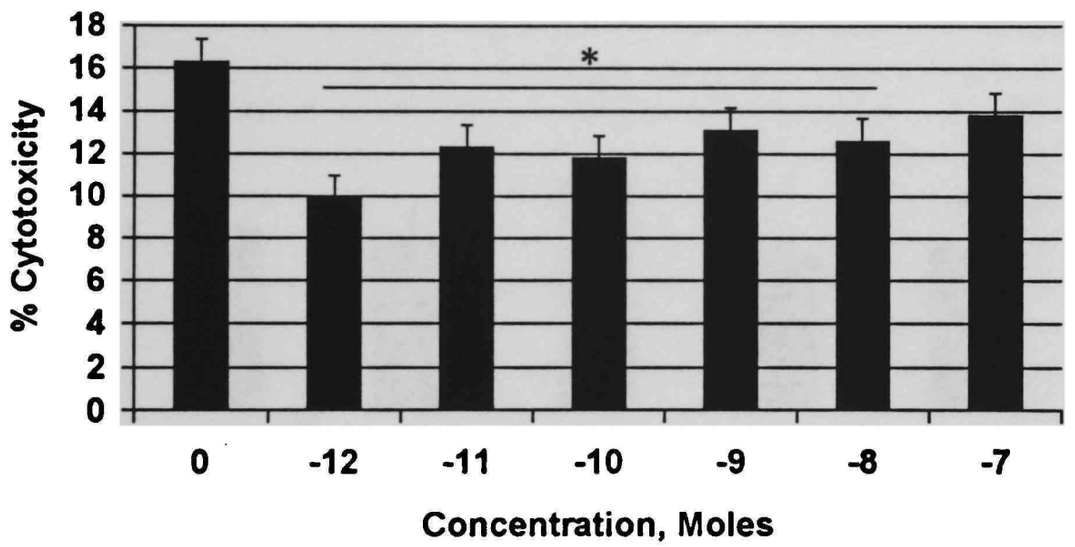

Figure. 3.1. Effects of concentration of β-endorphin on porcine NK activity. At all doses evaluated, except 10^{-7}, pig NK activity was suppressed ($P < .05$) by β-endorphin.

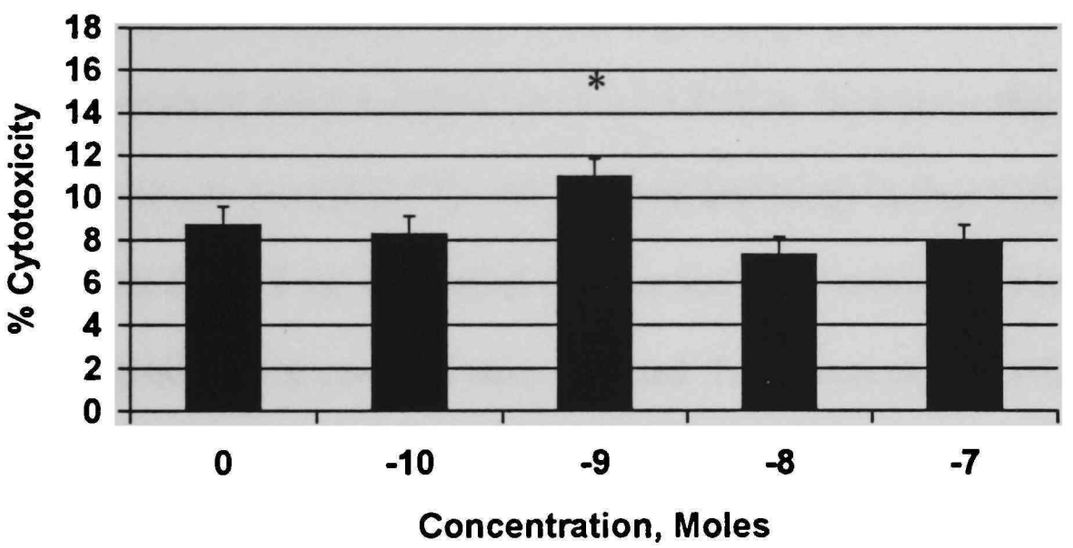

Figure 3.2. Effects of concentration of epinephrine on porcine NK activity. Pig NK activity was greater ($P < .05$) when incubated with 10^{-9} M than 10^{-10} M epinephrine.

CHAPTER IV

FINDING A CONSISTENT MEASURE OF ACUTE STRESS: BEHAVIORAL, ENDOCRINE AND IMMUNE MEASURES

Abstract

One hundred thirty-two weanling pigs were used to investigate the effects of three common stressors (and a control) and differing social status on behavior, immunity, plasma cortisol and blood chemical measures. Eleven blocks of 12 pigs each were evaluated. Each block contained four pens of three pigs, established at weaning, and examined for outcome of agonistic interactions. Pigs were labeled either socially Dominant (DOM), Intermediate (INT) or Submissive (SUB). Two weeks later, random pens of pigs experienced either a control treatment (CON) or for 4 hours they were shipped (SHIP), heat stressed (HEAT) with overhead heat lamps in their home pens, or cold stressed (COLD) by direct application of water and an air current. A battery of physiological and behavioral measures were collected. Treatments did not influence body weights, however, percentage weight change during SHIP was greater ($P < .001$) than for other treatments (.23, -2.9, .72, .91% ± .96 for CON, SHIP, COLD, HEAT, respectively). Serum glucose was elevated ($P = .02$) for SHIP pig compared with other treatments. Serum cortisol was decreased ($P = .03$) for HEAT compared with COLD and SHIP pigs. Social status influenced ($P < .01$) body weights at weaning and throughout the study with body weights heaviest for DOM pigs than INT and SUB pigs. Social status also influenced serum globulin, albumin/globulin (A/G) ratio, and cortisol concentrations. Globulin

decreased, while A/G increased in DOM pigs. SUB pigs showed an increase in cortisol compared to DOM and INT pigs. An interaction between social status and treatment occurred for NK cytotoxicity at 50:1 E:T (P = .06). Socially DOM and INT pigs had higher NK cytotoxicity than SUB, COLD pigs. An interaction was also observed between social status and stress treatment for lymphocyte blastogenesis under Phytohemagglutinin mitogen. Among HEAT pens, the INT pigs had lower lymphocyte proliferation than SUB pigs, but was not different from the DOM pigs. Among SHIP pens, the INT pigs had higher lymphocyte proliferation than either the DOM or SUB pigs. In conclusion, social status had large effects on plasma cortisol, globulin, A/G ratio, body weight and weight changes, although only acute shipping stress resulted in weight loss. Many immune and blood measures were not changed among acutely stressed pigs, however, the relationship between social status and LTA and NK cytotoxicity was disrupted during acute stress. Pig behavior was largely influenced across all stress treatments. COLD pigs had greater overall activity, including, feeding, standing and attacking and they had less lying behavior. The HEAT and SHIP pigs had depressed activity levels and spent more time lying. During acute stress, behavioral symptoms appears to be the most accurate and reliable indicator of stress.

Introduction

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects (Khansari et al., 1990). One response to acute stressors is activation of the hypothalamic-pituitary-adrenal

(HPA) axis, resulting in elevated CRH. CRH stimulates the anterior pituitary to release ACTH and other peptides. Elevated ACTH stimulates the release of glucocorticoids from the adrenal cortex into the blood of stressed farm animals (Dantzer and Mormede, 1983). Shipping (Nyberg et al., 1988; Parrot and Mission, 1989), social stress (Parrot and Mission, 1989), electrical stimulation and heat stress (Becker et al., 1985), and food and water deprivation (Houpt et al., 1983; Parrot and Mission, 1989) have been shown to increase plasma cortisol concentration in pigs. During the production and marketing of pigs, a number of stressful situations lead to production losses and pathological disease. Pigs are exposed to many environmental stressors (i.e., heat, cold, mixing, weaning, noise and shipping) that have been shown to increase disease susceptibility and impair immune function (Kelley, 1980, 1985).

While scientists have studied social, heat, cold and shipping stress, the wide variety of measures are bewildering. Pigs transported 4 hours had reduced immune function (NK cytotoxicity) particularly among submissive pigs and weight loss of 5% of their body weight (McGlone, 1993). Nyberg et al. (1988) suggested that transportation had long-term effects on cortisol concentrations, but McGlone et al. (1993) found only short-term effects of shipping on blood cortisol concentrations. Long-term cold stress in the nursery phase resulted in heavier adrenal glands, but had no effect on serum cortisol or antibody response (Kornegay et al., 1986). Exposure to an unpredictable and uncontrollable draft, caused a fourfold increase in exploratory activity that was expressed as aggressive behavior towards penmates (Scheepens et al., 1991). Morrow-Tesch et al. (1994) showed heat stress affected weight gain and immune measures in late nursery-stage pigs. The heat

stress treatment, however, had no effect on the duration of aggression or fighting or on the behavior of the pigs. The control pigs showed increased weight gain over time compared with the heat stress pigs. Several immune measures were altered by heat stress including leukocyte numbers and antibody titers. Stress has possible effects on behavior, growth, endocrine and immune measures. The objectives of this study was to determine the effects of relevant acute stressors (heat, cold and shipping) on pig performance, physiology and behavior.

Materials and Methods

Animals and Housing

Within each of 11 blocks, 12, 4-week-old pigs of the same sex (either gilts or barrows) and the same genotype (either PIC Camborough-15, or Yorkshire-Landrace cross) were weighed, weaned and randomly assigned to one of four pens. There were 3 non-littermate pigs per pen (44 pens total). The pigs were held in raised pens, measuring 1.2 X 1.2 m, with woven-wire mesh flooring. One six-hole feeder and one nipple-waterer was in each pen. Pigs were fed a 19% crude protein, nutritionally balanced, corn-soybean based diet and water ad libitum. Pigs were maintained on a constant 24 h light photoperiod throughout the entire experimental period. Once the animals were allotted to their appropriate pen, each pen was videotaped for 24 h for later viewing to establish dominance order (dominant, intermediate and submissive, as in McGlone et al., 1993). The pigs were allowed to acclimate to their new environment and penmates for 2 weeks before treatments began.

Each pen was randomly assigned as either a treatment (heat, cold, or ship) or control pen. One day prior to administration of treatments, anemometer (wind speed) measures and preliminary respiration rates were taken. The following day, each pen of pigs and the feeders were weighed and the pigs were subjected to their assigned stressor for 4 h. The heat stress pen was exposed to 4, 250-watt infrared heat lamps 1.09 m above the pen. Pigs in the cold stress pens were misted with water every 30 minutes and a oscillating fan produced a constant draft averaging 1.5 m/sec. The shipping pigs were placed in the back of a pickup truck with wheat straw bedding and the truck was driven for 4 h. Each group of pigs were videotaped during the stress period. Upon arrival of the shipping pigs, each feeder and pig was again weighed and bled via jugular venipuncture using 20 gauge, 2.54 cm. vacutainer needles while inverted on a V-trough. Two 7-mL heparinized tubes and one 7-mL serum tube were collected per pig. In addition, the pigs were injected subcutaneously with 1 mL of thrice-washed 40% sheep red blood cells (SRBC) to measure antibody response. The collected blood samples were centrifuged for 10 min to separate plasma or serum. Samples were assayed for NK, LTA, chemotaxis/chemokinesis and neutrophil phagocytosis, immediately after collection. Serum and plasma samples were also stored at -70°C in aliquots until they were later assayed for various measures. Five days post-treatment, the feeders and pigs were weighed and the pigs were bled via venipuncture using vacutainer needles and one 7-mL heparinized tube for antibody titers.

Natural Killer Cell Assay

A ^{51}Cr-release assay was performed to measure porcine NK cytotoxicity according to standard techniques described by Lumpkin and McGlone (1992). Porcine peripheral lymphocytes were used as effector cells and K562 chronic human leukemia tumor cell (American Type Culture Collection, Rockville Pike, MD) were used as the target cells. Whole blood was centrifuged at 850 x g for 20 minutes. The buffy coat was aspirated. The buffy coat was mixed with Roswell Park Memorial Institute (RPMI) 1640 (Sigma Chemical Co., St. Louis, MO) and layered over histopaque 1077 (Sigma Chemical Co., St. Louis, MO) and centrifuged at 400 x g for 30 minutes. The peripheral mononuclear cells (MNC) were washed once with RPMI. MNC cells were incubated for 2 h in order to remove the adherent cells. After 2 h incubation period, the non-adherent MNC were collected and washed in RPMI. The concentration of the non-adherent PMN were adjusted to 1 x 10^7 cells/mL in RPMI. Target cells were maintained in log growth phase prior to assay. Target cells were labeled with 200 μCi of inorganic ^{51}Cr (ICN Immunochemicals, Costa Mesa, CA) for 2 hours prior to the assay. Targets were washed several times and were resuspended in RPMI-10% fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, MO). A constant 10,000 targets per well were used in the assay. Samples were run in triplicates at effector (lymphocytes) to target (K562) ratios (E:T) of 12.5, 25, 50 and 100 to 1. The plates incubated for 18 h in a 5% CO_2 humidified chamber. One hundred microliters of supernatants were collected and counted on a gamma counter. Maximum release was determined by adding 7.5% Triton-X detergent (Sigma Chemical Co., St. Louis, MO) to the target cells to lyse all targets (Sigma

Chemical Co., St. Louis, MO). Spontaneous release was determined by incubating labeled targets with RPMI-10% FBS.

Percent cytotoxicity was calculated as the difference between the experimental release and the spontaneous release divided by the difference between the maximum release and the spontaneous release. Assays were considered valid if SR/MR was less than 30%.

Lymphocyte Transformation Assay (LTA)

The mitogen-induced lymphocyte proliferation assay was used to determine proliferation of T cells according to a modification of the method of Mosemann (1983) and as described in Morrow-Tesch et al. (1991). The buffy coat was placed in a 15-mL conical centrifuge tube and mixed with 10 mL of Rosewell Park Memorial Institute (RPMI) 1640. Ten milliliters of the buffy coat-RPMI mixture was layered onto 4 mL of histopaque 1077 (Sigma Chemical Co., St. Louis, MO) and centrifuged at 400 X g for 40 min at 25°C. The opaque interface containing the mononuclear cells was aspirated and transferred to a clean conical centrifuge tube. The opaque interface was washed in RPMI and centrifuged at 800 X g for 10 min. the supernatant was removed and the cells were resuspended in 1 mL of RPMI. The cells were diluted at 1:1500 and were counted using the Coulter counter (Hemo-W, Coulter Electronics, Hialeah, FL). The samples were diluted in RPMI supplemented with 10% fetal bovine serum (FBS) (Sigma Chemical) and a final cell concentration of 5 X 10^6 cells/mL was obtained.

One hundred microliters of diluted cells were added in triplicate to the 96-well flat-bottom sterile plate. Phyto-hemaglutinin (PHA; ICN, Costa Mesa, CA) at 0, 0.2, 2.0 and 20 μg/mL was added to each well in triplicate. The plates were incubated at 37°C in 5% CO_2 for 48 h. The cells were pulsed with 50 μL (1 μCi/well) of tritiated thymidine^{14} (^{3}H) (ICN, Immunochemicals, Costa Mesa, CA) diluted to 20 μL/mL. The plates were incubated for 24 h. After 72 h, cells were harvested on glass fiber filters. Each filter disk was placed in a scintillation vial. Five milliliters of scintillation fluid was added to each vial, samples were counted on a scintillation counter and cpm were calculated.

Neutrophil Chemotaxis and Chemokinesis

Ten mL of heparinized whole pig blood was mixed with 5 mL of Roswell Park Memorial Institute (RPMI) 1640 medium. The mixture was layered onto 3 mL of Histopaque 1077 and 3 mL of Histopaque 1119 (Sigma) and centrifuged at 700 X g for 30 min at 25°C. The opaque interface containing the mononuclear cells was aspirated and discarded. The polymorphonuclear cells (PMN) interface including some red blood cells (RBC) were aspirated and transferred to a clean, sterile conical centrifuge tube and washed one time in RPMI. Red blood cells were lysed using a hypertonic solution of ammonium chloride-tris base (0.1 mL of cells mL^{-1} of NH_4-tris base). Supernatants were discarded and the PMN pellet was washed twice in RPMI and centrifuged at 600 X g for 10 minutes. PMN's were counted using an electronic Coulter counter and cell concentration adjusted in RPMI to 2.5 X 10^5 cells per 50 μl.

A modified Boyden chamber (Neuro Probe, Cabin John, MD) was used to measure the migration of neutrophils towards RPMI (control; chemokinesis) or toward 10^{-8} M of recombinant human complement C5a (rhC5a; chemotaxis). RPMI (30 µl) or rhC5a was ed into the wells of the bottom chamber. The chamber was placed in an incubator mal equilibration of the bottom chamber. In the wells of the top chamber, 50 µl of pared and suspended cells in RPMI were pipetted. The chamber was incubated for 7°C in a humidified chamber (5% CO_2). The polycarbonate (pore size 5 µm; be, Cabin John, MD) filter was fixed and stained using LeukoStat I and II (Fischer Scientific, Houston, TX). The cells that migrated to the underside of the e counted in a blind fashion by the technician. Five fields per well were counted with a light microscope and duplicates were averaged.

l Phagocytosis and Oxidative Burst

flow cytometric assay was performed to measure neutrophil phagocytosis and urst using standard techniques described by Bohmer et al. (1992). To quantify is, equal volumes of Pansorbin 10% wt./vol. (*Staphylococcus aureus*, , San Diego, CA) and propidium iodine (PI; Calbiochem, San Diego, CA) and allowed to stand for 30 min at room temperature. The bacteria solution twice and resuspended in Hanks Balanced Salt Solution (HBSS, Sigma , St. Louis, MO) at 5% wt/vol.

antify oxidative burst, 500 µM 2', 7' dichlorofluorescein diacetate (DCFH- Kodak Co., Rochester, NY) was added to 440 µL of whole blood. The

mixture was placed in a 37°C shaking waterbath for 10 min to load the dye. Fifty microliters of the blood mixture was added to 1.5 mL of a lysing reagent (T = 0). After 5 min, the mixture was washed and resuspended in 200 μL HBSS. To the original blood mixture, 50 μL of the PI labeled Pansorbin was added and agitated in the waterbath. Ten minutes after the bacteria was added, a 50 μL sample was taken and added to the lysing reagent. The sample was washed twice and resuspended in 200 μL of HBSS. Each sample was placed on ice and analyzed on a flow cytometer (Becton Dickinson, Mountain View, CA). Standardization occurred using FITC and PI beads (Molecular Probes, Eugene, OR)

White Blood Cell Differentials

Blood smears were made from whole blood, fixed in methanol and stained with LeukoStat Solution I and II (Fisher Scientific, Houston, TX) for differential white cell counts. Total white blood cells (WBC) were counted using a Coulter cell counter (Hemo-W, Coulter Electronics, Hialeah, FL) at a 1:500 dilution of whole blood.

Antibodies to SRBC

The hemagglutination assay was performed in duplicate to determine pigs' antibody response to SRBC according to the methods of Blecha and Kelley (1981). Plasma samples were thawed and heat-inactivated in a 57°C water bath for 30 min. The heat-inactivated samples (200 μL) were placed in the first wells of each round-bottom plate. One hundred microliters of Phosphate Buffered Saline (PBS) were added to all

wells. Samples were diluted by removing 100 μL of each sample with the octapipette and serially diluting each well. To each well, 100 μL of 1% SRBC was added and the plates were agitated for 1.5 min. Plates were covered and incubated at room temperature for 24 h. The titers were determined by the sedimented cells forming a distinct pattern on the bottom of the wells. The highest dilution giving a positive reaction determined the titer.

Cortisol

Cortisol concentrations were determined using the radioimmunoassay procedure of Coat-A-Count (DPC, Diagnostic Products Corporation, Los Angeles, CA). Four plain (uncoated) 12 X 75 mm polypropylene tubes were labeled with total counts (T) and nonspecific binding (NSB) in duplicate. The cortisol antibody-coated tubes were labeled in duplicate with the calibrators A-F and sample numbers. Twenty-five microliters of the calibrator A was pipetted into the NSB and A tubes. Each calibrator and plasma sample was pipetted (25 μL) into their appropriate tube. One milliliter of ^{125}I-labeled cortisol was added to each tube and vortexed. The tubes were incubated in a waterbath at 37°C for 45 min. The tubes were thoroughly decanted and counted for 1 min in a gamma counter. Standard curves were calculated and used to determine cortisol concentrations from plasma samples.

Haptoglobin

Serum samples were sent to the Texas Veterinary Medical Diagnostic Laboratory (TVMDL) in Amarillo, TX for the determination of haptoglobin by an automated indirect

assay. The procedure described here is based upon the method of Roy et al. (1969), as modified by Elson (1974). This assay exploits the potential of haptoglobin-bound cyanomethemoglobin to resist the effects of incubation in an acid medium on either, the Soret-band optical absorbance or the peroxidase activity of unbound cyanomethemoglobin (HICN). The resistance of HICN to the loss of optical absorbance in the Soret band at pH 3.7 is proportional to serum haptoglobin concentration in mg/dL of HICN combining power. This method was developed for the Abbott Series II automated biochemical analyzer and uses primary and secondary wavelengths of 404 and 380 nm.

Fibrinogen

Plasma samples were sent to TVMDL in Amarillo, TX for the determination of fibrinogen. A microcapillary tube was utilized to draw up the plasma by capillary action. The tube was plugged and placed vertically in an empty critoseal holder. The critoseal holder was placed in a 56°C waterbath covering the whole plasma column of the tubes. The samples were immersed in the waterbath for 3 min and retrieved. The tubes were micro-centrifuged for the maximum packing time of the centrifuge. After centrifugation, a reader determined the height of the fibrinogen plug and used the formula: fibrinogen (mg/dL)= fibrinogen plug (%)/100 - PCV (%) X 10,000.

Alpha$_1$-acid Glycoprotein (AGP)

AGP concentrations were determined using an RID test kit (DTI, Development Technologies International, Inc., Frederick, MD). Five microliters of standard solutions

A, B and plasma samples were pipetted to a separately identified well of the test plates. The plate was securely covered and placed in a 37°C, humidified incubator for 24 to 48 h. After incubation, plates were removed and placed over a source of illumination to clearly see precipitin rings. The external diameter of the rings were measured to the nearest 0.1 mm using the scale provided. Using the measurements from standards A and B, a reference curve was plotted to determine the concentration (μg/mL) of the test samples.

Serum Chemistries

Serum samples were sent to TVMDL in Amarillo, TX for the determination of blood chemistry constituents. All blood chemical measures were analyzed on an Abbott Spectrum Spectrophotometer (Abbott Laboratories Diagnostics Division, Abbott Park, IL).

Total Protein

Abbott's Quickstart procedure was utilized to determine total protein in serum. Polypeptides containing at least two peptide bonds react with biuret reagent. In an alkaline solution, cupric ion forms a coordination complex with protein nitrogen with very little difference between albumin and globulin on a protein-nitrogen basis. Samples were assessed with a spectrophotometer.

Albumin

The A-Gent Albumin test was used to determine albumin concentration in serum. This test is based on the ability of albumin to bind to bromcresol green to form a complex which absorbs at 630 nm. The presence of a surfactant prevents the precipitation of the albumin-BCG complex and afford linearity.

Calcium

Abbott's QuickStart Calcium was used to determine the serum concentration of calcium. Serum calcium forms a colored chromophore with o-cresolphthalein complexone (o-CC) in alkaline solution. Samples were assessed with a spectrophotometer.

Phosphorus

To determine serum phosphorus, the Abbott Spectrum phosphorus test was utilized. Inorganic phosphorus reacts with molybdate in acidic solution to form phosphomolybdate. This formation of the phosphomolybdate complex is directly proportional to the total phosphorus content of the sample. Samples were assessed with a spectrophotometer.

Glucose

Abbott's QuickStart Glucose was used for the quantitation of serum glucose. In the presence of hexokinase, glucose is phosphorylated to form glucose-6-phosphate. Glucose-6-phosphate in the presence of glucose-6-phosphate dehydrogenase reduces

NAD^+ to NADH. The change in absorbance at 340/380 nm is proportional to the amount of glucose present in the sample.

Urea Nitrogen

Urea nitrogen was determined using Abbott Spectrum test. Urea is hydrolyzed to carbon dioxide and ammonia by urease. Ammonia subsequently reacts with α-ketoglutarate in the presence of glutamate dehydrogenase (GLDH) with the concomitant oxidation of NADH to NAD^+. The oxidation of NADH is measured by spectrophotometry at 364/652 nm and is proportional to the amount of ammonia which is, in turn, related to the amount of urea initially present in the sample.

Creatinine

Creatinine was measured using the Abbott Spectrum test. Creatinine reacts with alkaline picrate to form a colored complex. The amount of color complex formed is directly proportional to the concentration of creatinine in serum. Samples were assessed with a spectrophotometer.

Total Bilirubin

Total bilirubin was measured using the Abbott Spectrum test. Bilirubin combines with the diazonium salt of 2,4-dichloroaniline in the presence of sulfamic acid and surfactants to form azobilirubin, a pink chromophore measured by spectrophotometry at 548 nm.

Alkaline Phosphatase

Alkaline Phosphatase was measured using the RefLab test (Medical Analysis System, Inc. Camarillo, CA). ALP catalyzes the hydrolysis of colorless p-nitrophenyl phosphate (p-NPP) to give p-nitrophenol and inorganic phosphate. The rate of absorbance increase at 405 nm is directly proportional to the ALP activity in the sample.

Creatine Kinase

Abbott QuickStart test was utilized to determine CK concentration. This method is based on the CK catalyzed formation of adenosine triphosphate (ATP) from creatine phosphate and adenosine diphosphate (ADP). The ATP enters a hexokinase (HK)-catalyzed reaction which yields glucose-6-phosphate and regenerates ADP. The glucose-6-phosphate is then oxidized in a reaction with nicotinamide adenine dinucleotide phosphate (NADP) by glucose-6-phosphate dehydrogenase (G6PDH). The change in absorbance due to the formation of NADPH is measured bichromatically at 340/380 nm and is directly proportional to the CK activity.

Alanine Aminotransferase

The Abbott Spectrum test was used to determine ALT concentration. ALT catalyzes the transamination of L-alanine and α-ketoglutarate. Pyruvate is formed in the transaminase reaction and is measured indirectly by adding excess lactic dehydrogenase (LDH) and NADH to produce lactic acid and NAD. The decrease in absorbance in the

ultraviolet region as NADH is oxidized to NAD is directly proportional to the ALT activity of the sample. Samples were assessed with a spectrophotometer.

Aspartate Aminotransferase

The Abbott Spectrum test was used to measure AST. AST catalyzes the transamination of L-aspartate and α-ketoglutarate. The oxaloacetate formed in the transaminase reaction is measured indirectly by adding excess malic dehydrogenase (MDH) and NADH to produce malate and NAD. The decrease in absorbance in the ultraviolet region as NADH is oxidized to NAD is directly proportional to the AST activity of the sample. Samples were analyzed with a spectrophotometer.

Cholesterol

Serum cholesterol was measured using the Abbott Spectrum test. Cholesterol esters in serum are hydrolyzed to free cholesterol by cholesterol esterase. The cholesterol produced is oxidized by cholesterol oxidase in a reaction that results in the formation of hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine (4-AA) and phenol in the presence of peroxidase to yield a quinoneimine dye which absorbs at 500 nm.

Immunoglobulin G

IgG concentrations were determined using an radioimmunodiffusion test kit (DTI, Development Technologies International, Inc., Frederick, MD). Five microliters of standard solutions and diluted plasma samples were pipetted to a separately identified well

of the test plates. The plate was securely covered and placed in a 37°C, humidified incubator for 48 to 72 h. After incubation, plates were removed and placed over a source of illumination to clearly see precipitin rings. The external diameter of the rings were measured to the nearest 0.1 mm using the scale provided. A reference curve was plotted using the diameters measured from standard solutions. From the reference curve, the IgG concentration of each diluted test sample was calculated by multiplying the concentration read from the curve by the dilution factor to obtain the actual concentration, in mg/mL.

Corticosteroid Binding Globulin (CBG)

Serum samples were sent to Dr. Henry Kattesh at the University of Tennessee for the determination of CBG concentrations. A direct ELISA method was performed. Isolation and purification of porcine-CBG (pCBG) from serum was performed by affinity chromatography and HPLC-DEAE anion exchange techniques. pCBG (12 ng/well) was immobilized to microtiter plates and standards or samples added along with rabbit antiserum developed against the purified pCBG. Goat anti-rabbit IgG-alkaline phosphatase conjugate was added followed by p-NPP substrate. The resultant color development was read at 405 nm wavelength.

Behavior

Initially, pigs were videotaped for 24 h to establish dominance order. Videotaping was recorded at .8 frames/sec and viewed at 2.4 frames/sec. (Arnold-Meeks and McGlone, 1986). Two weeks later, during treatment exposure, the heat, cold and control

pigs were videotaped for 4 h at .8 frames/sec and viewed at 2.4 frames/sec. The pigs which were shipped were videotaped at real time for 4 h and viewed at real time. Tapes were analyzed by trained observers utilizing a sequence duration program described by McGlone et al. (1985). The behaviors recorded during the 4-h stress period were standing, feeding, drinking, sitting, attacking, being attacked and lying.

Statistical Analysis

All analyses were performed using SAS (1985) software. Analysis of variance was performed using the GLM procedure and means were separated with the predicted difference test. A randomized complete block design was utilized with a 4 X 3 factorial arrangement of treatments. Main effects included stress treatments and social status and interactive effects were stress treatments x social status. Correlation analyses were calculated overall to seek possible relationships among stress measures and calculated within treatments to determine relationships between stress treatments and measures to generate hypotheses for future work.

Results

Behavior

Pig behavior during stress was greatly influenced by stressor treatments, but not by social status (Table 4.1). Lying behaviors were statistically different for each treatment group. Heat stressed and shipped pigs exhibited more lying behavior (47.7 and 53.0 min/hr, respectively) than control or cold-stressed pigs. Cold-stressed pigs had the least

amount of lying duration, the greatest standing and sitting durations and thus were the most active pigs. Cold-stressed animals spent more time feeding and the heat-stressed pigs spent less time feeding (P = .0001), compared with control pigs. Cold-stressed and control-treated pigs showed similar duration of attack behaviors but more than heat stressed pigs.

Serum Chemistries and Acute Phase Proteins

A stress effect was significant (P = .02) for serum glucose concentration (Table 4.2). Shipped pigs showed an increase in glucose compared to control pigs (116.4 and 98.3, respectively). All other serum chemistry measures were not different among treatments.

Social status significantly influenced albumin/globulin ratio. Socially dominant pigs exhibited a trend for reduced serum globulin concentrations (Figure 4.1) and a higher albumin/globulin ratio (Figure 4.2) than pigs of lower social status. Social status did not have a significant effect on other serum chemistry measures.

Immune and Cortisol Measures

NK cytotoxicity was largely not influenced by stress treatment, however, cold stress elevated (P < .05) NK cytotoxicity at an effector:target ratio of 50:1 compared with control and shipped pigs (Table 4.3). An interaction between social status and treatment was observed for NK at 50:1 E:T (P = .06). Socially dominant and intermediate pigs had

higher NK cytotoxicity than submissive, cold stress pigs (Figure 4.3). Other stressors and pigs of each social status had statistically similar NK cytotoxicity.

Numbers of WBC, differential leukocyte counts, PCV and hemoglobin concentration were not influenced by stress treatment or social status.

The stressor treatments had a significant interaction with social status for lymphocyte proliferation (Figure 4.4). Socially intermediate, heat stressed animals had lower lymphocyte proliferation than submissive, heat stressed pigs. Socially intermediate, shipped pigs had higher lymphocyte proliferation compared to both dominant and submissive shipped pigs. Pigs of each social status had similar lymphocyte proliferation when in control and cold stress conditions.

Heat stressed pigs had lower cortisol (33.0 ng/mL; Table 4.3). compared to cold stressed and shipped animals (53.2 and 50.4 ng/mL, respectively). Control animals and heat stressed pigs had statistically similar values for cortisol. Social status also had an effect on cortisol (P =.06; Figure 4.5). Submissive pigs had higher cortisol concentrations (52.0 ng/mL) than intermediate pigs (39.7 ng/mL). Socially dominant pigs had an intermediate level of plasma cortisol (42.6 ng/mL).

Pig Performance

Pigs within each treatment had similar body weights at weaning and for their final weight (Table 4.4). Treatments did affect weight change during the 4-h stress period (P =.02). Shipped pigs lost 2.9% of their body weight while other treatment groups did not

significantly change body weight during the 4-h stress period. Acute stressors did not influence ADG, feed intake or gain:feed ratio before or after the stressor.

Social status had significant effects on weaning weight, pre-stress weight, post-stress weight, post-stress ADG and final weight (Table 4.5). Socially dominant pigs were heavier at the start and throughout the study period. Dominant pigs also gained more weight per day than socially intermediate and submissive pigs.

Correlation Analyses

Correlation analyses are presented in Table 4.6. Only correlation coefficients that were highly significant ($P < .01$) are presented. Antibody titers to SRBC were positively correlated with ADG ($r = .23$ to $.29$). Interestingly, antibody titers were positively correlated with neutrophil chemokinesis and negatively correlated with neutrophil chemotaxis.

ADG and body weights at each age were positively correlated with lymphocyte proliferation ($r = .26$ to $.29$) and % lymphocytes ($r = .24$ to $.30$). ADG was negatively correlated with standing ($r = -.24$) and positively correlated with lying ($r = .28$). Duration of attack, feeding, standing and sitting were correlated with each other (Table 4.6).

Serum glucose was positively correlated with lying ($r = .34$), cortisol concentration ($r = .38$) and post-stress average daily gain ($r = .33$), yet negatively correlated with drinking ($r = -.36$). Calcium and cholesterol concentrations were negatively correlated with sitting, while CBG concentrations were positively correlated with sitting. Urea concentrations were positively correlated with NK cytotoxicity at 25:1 (E:T) and cortisol,

but negatively correlated with pre-stress average daily gain. Creatine kinase was positively correlated with both LTA 20 (r = .32) and percentage of lymphocytes (r = .36). α_1-acid glycoprotein was positively correlated with NK cytotoxicity at both 25:1 and 50:1 (E:T). Cortisol concentrations were positively correlated with total protein and albumin. Total protein was also positively correlated with lymphocyte % (r = .43), yet negatively correlated with pre-stress average daily gain (r = -.34). Globulin was positively correlated with lymphocyte % (r = .50), but negatively correlated with neutrophil % (r = -.34). Alkaline phosphatase concentrations were positively correlated with neutrophil phagocytosis and pre-stress average daily gain. Phosphorus, body weights and pre-stress ADG were positively correlated with each other. Creatinin was positively correlated with all body weights, except during stress treatments and negatively correlated with pre-stress ADG. Pre-stress ADG was positively correlated with CBG concentrations (r = .53), yet negatively correlated with haptoglobin (r = -.33). CBG was also positively correlated with all measures of body weight.

Discussion

Cold Stress

Scheepens (1991) suggested that cold stress increased aggressive behavior in nursery pigs, including tail, ear, and flank biting compared to control pigs. This research showed that the cold stressed and control treatments had similar aggressive instances, while the heat and shipping stressed pigs were less likely to engage in aggressive behavior. Standing and feeding behavior were also increased among cold-stressed pigs.

Determinants of stress for the three stressors are presented in Table 4.7. Increased feeding did not lead to a higher feed intake which opposes work suggesting that feed intake was enhanced proportionally to the degrees of cold (Cabanac, 1974). Because pigs showed clear behavioral signs of cold stress, they may not have been actually ingesting feed while their head was in the feeder. Because of the constant draft, they may have their heads in the feeders to avoid the cold air. Other studies have shown that by decreasing temperature, growth rate was suppressed (Le Dividich, 1981). This also is inconsistent with the present study of acute stress. Growth was not affected by acute cold stress. A study examining fluctuating temperature found that growth, feed intake and feed-conversion efficiency was similar compared to control pigs (Minton et al., 1988). Overall, during the 4-h stress period, the cold stress pigs demonstrated a significantly higher activity level than either of the other treatments or the control pigs. The behavioral symptoms shown by the cold-stressed pigs included group huddling, shivering, shelter seeking and overall increased activity.

Cold stress elevated NK cytotoxicity. However, a significant interaction occurred between social status and treatment. Socially dominant and intermediate cold stressed pigs had elevated NK cytotoxicity compared to submissive cold stressed pigs. Studies show that acute cold stress results in an elevation of glucocorticoids, particularly cortisol (Webster, 1974). Although not significant, cortisol was highest among cold stressed pigs. The cold stressed pigs had increased cortisol compared to heat stressed pigs, but did not differ from control pigs' plasma cortisol. Minton et al. (1988) found that fluctuating temperatures did not affect serum cortisol, plasma glucose or antibody response.

Heat Stress

Pigs which were heat stressed generally had a depressed activity level. The heat stressed pigs mainly engaged in lying behavior, thus having significantly lower feeding and standing periods. Unlike the cold stress and control treatment, heat stress had no effect on aggression in pigs. Morrow-Tesch et al. (1994) found similar results in chronic heat-stressed pigs.

A significant interaction occurred between social status and heat stress for lymphocyte proliferation. Socially intermediate pigs had lower lymphocyte proliferation than submissive, heat stressed pigs. Although statistically similar with the control treatment, compared with the cold- and shipping-stressed pigs, cortisol concentration was decreased in the heat-stressed pigs. Previous studies have found heat stress to decrease feed intake and thus weight gain (Seymour, 1964; McGlone et al., 1987; Christon, 1988). Although feeding behavior was decreased in the heat stressed pigs, feed intake, gain:feed ratio, and weight gain were not different between control pigs or the other treatments.

Research has found that heat and social stress interact in their effect on the pigs' immune system. Chronic heat stress resulted in increased neutrophil numbers and decreased antibody production (Morrow-Tesch et al., 1994). These findings did not correspond with the present study of acute heat stress in that neutrophil number and antibody response were not effected by heat stress. During the 4-h period, the heat stressed pigs expressed symptoms such as: spreading out among the pen, panting and increased respiratory rates.

Shipping Stress

Overall, the pigs which were shipped had a decrease in activity level. The pigs did not have access to neither feed nor water during the 4-h stress period, so these activities were not relevant. The pigs spent the majority of the period lying.

The shipped pigs had a significant elevation in serum glucose. A study conducted by Cole et al. (1988) also found an elevation in serum glucose in relation to transport duration in calves. In addition to glucose; enzymes, iron, urea nitrogen, β-globulin, and urea nitrogen-to creatinin ratio determinations were also affected linearly by transport duration (Cole et al., 1988). This was not found in the present study, probably because the shipping experience lasted only 4 hours. Research has shown that shipping reduced NK cytotoxicity, particularly among submissive pigs (McGlone, 1993). This however, was not the case for this particular study. NK cytotoxicity was not affected by shipping. A significant interaction occurred between social status and shipping for lymphocyte proliferation. Socially intermediate shipped pigs had greater lymphocyte proliferation compared to dominant and submissive pigs.

A significant weight loss occurred during the stress period for the shipping pigs compared to the other treatments and the control pigs. During the 4-h stress period, ADG was also significantly decreased for the shipping treatment. This is consistent with past research. McGlone et al. (1993) found a 5% body weight loss among shipped pigs. The behavioral symptoms expressed by the shipped pigs included: chewing straw, weight shifting and huddling.

Social Status Effects

Physiological determinants of social status are presented in Table 4.8. Serum globulin was decreased in the socially dominant pigs, while albumin/globulin ratio was enhanced. Socially submissive pigs had elevated cortisol which is consistent with past research. Arnone and Dantzer (1980) have shown that socially subordinate pigs have slightly elevated blood glucocorticoids when placed in a new environment.

Socially dominant pigs were significantly heavier than social subordinates at weaning, before and after stress, and 5 d after stress upon completion of the study. Morrow-Tesch et al. (1994) found similar information. In their study, small differences in body weight led to the heavier pigs becoming socially dominant. All of the pigs gained weight over time, but the dominant pigs' weight gain was greater (Morrow-Tesch et al., 1994).

General

Throughout history, attempts to define measures of stress have been difficult and controversial. The endocrine system was thought to be an appropriate indicator of stress, yet has recently been discredited for many reasons. For example, stressful situations do elevate some stress hormones, yet many times stress hormones are elevated even though an individual may not be experiencing stress, as in a disease situation. Plasma cortisol was not consistently changed by stress. In fact, it tended to decrease among heat stressed pigs.

Certain stressors such as heat, cold and shipping have been studied individually. Because of the lack of agreement on the appropriate or "best" measures for stress, for this

study, a battery of physiological, behavioral, endocrine and immune measure were conducted. Although there were several treatment, social status and interactive effects, there were no consistent effects for each stressor, except one. In every treatment, the pigs' behavioral activity level changed. For example, the heat and shipping stressed pigs exhibited a depressed activity level, while the cold stressed pigs' activity increased. The ramifications of this study are that pigs and animals in general, respond with different behaviors to different stressors. Other than behavioral indicators, no one measure is consistent across each stressor. Physiological changes were stressor-specific while behavior was reliably and predictably changed in each stressor.

Table 4.1. Behavior of pigs during acute stress or control treatment. Table values are least squares means ± SE (min/h) and represent a 4-h period. P-value column refers to treatment effects.

Measure	Cold	Heat	Ship	Control	P-value
Feed	18.0 ± 1.1^{a}	5.7 ± 1.1^{b}	N/A	12.0 ± 1.1^{c}	.0001
Drink	$1.1 \pm .35$	$.70 \pm .35$	N/A	$.50 \pm .35$	.23
Stand	19.0 ± 1.3^{a}	5.8 ± 1.3^{b}	$6.9 \pm 1.5^{b,c}$	10.4 ± 1.3^{c}	.0001
Sit	$.85 \pm .24^{a}$	$.16 \pm .24^{b}$	$.55 \pm .27^{a,b}$	$.06 \pm .24^{b}$	.07
Attacking	$.12 \pm .03^{a}$	$.01 \pm .03^{b}$	$.03 \pm .04^{a,b}$	$.12 \pm .03^{a}$	.04
Being Attacked	$.06 \pm .03$	$.005 \pm .03$	$.005 \pm .04$	$.09 \pm .03$	.17
Lying	20.9 ± 1.8^{a}	47.7 ± 1.8^{b}	53.0 ± 2.0^{c}	36.9 ± 1.8^{d}	.0001

^{a,b,c,d}Means with uncommon superscripts differ $P < .05$.
N/A: not applicable because feed and water were not offered.

Table 4.2. Blood serum chemistries of pigs during acute stress or control treatment. Table values are least-squares means ± SE. P-value column refers to treatment effect.

Measure	Cold	Heat	Ship	Control	P-value
Total protein, g/dL	5.0 ± .17	5.0 ± .18	5.1 ± .18	5.1 ± .18	.92
Albumin, g/dL	2.8 ± .09	2.8 ± .09	2.8 ± .09	3.0 ± .09	.49
Calcium, mg/dL	10.3 ± .23	10.3 ± .24	10.3 ± .24	10.8 ± .24	.39
Phosphorus, mg/dL	6.77 ± .22	6.34 ± .23	7.01 ± .23	6.54 ± .24	.23
Glucose, mg/dL	98.2 ± 4.5[a]	100.7 ± 4.7[a]	116.4 ± 4.7[b]	98.3 ± 4.87[a]	.02
Urea, mg/dL	19.6 ± 1.1	18.8 ± 1.1	17.5 ± 1.1	18.6 ± 1.1	.61
Creatinin, mg/dL	1.2 ± .05	1.2 ± .05	1.2 ± .05	1.2 ± .05	.63
Total bilirubin, mg/dL	.32 ± .06	.27 ± .06	.39 ± .06	.41 ± .06	.41
Alkaline phosphatase, U/L	333.9 ± 19.6	292.7 ± 20.5	308.4 ± 20.5	317.6 ± 20.6	.53
Creatine kinase, IU/L	891.1 ± 453.1	895.1 ± 475.2	1425.4 ± 473.4	1530.0 ± 477.7	.67
Alanine aminotransferase, U/L	34.2 ± 1.6	29.9 ± 1.6	32.1 ± 1.6	30.7 ± 1.6	.26
Aspartate aminotransferase, U/L	45.1 ± 10.2	53.5 ± 10.7	56.6 ± 10.7	67.5 ± 10.8	.52

Table 4.2 (Continued)

Measure	Cold	Heat	Ship	Control	P-value
Cholesterol, mg/dL	58.1 ± 3.2	57.5 ± 3.4	60.9 ± 3.4	60.0 ± 3.4	.90
Sodium, mEq/L	148.9 ± 4.6	139.4 ± 5.4	148.7 ± 5.2	142.5 ± 5.6	.48
Potassium, mEq/L	5.5 ± .23	5.3 ± .26	5.4 ± .26	5.6 ± .28	.81
Chlorine, mEq/L	109.4 ± .69	108.2 ± .80	108.3 ± .78	106.7 ± .84	.11
Sodium/Potassium Ratio	26.1 ± .89	27.1 ± 1.0	27.2 ± 1.0	26.0 ± 1.1	.75
Globulin, g/dL*	2.1 ± .14	2.2 ± .15	2.2 ± .15	2.2 ± .15	.83
Albumin/Globulin Ratio*	1.5 ± .09	1.3 ± .09	1.4 ± .09	1.5 ± .09	.74
Hemoglobin, mg/dL	68.3 ± 16.9	62.3 ± 17.7	95.9 ± 17.6	94.2 ± 17.8	.42
Hemolysis, mg/dL	52.1 ± 13.2	61.9 ± 14.2	94.5 ± 14.1	71.8 ± 14.2	.17
Fibrinogen, mg/dL	110.2 ± 6.6	105.3 ± 7.1	98.7 ± 7.1	107.7 ± 7.2	.68

Table 4.2 (continued)

Measure	Cold	Heat	Ship	Control	P-value
Haptoglobin, mg/dL	110.3 ± 16.2	102.5 ± 17.5	80.5 ± 17.4	89.7 ± 17.6	.62
α-1-acid-glycoprotein, μg/mL	853.7 ± 317.6	837.6 ± 342.0	1600.1 ± 340.8	803.8 ± 343.8	.30
CBG, ng/mL	1604.3 ± 123.5	1530.1 ± 133.0	1671.9 ± 132.5	1587.4 ± 133.7	.90

*Dominance effect, $P < .05$

[a,b]Means with uncommon superscripts differ $P < .05$

Table 4.3. Immune measures and plasma cortisol for pigs during acute stress or control treatment. Table values are least squares means ± SE. P-value column refers to treatment effect.

Measure	Cold	Heat	Ship	Control	P-value
NK at 12:1 E:T	4.2 ± .72	4.7 ± .72	4.0 ± .77	4.7 ± .79	.88
NK at 25:1 E:T	6.5 ± .82	5.7 ± .80	4.7 ± .87	5.9 ± .89	.53
NK at 50:1 E:T*	8.0 ± .93[a]	5.9 ± .91[a,b]	4.2 ± .98[b]	5.1 ± 1.0[b]	.04
NK at 100:1 E:T	5.3 ± .69	3.9 ± .67	3.5 ± .73	4.5 ± .74	.32
WBC, %	19.2 ± .97	20.0 ± .97	18.7 ± 1.0	19.3 ± 1.0	.84
Hemoglobin, %	10.4 ± .27	10.2 ± .27	9.9 ± .29	10.3 ± .29	.68
PCV, %	37.8 ± .96	37.0 ± 1.1	38.7 ± 1.1	36.7 ± 1.1	.55
Eosinophil, %	8.0 ± .47	8.1 ± .47	8.0 ± .50	7.9 ± .51	.99
Neutrophil, %	41.8 ± 2.6	37.8 ± 2.6	43.0 ± 2.8	39.2 ± 2.9	.51
Lymphocyte, %	43.4 ± 2.6	47.8 ± 2.6	47.2 ± 2.8	47.5 ± 2.9	.61
Monocyte, %	14.1 ± 1.4	12.4 ± 1.4	9.8 ± 1.5	11.5 ± 1.5	.18
Other, %	1.3 ± .23	.87 ± .23	.74 ± .25	.97 ± .26	.45

Table 4.3 (Continued)

Measure	Cold	Heat	Ship	Control	P-value
Neutrophil:Lymphocyte	1.4 ± .19	.94 ± .19	1.3 ± .20	1.1 ± .20	.30
LTA 20, µg/mL PHA*	75553 ± 5760.2	82887 ± 5633.4	75529 ± 6085.9	88144 ± 6237.0	.39
LTA 2, µg/mL PHA	87894 ± 7438.2	92129 ± 7274.5	102665 ± 7858.9	84818 ± 8053.9	.41
LTA 0.2, µg/mL PHA	77136 ± 7352.9	79502 ± 7191.1	80310 ± 7768.8	84566 ± 7961.6	.92
LTA 0, µg/mL PHA	65231 ± 5347.1	75069 ± 5229.4	78876 ± 5649.6	69548 ± 5789.7	.32
Hemagglutination	3.4 ± .17	3.6 ± .17	3.2 ± .17	3.5 ± .17	.22
Chemotaxis	62.1 ± 6.1	52.7 ± 6.7	65.7 ± 6.9	59.3 ±	.58
Chemokinesis	35.1 ± 4.5	34.5 ± 4.9	28.9 ± 5.0	34.8 ± 5.0	.79
Neutrophil Phagocytosis, %	14.0 ± 2.4	13.8 ± 2.8	11.8 ± 2.8	14.5 ±2.4	.90
Oxidative Burst, %	1.7 ± .97	2.3 ± 1.1	2.9 ± 1.1	1.8 ± .97	.84
Cortisol, ng/mL**	53.2 ± 4.8[a]	33.0 ± 5.0[b]	50.4 ± 5.4[a]	43.2 ± 5.0[a,b]	.03
Immunoglobulin G, mg/mL	8.0 ± .71	8.3 ± .71	7.0 ± .81	6.7 ± .78	.34

[a,b,c]Means with uncommon superscripts differ P < .05. *Treatment by dominance effect, P < .05. **Dominance effect, P < .05.

Table 4.4. Performance measures of pigs during acute stress or control treatment. Table values are least squares means ± SE. P-value column refers to treatment effect.

Measure	Cold	Heat	Ship	Control	P-value
Body weight, kg					
Weaning weight**	7.0 ± .29	7.5 ± .31	7.1 ± .31	7.1 ± .31	.70
Pre-stress weight[c]**	8.9 ± .37	9.6 ± .39	9.0 ± .39	9.3 ± .39	.56
Post-stress weight[d]**	9.0 ± .37	9.7 ± .39	8.7 ± .39	9.4 ± .39	.34
% Weight change	.72 ± .92[a]	.91 ± .98[a]	-2.9 ± .98[b]	.23 ± .98[a]	.02
Final weight[e]**	10.7 ± .43	11.6 ± .46	10.6 ± .46	11.0 ± .46	.44
ADG, kg/d					
Pre-stress[f]	.14 ± .01	.15 ± .02	.13 ± .02	.16 ± .02	.51
During stress[g]	.09 ± .08[a]	.10 ± .09[a]	-.24 ± .09[b]	.02 ± .09[a]	.02
Post-stress[h]	.28 ± .02**	.31 ± .02	.31 ± .02	.27 ± .02	.32
Feed Intake, kg/d					
Pre-stress[f]	.28 ± .01	.30 ± .01	.29 ± .02	.30 ± .01	.76
During stress[g]	.25 ± .06	.14 ± .06	---	.29 ± .06	.07
Post-stress[h]	.67 ± .05	.70 ± .05	.74 ± .06	.64 ± .05	.63
Gain:Feed ratio					
Pre-stress[f]	.56 ± .06	.58 ± .06	.76 ± .09	.55 ± .06	.25
Post-stress[h]	.51 ± .04	.53 ± .04	.51 ± .06	.46 ± .04	.68

**Dominance effect, $P < .05$.
[a,b]Means with uncommon superscripts differ $P < .05$.
[c]Pretreatment, 14 d after weaning
[d] At the end of a 4-h treatment period
[e]5 d after treatment
[f] 14-d period after weaning
[g] Weight change during the 4 hour treatment period
[h] 5-d period after stress

Table 4.5. Social status effects on pig performance. Table values are least squares means ± SE. Other measures listed in Table 4.4 were not significantly influenced by social status. P-value column refers to dominance effect.

Measure	Dominant	Intermediate	Submissive	P-value
Weaning weight, kg	7.8± .26[a]	6.8 ± .26[b]	6.9 ± .26[b]	.02
Pre-stress weight, kg	10.0 ± .33[a]	9.0 ± .33[b]	8.6 ± .33[b]	.01
Post-stress weight, kg	10.1 ± .33[a]	8.9 ± .33[b]	8.6 ± .33[b]	.01
Final weight, kg	12.0 ± .39[a]	10.4 ± .39[b]	10.4 ± .39[b]	.004
Post-stress ADG, kg/d[c]	.40 ± .02[a]	.31 ± .02[b]	.35 ± .02[a,b]	.03

[a,b]Means with uncommon superscripts differ P < .05.
[c] 5-d period after stress

Table 4.6. Correlation analyses.

Measure 1	Measure 2	N	r
Antibody titer	Average Daily Gain	130	.23*
Antibody titer	Average Daily Gain 1	130	.29*
Antibody titer	Chemokinesis	96	.58*
Antibody titer	Chemotaxis	96	-.33*
Antibody titer	Eosinophil, %	127	.31*
Antibody titer	NK 12	114	-.30*
Antibody titer	Total white blood cells	128	-.33*
Chemokinesis	Drink	91	.28*
Chemokinesis	NK 12	95	-.30*
Chemotaxis	Chemokinesis	97	-.37*
Hemoglobin	Oxidative Burst	44	.41*
Total white blood cells	Chemokinesis	97	-.47*
NK 100	LTA 0.2	115	.25*
Calcium, mg/dL	Sit	65	-.63*
Glucose, mg/dL	Drink	65	-.36*
Cholesterol, mg/dL	Sit	64	-.39*
CBG, ng/mL	Sit	62	.51*
Glucose, mg/dL	Lying	65	.34*
Urea, mg/dL	NK 25	66	.34*
Sodium/Potassium ratio	LTA 20	55	-.38*

Table 4.6 (continued)

Measure 1	Measure 2	N	r
Alanine aminotransferase, U/L	NK 100	66	-.32*
Creatine Kinase, IU/L	LTA 20	66	.32*
Potassium, mEq/L	LTA 20	55	.40*
$alpha_1$-acid glycoprotein, μg/mL	NK 25	66	.31*
$alpha_1$-acid glycoprotein, μg/mL	NK 50	66	.33*
Total protein, g/dL	Cortisol	64	.34*
Albumin, g/dL	Cortisol	64	.38*
Glucose, mg/dL	Cortisol	64	.38*
Urea, mg/dL	Cortisol	64	.38*
Alanine aminotransferase, U/L	LTA 0	66	-.37*
Hemoglobin, mg/dL	Eosinophil, %	67	.39*
Globulin, g/dL	Neutrophil, %	67	-.34*
Globulin, g/dL	Lymphocyte, %	67	.50*
Total protein, g/dL	Lymphocyte, %	67	.43*
Total bilirubin, mg/dL	Eosinophil, %	67	.38*
Creatine kinase, IU/L	Lymphocyte, %	67	.36*
Aspartate aminotransferase, U/L	Eosinophil, %	67	.51*
Aspartate aminotransferase, U/L	Antibody titer	68	.34*
Alkaline phosphatase, U/L	Neutrophil phagocytosis	19	.59*
Phosphorus, mg/dL	Weight 1	68	.32*

Table 4.6 (continued)

Measure 1	Measure 2	N	r
Phosphorus, mg/dL	Weight 2	68	.32*
Phosphorus, mg/dL	Weight 3	68	.31*
Phosphorus, mg/dL	Average Daily Gain 1	68	.45*
Total protein, g/dL	Average Daily Gain 1	68	-.34*
Albumin, g/dL	Average Daily Gain 1	68	-.38*
Urea, mg/dL	Average Daily Gain 1	68	-.45*
Creatinin, mg/dL	Weight 0	68	.44*
Creatinin, mg/dL	Weight 1	68	.31*
Creatinin, mg/dL	Weight 3	68	.33*
Creatinin, mg/dL	Average Daily Gain 1	68	-.39*
Alkaline phosphatase, U/L	Average Daily Gain 1	68	.55*
Haptoglobin, mg/dL	Average Daily Gain 1	69	-.33*
CBG, ng/mL	Average Daily Gain 1	69	.53*
CBG, ng/mL	Weight 1	69	.46*
CBG, ng/mL	Weight 2	69	.46*
CBG, ng/mL	Weight 3	69	.43*
Glucose, mg/dL	Average Daily Gain 3	68	.33*
Alanine aminotransferase, U/L	Average Daily Gain 3	68	.33*
Sodium, mEq/L	Average Daily Gain 3	57	.34*
Packed Cell Volume, %	Oxidative Burst	44	.41*

Table 4.6 (continued)

Measure 1	Measure 2	N	r
Average Daily Gain 1	LTA 20	115	.29*
Average Daily Gain 1	Oxidative Burst	44	-.45*
Average Daily Gain 3	LTA 20	114	.26*
Average Daily Gain 3	Lying	120	.28*
Average Daily Gain 3	Stand	120	-.24*
Weight 0	Average Daily Gain 3	131	.21*
Weight 0	Lymphocytes, %	128	.24*
Weight 0	Total white blood cells	129	.23*
Weight 1	LTA 20	115	.28*
Weight 1	Lymphocytes, %	128	.30*
Weight 2	LTA 20	114	.26*
Weight 2	Lymphocytes, %	127	.31*
Weight 3	LTA 20	114	.29*
Weight 3	Lymphocytes, %	127	.27*
Attack	Feed	121	.30*
Lying	Feed	121	-.87*
Feed	Stand	121	.53*
Sit	Attack	121	.30*
Stand	Attack	121	.29*

*P < .01

Table 4.7. Determinants of stress for three common stressors. Table entries refer to significant differences relative to control pigs.

Category	Cold stress	Heat stress	Shipping stress
Behavior	↑ head in feeder	↓ feeding	N/A
	↑ standing	↓ standing	↓ standing
	↓ lying	↑ lying	↑ lying
Endocrine and Chemistries			↑ Glucose concentration
Immune	DOM & INT pigs had ↑ NK cytotoxicity		
		INT pigs had ↓ LTA	INT pigs had ↑ LTA
Performance			Weight loss
			↓ ADG during stress

Table 4.8. Physiological determinants of social status. Behavioral effects of social status were not significant for established social groups.

Category	Dominant	Intermediate	Submissive
Endocrine and Chemistries	↓ Globulin concentration ↑ Albumin/Globulin ratio		↑ Cortisol concentration
Immune	↑ NK cytotoxicity when cold	↑ NK cytotoxicity when cold ↓ LTA when heated ↑ LTA when shipped	
Performance	↑ Weaning weight ↑ Pre-stress weight ↑ Post-stress weight ↑ Final weight ↑ Post-stress ADG		

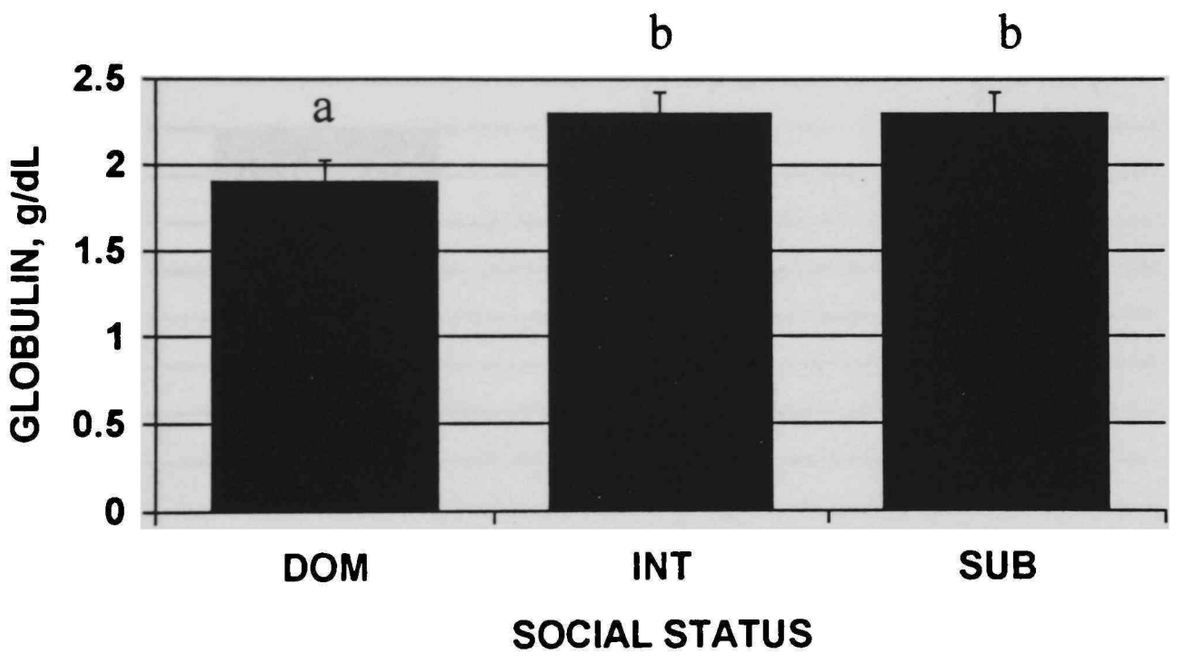

Figure 4.1. Social status effects on serum globulin concentration (P = .06). Least square means with a different letter (a,b) differ P < .05.

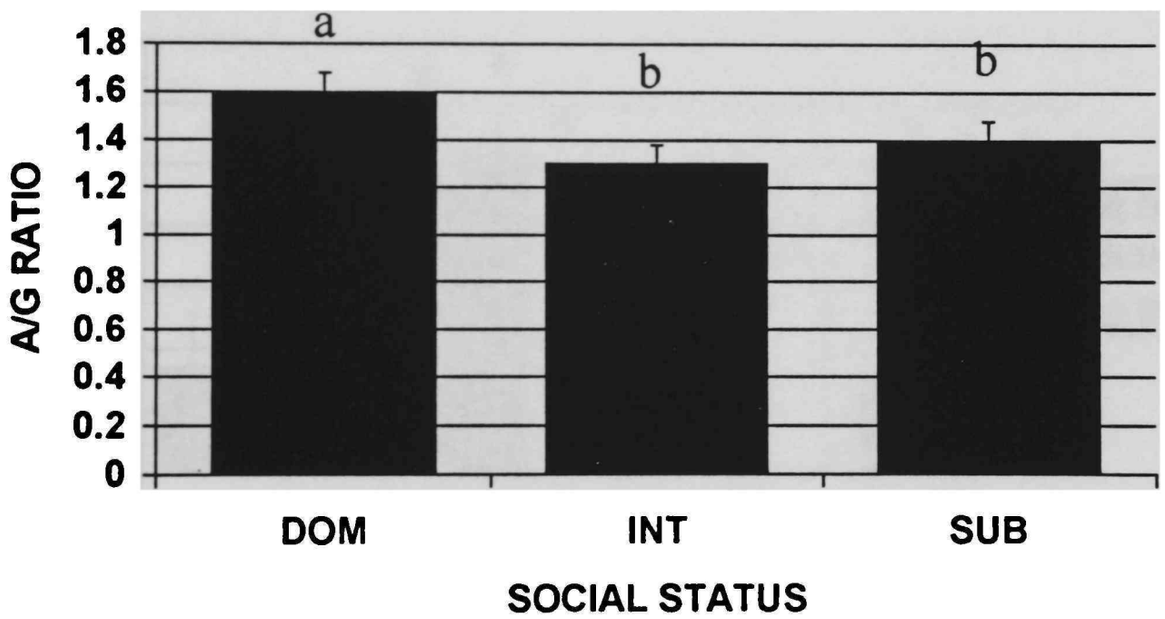

Figure 4.2. Social status effects on serum Albumin/Globulin ratio (A/G; P = .02). Least square means with a different letter (a,b) differ P < .05.

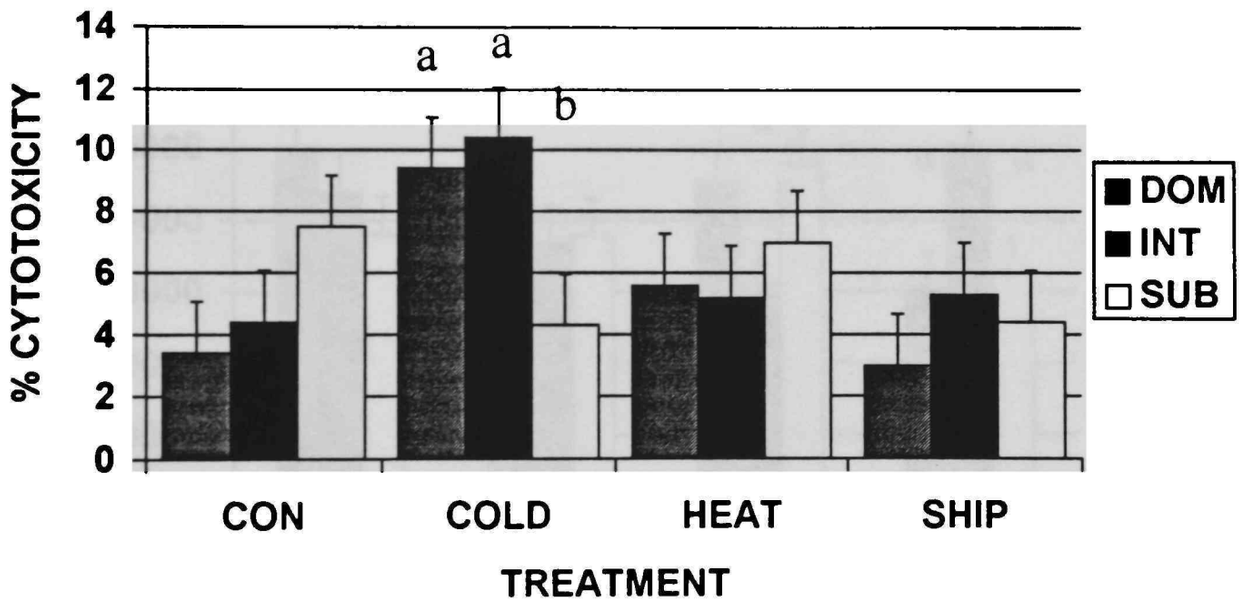

Figure 4.3. The relationship between NK cytotoxicity (E:T= 50:1), stress treatment and dominance. Least squares means with a different letter (a,b) differs $P < .05$. Treatment by dominance effect $P = .06$.

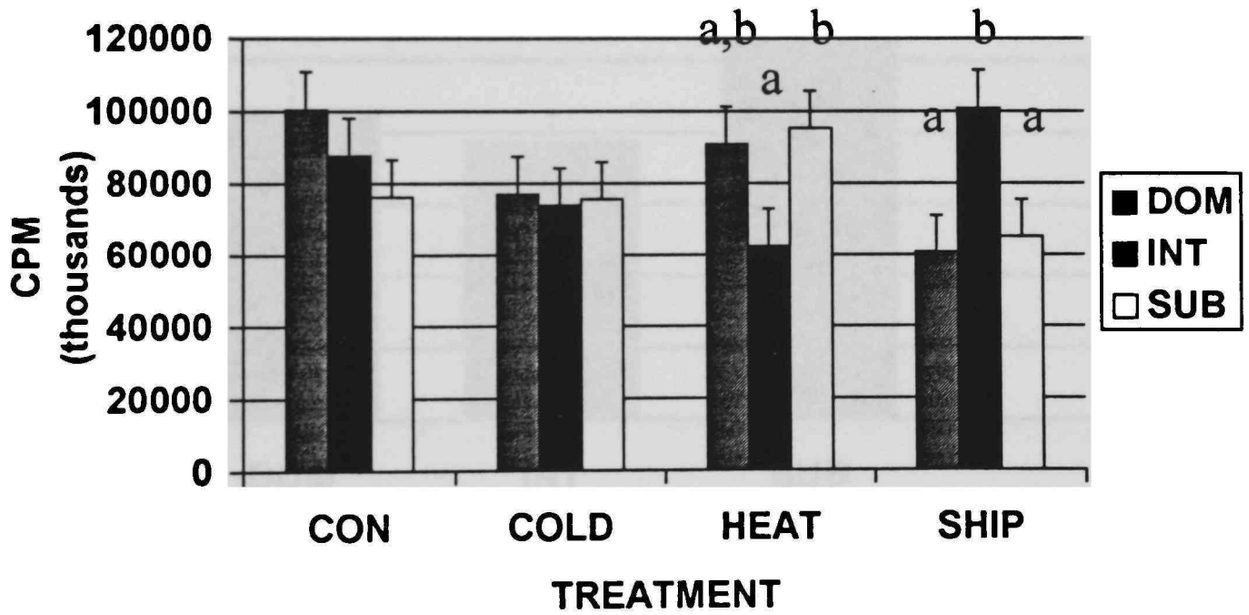

Figure 4.4. Relationship between lymphocyte proliferation (cpm of tritiated thymidine uptake), treatment and dominance. Least square means with a different letter (a,b) differs $P < .05$. Treatment by dominance effect $P = .01$.

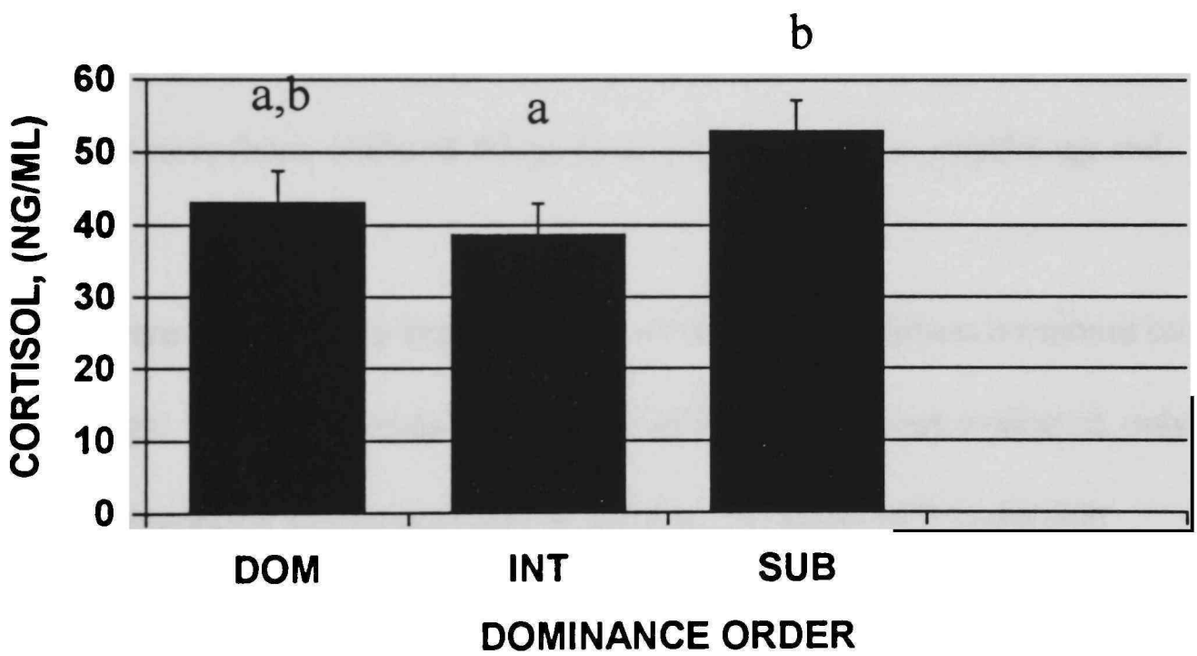

Figure 4.5. Social status was significant (P = .06) for cortisol. Least square means with a different letter (a,b) differ P < .05.

CHAPTER V

OVERALL DISCUSSION AND CONCLUSIONS

Stress is known to suppress the immune system, alter the behavior, and cause variation in the performance of humans and animals. Several hormones and neurotransmitters have been proposed as mediators of the stress response and stress-induced immunosuppression (i.e., CRH, ACTH, cortisol, β-endorphin and catecholamines; Goulub and Gershwin, 1985; Griffin, 1989; Peterson et al., 1991; Hauger et al., 1993). The ambiguity of stress effects on animal physiology remains evident today. Therefore, it was the intent of this research to determine if physiological concentrations of various hormones had effects on *in vitro* NK cytotoxicity in pigs and to determine the effects of three relevant stressors (heat, cold and shipping) on pig performance, physiology and behavior.

Studies were conducted to examine the *in vitro* effects of various hormones on NK cytotoxicity in pigs. These studies determined that of all the hormones evaluated, only β-endorphin and epinephrine altered porcine NK activity. All doses of β-endorphin decreased NK cytotoxicity, suggesting that β-endorphin may be a potent suppressor of porcine NK activity. These findings sharply contrast with various studies that have reported that *in vitro* β-endorphin enhances NK cytotoxicity. For example, both human and mouse NK cells have been shown to be enhanced by β-endorphin (Mandler et al., 1986; Williamson et al., 1988; Carr and Blalock, 1990; Gatti et al., 1993). This may suggest that the pig NK cell is opioid sensitive, rather than glucocorticoid sensitive.

Epinephrine appeared to enhance NK cytotoxicity, although dose-dependent. This is consistent with a study conducted in humans where *in vivo* administration of epinephrine increased NK activity (Tonneson et al., 1984).

The second study sought to determine the effects of three common stressors (heat, cold and shipping) on pig performance, physiology and behavior. Pig behavior rather than physiology was most consistently affected by the treatments evaluated. The cold stressed and control treatments had similar aggressiveness, while the heat and shipping stressed pigs were less likely to engage in aggressive behavior. In past studies, heat stress also did not affect aggressive behavior (Morrow-Tesch, 1994). Among the cold-stressed pigs, standing and feeding behavior was increased, while the heat- and shipping-stressed pigs' lying behavior increased. Studies show that cold stress leads to an enhanced feed intake (Cabanac, 1974) which is inconsistent with the findings from the present study. Although the cold-stressed pigs feeding behavior increased, neither feed intake nor gain:feed ratio was improved. Therefore, the pigs which were cold stress may not have actually been eating, but using the feeder to shelter themselves from the constant draft. Besides weight loss in shipped pigs during the 4-h period, pig performance was not affected by either of the stressors. However, socially dominant pigs tended to be heavier throughout the study compared with intermediate and submissive pigs.

In cold-stressed pigs, NK cytotoxicity was elevated overall and socially dominant and intermediate cold-stressed pigs had enhanced NK activity compared to submissive pigs. Socially intermediate, heat-stressed pigs had lower lymphocyte proliferation than submissive, heat-stressed pigs. In the shipped treatment, socially intermediate pigs had

greater lymphocyte proliferation compared to dominant and submissive pigs. Plasma cortisol concentration was lowered in the heat-stressed pigs compared to cold-stressed and shipped-pigs, but not different than the control pigs. Serum glucose was enhanced in the shipped pigs compared with the other treatments and the control pigs. This is consistent with past research indicating that shipping increases glucose concentration in calves (Cole et al., 1988).

These data indicate that stress is not easily measured by a few simple assays. Each stressor affects the treated animals differently and with different consequences. Certain behavioral changes, such as overall activity level, appears to be an important indicator that an animal may be experiencing stress. Being able to recognize the consequences of stress and understanding the impact of environmental stimuli on disease susceptibility would enable one to optimize the use of preventive veterinary care. For acute stress, behavioral symptoms seem the most consistent and reliable indicator of stress. Chronic stressors may (almost certainly do) have different symptoms.

Future Studies

Stress effects are yet to be understood, thus many new investigations are possible. The first addition to this project should be the determination of the catecholamines, norepinephrine and epinephrine. Catecholamine determination was attempted for this study, however, was not successful. Difficulty during the extraction phase was more than likely the probable cause of failure. In the first study, epinephrine was dose-dependent in enhancing NK activity in vitro. Possibly, epinephrine released in the cold-stressed pigs

resulted in the subsequent increase in NK activity. Epinephrine also promotes glycogenolysis which is the breakdown of glycogen stored in the liver and skeletal muscle to generate glucose as needed as a metabolic substrate. The shipping pigs had an elevation of serum glucose concentration. Possibly epinephrine levels were increased as well.

β-endorphin should also be determined when an appropriate assay is available. It would be interesting to determine the concentrations of other acute phase proteins, such as C-reactive protein. CRP was reported to represent a significant APP in pigs (Buerger et al., 1992). Cytokine measurement may also be considered. APP would seem to be a better measurement because they remain in circulation at least 24 hours and sometimes 2 to 3 days, while cytokines are cleared within a few hours (Hol et al., 1987).

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