To the Graduate Council:

I am submitting herewith a thesis written by David B. Kersten entitled "The Effect of T-2 Mycotoxin on (*Colineus virginianus)* Adult Bobwhite Quail." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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THE EFFECT OF T-2 MYCOTOXIN ON ADULT BOBWHITE QUAIL (*Colineus virginianus)*

A Thesis
Presented for the
Master of Science
Degree

The University of Tennessee, Knoxville

David Brian Kersten
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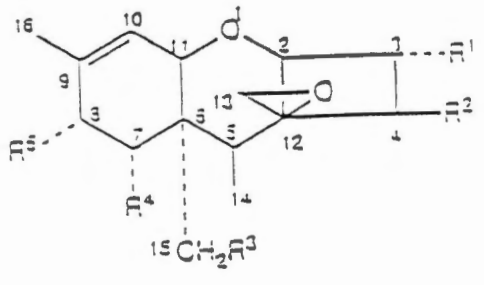
PART I

LITERATURE REVIEW

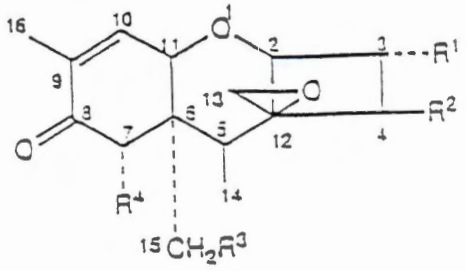
Trichothecene mycotoxins are a chemical group of fungal metabolites characterized by a tetracyclic 12, 13-epoxy-trichothecene skeleton (Steyn, 1980). A total of 148 trichothecenes, 83 non-macrocyclic and 65 macrocyclic, have been isolated from fungal cultures and plants (Grove, 1988). While a number of taxonomically unrelated fungi are capable of producing trichothecene's, the most common source is the species *Fusarium* (Uneo, 1983). Trichothecene's are end products of secondary metabolism and are byproducts of normal fungi metabolism. These end products play no obvious role in the economy of the organism, and are restricted to lower life forms (Betina, 1984). Secondary metabolism is often the mechanism by which organisms dispose of toxic byproducts of primary metabolism: the energy forming processes of the fungi (Betina, 1984). When levels of metabolic byproducts approach toxic levels, primary metabolic pathways shut down and secondary metabolic pathways start, often utilizing the same enzymatic pathways as primary metabolism (Steyn, 1980).

As a group, the trichothecenes have a variety of biologic effects which include growth inhibition of other fungi, and toxicity to plants, humans, and animals. Trichothecene mycotoxins can be divided into four categories according to similarity of functional groups (Uneo, 1977). Type A trichothecene's have a functional group other than a ketone at carbon 8. This is the largest group of trichothecene's and include T-2 toxin and diacetoxyscirpenol (DAS). Type B trichothecene's have a carbonyl function at carbon 8 and include 4-deoxynivalenol (DON) and nivalenol (NIV). Type C trichothecene's are characterized by a second epoxide group at carbon 7,8 or carbon 9,10 and Type D trichothecene's contain a macrocyclic ring system between carbon's 4 and 15 with two ester linkages (Nakadate & Uneo, 1990).

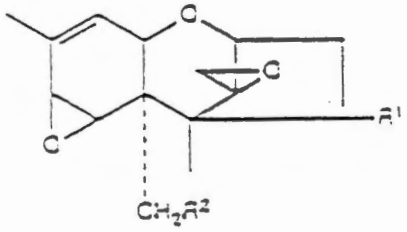
Type A Trichothecenes



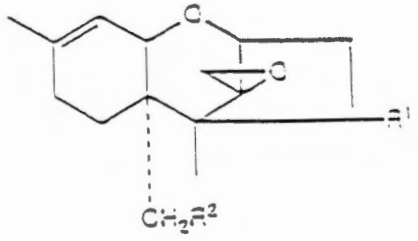
Type B Trichothecenes



Type C Trichothecenes



Type D Trichothecenes



T-2 Type A toxin is identified as 3-hydroxy-4, 15-diacetoxy-8-(3-methylbutyryloxy)-12,13-delta 9 tricothecene (Uneo, 1979). Because of its acute toxicity, immunosuppressive effects and potential use as a biological-chemical warfare agent, T-2 mycotoxin is one of the more thoroughly investigated mycotoxins (Corrier, 1991).

A Historical Perspective

The production of mycotoxins depends upon available substrates, temperature, humidity, and duration of incubation time (Uneo, 1983). Specifically, the yield of T-2 toxin was higher at

lower temperatures (7° C) than at higher temperatures (14-20° C; Giligan *et al.*, 1966). Cereal grains are often infected by *Fusarium* because of the practice of storing improperly dried grains in cool, dark silo's which make ideal habitat for these fungi (Joffe, 1986).

Mycotoxins have been implicated in the 10 plagues of Egypt, and other serious blights and plagues of ancient times (Schoental, 1984). Historical descriptions of the victims and climatic conditions during the 10 plagues indicate mycotoxins were prevalent in foodstuffs (Schoental, 1984). While such inferences are pure speculation, it is interesting to note that mycotoxins have also been implicated in Mozart's death, and King George the Third's well documented madness (Schoental 1983). Correlation's have been made between mycotoxins and the great plagues of Europe during the 12th century (Matossian, 1989). In fact, many victims displayed characteristics of mycotoxin poisoning rather than those of the bubonic plague (Matossian, 1989).

While modern agriculture aims to prevent fungal infestation, many underdeveloped countries still utilize ancient farming practices which expose grains to conditions conducive to fungal growth. Because of this, in the twentieth century, mycotoxins continue to be a problem often reaching epidemic proportions in underdeveloped countries (Forgacs and Carll, 1962).

In Russia, poisoning by cereal grains was endemic in many rural communities during the early 1900's (Forgacs and Carll, 1962). Medical records indicated that 10% of the population of Orenburg, Siberia, suffered from symptoms of a mycotoxicosis termed Alimentary Toxic Aleukia (ATA; Mayer, 1953). The fungi *Fusarium sporotrichiodes*, known to produce trichothecene mycotoxins, was isolated from toxic millet and shown responsible for the outbreak (Uneo, 1983). This fungi was able to produce toxic compounds on various grains which varied with climate. Greatest toxicity was observed during heavy snows of winter, and after frequent alternate freezes-thaws in the spring (Joffe, 1986). Patients suffered from a variety of symptoms including aplastic anemia (a lack of erythrocyte development resulting in a reduced cell numbers), hemorrhagic

aleukia (the escape of leukocytes from the blood), and agranulocytosis (marked reduction in granulocytes accompanied by throat, gastrointestinal tract, and skin lesions; Mayer, 1953). Alimentary toxic aleukia (ATA) is a non-contagious, non-infective, often fatal disease. The clinical symptoms developed in 4 stages stemming from pathologic changes in the bone marrow and other hematopoeitic (blood forming) compartments (Mayer, 1953). In the first stage, mild irritation of the mucous membranes and gastro-intestinal tract was followed by gastroenteritis, diarrhea, nausea and vomiting. The second stage was called the incubation period or leukopenic stage. It involved a symptom-less stage ranging from 2 weeks to 2 months during which the toxin exerted its destructive action upon the bone marrow. The most profound symptom of this stage was a dramatic drop in the absolute number of leukocytes. The third stage was the symptomatic stage during which the results of bone marrow depletion were seen. These symptoms included hemorrhagic diathesis, necrotic angina, a septic state and continuing leukopenia. The fourth stage was a period of recovery during which the body repaired damage from the toxin, and required as little as two weeks or as long as two months. Widespread outbreaks of ATA were attributed to both poor agricultural practices, and refusal of farmers to grow crops such as potatoes which are more resistant to mycotoxin infestation (Mayer, 1953).

In the United States during the 1960's, the development of toxicoses in farm animals after ingestion of moldy grains became a problem (Uneo, 1980). Corn harvested in the fall was often stored in cribs without proper drying which allowed mold to grow throughout the winter. Animals ingesting this moldy corn developed diarrhea, vomiting, refused food, and developed hemorrhagic toxicosis similar to ATA (Forgacs and Carll, 1962). Laboratory analysis of the moldy corn indicated trichothecene mycotoxins to be responsible.

The most recent outbreak of tricothecene mycotoxicosis was reported in 1987 in the Kashmir Valley, India where thousands of residents were afflicted with gastrointestinal (GI)

disorders (Bhat *et al.*, 1989). While GI disorders are not a rare occurrence in underdeveloped countries, the magnitude of the outbreak, and fact that the victims came from all economic classes suggested an epidemic. An investigation linked the disorders to locally produced bread made from moldy grains (Bhat *et al.*, 1989). Laboratory analysis of the grain indicated the trichothecene mycotoxins as the cause of the disorders. The severity of mycotoxicosis and the prevalence with which it occurs worldwide make mycotoxins an area worthy of investigation.

Trichothecene Mycotoxin Effects

Ingestion of mycotoxins cause a variety of detrimental effects on reproductive performance, immune function, and hematological parameters (Chi *et al.*, 1977a; Corrier, 1991; Mann *et al.*, 1983). Trichothecene mycotoxins are considered to be potent inhibitors of macromolecule (protein) synthesis in eukaryotic cells (Uneo, 1979). In addition to its ability to inhibit protein synthesis, T-2 toxin has also been observed to induce single strand breaks in the DNA of lymphoid cells (LaFarge-Frayssinet *et al.*, 1981). T-2 toxin is extremely cytotoxic and induces cellular damage to tissues characterized by the destruction of actively dividing cells in thymus, testis, intestine, spleen, liver, muscle and heart (Uneo, 1979; Thompson and Wannemacher, 1990).

Growth and Reproduction

Mycotoxin contamination of feed grains is difficult to regulate, and as a consequence of this, decreased growth and reproductive performance in farm animals has been reported. In all studies where T-2 toxin was ingested in food, test animals showed a decrease in overall body

weight reflecting an inability or lack of desire to ingest food (Sharlin *et al.*, 1981; Kubena *et al.*, 1995; Wyatt *et al.*, 1973; Speers *et al.*, 1977; Allen *et al.*, 1983). One of the main side effects of T-2 ingestion is the formation of lesions throughout the oral tract, which makes feeding difficult and painful. These lesions are the result of the caustic effects manifested by extensive necrosis of the oral mucosa and skin in contact with the toxin (Chi and Mirocha, 1978). This inflammatory response is thought to be the result of increased blood vessel permeability (Guarino *et al.*, 1968). Chemical analysis of inflammatory exudate revealed lower concentrations of sodium and proteins, and greater amounts of potassium, calcium and phosphorus than in the serum. Through increased permeability, fluids are allowed to leak into the subcutaneous space causing swelling and inflammation. This leads to the formation of oral lesions making feeding difficult and painful. The lesions consist of a fibrinous surface layer and a heavy infiltration of the underlying tissues by granular leukocytes. Secondary invasion of lesions by oral bacteria *Escherichia coli* and *Staphylococcus epidermis* lead to necrosis of the infected tissue (Nakadate & Uneo, 1990).

In studies utilizing either either quail or chickens significant reductions in both body weight and reproductive performance have been reported. Ruff *et al.* (1992) fed bobwhite and Japanese quail diets containing 4, 8 and 16 mg T-2/kg feed. In bobwhite quail, body weight was significantly reduced ($P \leq .05$) by 8 and 16 mg/kg diets by one week of age as compared to controls. In Japanese quail, body weight was reduced by 16 mg/kg diets by 1 week of age as compared to controls suggesting Japanese quail are slightly more resistant to T-2 toxin. In a similar study, Huff *et al.* (1992) fed day old ring-neck pheasants diets containing 4, 8 and 16 mg T-2 toxin/kg feed. Body weight gain was less ($P \leq .05$) among birds fed 16 mg/kg diet as compared controls. Body weight depression, growth retardation, and reduced food intake has also been observed in broiler chickens fed 4, 8, and 16 mg T-2 toxin/kg feed (Wyatt *et al.*, 1973; Kubena *et al.*, 1989). Toxin

administered to laying hens at a concentration of 20 mg/kg feed reduced egg production and shell thickness (Wyatt *et al.*, 1975). In a similar study, Chi *et al.* (1977a) reported that feed consumption, egg production, hatchability of fertile eggs, and egg shell thickness were significantly decreased in laying hens fed 8 mg T-2 toxin/kg feed as compared to control birds fed no toxin. Tobias *et al.* (1992) reported a dose dependent reduction in egg production and hatchability from a dose of 1, 5, and 10 mg toxin/kg food. Likewise, Speers et al. (1977) observed loss of body weight, decreased feed consumption and decreased egg production in laying hens fed 8 and 16 mg / kg feed.

In turkey females, T-2 and zearalenone mycotoxins, accounted for decreased body weight, egg production, egg size and weight, and hatchability of fertile eggs at levels of 5 mg / kg feed (Allen *et al.*, 1983). Similarly, female turkey poults fed T-2 toxin at a rate of 5 mg/kg feed from day 1 - 21 of age showed a 26% reduction in body weight gain versus control birds (0 mg / kg; Kubena *et al.*, 1995). Chi *et al.* (1977b) reported that the single oral LD_{50} dose (the acute lethal dose of toxin that results in 50% mortality of the affected population) of T-2 toxin for one day old broiler chicks was 5 mg/kg body weight, while it was 5.0 and 6.3 mg/kg body weight for 8-week old broiler chicks and laying hens respectively. Death occurred within 48 hours of toxin administration. In White Leghorn roosters, ingestion of mycotoxins decreased sperm numbers and testicle weight, but not fertilization rate (Sharlin *et al.*, 1980). It was concluded that effects observed were due to body weight loss rather than a direct effect of the toxin. Lowered sperm numbers were due to diminished Leutinizing Hormone (LH) levels and subsequent suppression of leydig cell function caused by malnutrition (Sharlin *et al.*, 1981).

Mycotoxicosis is not restricted to poultry. T-2 toxicosis in swine is often more profound than in poultry. The LD_{50} of T-2 toxin was reported at 1.21 +/- 0.15 mg/kg body weight in normal, healthy, crossbred pigs weighing from 3 to 50 kilograms (Weaver *et al.*, 1978).

Intravenous administration of 4 or 8 mg T-2 toxin/kg body weight to pigs resulted in shock characterized by reduced blood pressure and cardiac output, and increased plasma epinephrine and norepinephrine (Lorenzana *et al.*, 1985). Intravenous injection of 0.41 and 0.21 mg/kg body weight T-2 mycotoxin caused abortion in sows within 48-80 hours. Gross and microscopic examination of fetuses revealed no explanation for the abortions (Weaver *et al.*, 1978). Suppression of erythrocyte and leukocyte populations as well as antibody formation resulted after growing pigs were fed 0.5, 1.0, 2.0 or 3.0 mg / kg T-2 toxin (Rafi *et al.*, 1995a). Rafi *et al.* (1995b) observed feed refusal, decreased weight gain, and decreased plasma glucose levels in T-2 treated pigs versus controls ($P \leq .05$). Similar results were observed in rabbits dosed with T-2 toxin (Niyo *et al.*, 1988).

Immunosuppression

Formation of effector cells of the immune system involves the formation of huge number of cells, of which only a few survive the selection process (Kuby, 1992). Immune cells must be able to differentiate between self antigen and foreign antigen, however 98% of the cells produced cannot do this, and are killed. In order to get viable immune cells much cell division must occur, making the immune system extremely sensitive to T-2 toxin. In healthy animals recognition of a foreign antigen is followed by activation of B cells which produce antibodies against the antigen (Kuby, 1992). The immune response is also characterized by cytotoxic T cells which phagocytize antigen-antibody complex's, and T-helper cells which secrete cytokines and other factors to aid this response. When toxins prevent activation of these effector cells, the body is rendered defenseless and the results devastating (Smith *et al.*, 1994). In the case of Alimentary Toxic Aleukia (ATA),

the disease is characterized by a lack of white blood cells, resulting from T-2 toxin's selective targeting of hematopoeitic compartments of the bone marrow and spleen.

Immunosuppressive properties of the trichothecene mycotoxins have been documented in many species. Aerosol treatment of mice with high dosages of T-2 toxin resulted in lymphoid necrosis of the thymus, spleen and lymph nodules of the intestinal tract within one hour of exposure (Thurman *et al.*, 1988). In mice, extreme sensitivity of lymphoid tissue to T-2 toxin has been reported due to low cellular levels of detoxification enzymes such as glutathione transferase (Rosenstein and LaFarge-Frayssinet, 1983). Ingestion of T-2 toxin was followed by thymic atrophy, lymphocytolysis, reduction of circulating numbers of T and B lymphocytes, and decreased antibody levels (Holladay *et al.*, 1995). Necrosis and depletion of lymphocytes in the thymus, bursa of Fabricius, and spleen has been shown in chickens treated with T-2 toxin (Wyatt *et al.*, 1973). Mice orally gavaged for 5 days with 0.75, 1.25 or 1.75 mg/kg body weight T-2 toxin showed profound thymic atrophy two days after cessation of treatment (Smith *et al.*, 1994). These same animals also exhibited bone marrow hypocellularity, indicating that multiple hematopoetic compartments may be targeted (Smith *et al.*, 1994; Wyatt *et al.*, 1973). Decreases in circulating T and B lymphocytes suggest that the precursors of these cells are highly sensitive targets of T-2 toxin exposure (Holliday *et al.*, 1995; Smith *et al.*, 1994). Holt and DeLoach (1988) suggest there may be a direct cytotoxic effect on the lymphoid cells.

T-2 effects on serum immunoglobulin (Ig) proteins have also been studied. The proteins IgA, IgM and complement proteins all play a role in defense against extracellular bacteria. Complement is defined as a group of serum proteins that participate in an enzymatic cascade, ultimately resulting in the formation of a cytolytic membrane attack complex and subsequent lysis of extracellular bacteria (Kuby, 1992). In calves, T-2 treatment lowered IgA, IgM and complement protein levels (Mann *et al.*, 1983). Treatment of mice with T-2 toxin increased their

susceptibility to infection by a variety of infectious agents including *Listeria monocytogenes* (Corrier and Ziprin, 1987). Decreased numbers of bacteria fighting immunogobulins are a direct result of the ability of T-2 toxin to inhibit protein synthesis (Mann *et al.*, 1983). In other studies, antibody response to sheep red blood cells was significantly decreased in mice exposed to T-2 toxin (LaFarge-Frayssinet et al., 1979). Decreased immune response to foreign red blood cells is an indication that the immune system is not functioning properly (Kuby, 1992). Other studies indicate that T-2 toxin may suppress tumor defense mechanisms and impair host control of tumor cell growth (Schoental *et al.*, 1979).

Hematology

Clotting factors are also known targets of T-2 toxin. In rats, clotting times were prolonged after ingestion of T-2 toxin (Kosuri *et al.*, 1971). Similarly in young chickens, platelet factors VII and X as well as prothrombin were all reduced by T-2 toxin treatment (Doerr *et al.*, 1981). In calves, injection of a single dose of T-2 toxin (0.25 mg/kg body weight) produced significant reductions in platelet factors VII, IX, X, XI and fibrinogen values (Gentry and Cooper, 1983). In a similar study, administration of T-2 toxin to calves at a dosage of 0.2 mg/kg body weight resulted in prolonged prothrombin time and marked neutropenia (Patterson *et al.*, 1979).

Protein Synthesis

One site of action of T-2 toxicity is thought to be at the cell membrane. T-2 toxin is an amphipathic molecule which distributes to the cell membrane in erythrocytes (Gyongyossy-Issa *et al.*, 1986; DeLoach *et al.*, 1987). It has been shown to have multiple effects on cell membrane

function by altering amino acid, nucleotide and glucose uptake as well as affecting calcium and potassium channel activities (Bunner and Morris, 1988). T-2 toxin may cause its effects by interfering with cell signaling pathways thereby preventing normal cell function. Whether these changes to cell membrane function contribute to cellular destruction, or the inhibition of protein and DNA synthesis is still being debated. However, any changes to cell membrane function induced by T-2 toxin can be considered to be a significant effect.

Tissue culture studies of normal human fibroblasts treated *in vitro* with T-2 toxin showed a dose-dependent reduction in protein and scheduled DNA synthesis, as well as impairment of unscheduled DNA repair (Oldham *et al.*, 1980). Treatment of mice with a single intraperitoneal dose of T-2 toxin (0.75 mg/kg body weight), resulted in inhibition of protein synthesis in cells obtained from the bone marrow, spleen and thymus (Rosenstein and Lafarge-Frayssinet, 1983). Treatment of rat hepatocytes with 0.01 ng/ml T-2 toxin produced a 75% inhibition of protein synthesis (Trusal and O'Brien, 1986).

Protein synthesis consists of 5 steps: activation, initiation, elongation, termination, and processing. This process involves the cooperation of over 300 macromolecules (Lehninger, 1993). Activation involves the covalent attachment of each of the 20 amino acids to a specific transfer-RNA (tRNA) which is catalyzed by aminoacyl tRNA synthetases, each specific for one amino acid and it's corresponding tRNA. Initiation is the process whereby messenger-RNA (m-RNA) bearing the code for the polypeptide to be made binds the smaller of the two major ribosomal subunits. This is followed by binding of the initiating aminoacyl-tRNA and the large ribosomal subunit to form an initiation complex. Elongation is characterized by lengthening of the polypeptide chain by covalent attachment of successive amino acid units. Each amino acid unit is carried to the ribosome and correctly positioned by its tRNA which base-pairs to its corresponding codon in the mRNA. Termination of the polypeptide chain is signaled by a termination codon on the mRNA

sequence. The polypeptide chain is then released from the ribosome, aided by proteins called release factors. The terminal step called folding and processing is accomplished when the polypeptide folds into its proper three-dimensional conformation in order to achieve biologic activity (Lehninger, 1993). In the elongation stage of translation, peptidyl transferase catalyzes peptide bond formation between newly synthesized amino acids. This enzyme plays a key role in the initiation of protein synthesis, elongation and termination of polypeptide chains and proteins (McLaughlin *et al.*, 1977).

As a group, the trichothecene's appear to inhibit protein synthesis by interacting with the peptidyl transferase center on the large, 60S ribosomal subunit and inhibit the transpeptidation of the peptide bond formation at the transcription site (Schindler, 1974). There is some evidence that T-2 toxin suppresses activity and production of DNA polymerases. In a cell line derived from a patient with acute lymphoblastic leukemia, the enzymatic activity of alpha and beta terminal deoxynucleotidyl transferases was strongly inhibited by treatment with high dosages of T-2 toxin (Munsch and Muller, 1980). *In vitro* studies with human fibroblasts have shown T-2 toxin to cause damage to the nuclei, rough endoplasmic reticulum and associated ribosomes; the locations of DNA and protein synthesis (Oldham *et al.*, 1980). Rosenstein and Lafarge-Frayssinet (1983) described the depression of DNA synthesis *in vivo* and *in vitro*. Three treatment schedules of a single dose, three daily doses, or seven daily doses of 0.75 mg toxin/kg body weight inhibited DNA synthesis in cell cultures from the spleen, thymus and bone marrow of treated mice. It has been suggested that T-2 toxin's ability to prevent DNA synthesis may be through altered membrane function and prevention of the uptake of the amino acid precursors required for DNA synthesis (Bunner and Morris, 1988). However, the mechanism of inhibition of nucleic acid synthesis in the *in vivo* system is not fully understood.

Detoxification

The methods currently available to decontaminate grains infested with T-2 toxin are of great interest. The 12,13-epoxide of the trichothecenes is essential for their biological activity (Jemmali, 1990). The de-epoxidation of DON and T-2 by rumen-microorganisms and in mammalian systems results in loss of toxicity.

There are some studies directed at creating antibodies against T-2 toxin as a way to neutralize the toxin *in vivo*. Feuerstein *et al.* (1985) used a mouse monoclonal antibody against T-2 to neutralize the inhibitory effect on protein synthesis *in vitro*. The antibody also provided rats with protection against lethal toxemia. Further studies report that monoclonal anti-T-2 antibody can promote the efflux of T-2 toxin out of the cell and also restore protein synthesis in poisoned cells in vitro (Hunter *et al.*, 1990). *In vivo* studies have demonstrated the ability of the antibody to pull the toxin out of poisoned tissues and into plasma (Hunter *et al.*, 1990). Minervini *et al.* (1994) utilized a the murine monoclonal antibody specific to T-2 toxin to neutralize the inhibition of blastogenesis in human lymphocytes. This neutralization indicated a true antigen-antibody interaction and holds promise for therapy for humans suffering from T-2 toxicosis.

There have been some studies performed on the effects of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on T-2 toxin induced hematopoietic injury in mice. Ohtani *et al.* (1993) observed that administration of rhG-CSF along with T-2 toxin prevented the characteristic suppression of leukocyte populations. The characteristic depression of bone marrow and spleen cells was also counteracted by administration of rhG-CSF. Similarly, simultaneous administration of rhG-CSF with T-2 toxin reduced mortality in a dose dependent manner. Although treatment with rhG-CSF appears to counteract the effects of T-2 toxin, the expense and availability of rhG-CSF makes it an impractical treatment regime.

There has been some research into bioflavonoids and the protective effects they may provide against T-2 mycotoxicosis. Bioflavonoid is a generic term for a group of aromatic oxygen heterocyclic compounds derived from 2-phenylbenzopyran or its 2,3-dehydro derivitives (Dorland's Medical Dictionary, 1981). They are widely distributed in a variety of plants. Bioflavonoids have a variety of effects on mammalian tissues and organs including increased production of detoxifying enzymes (Wood *et al.*, 1986), anti-inflammatory effects (Gabor, 1986), enhancement of lymphocyte proliferation (Berg and Daniel, 1988), and stabilization of cell membranes (Price and Middleton, 1986). Markham *et al.* (1987) performed an *in vivo* and *in vitro* study to assess the protective effects of quercetin, a bioflavonoid. Their results showed that murine thymocytes cultured with T-2 toxin and quercetin resulted in a 40% reduction of cytotoxicity as compared to cells cultured with T-2 toxin alone. Mice injected with quercetin prior to intraperitoneal injection with T-2 toxin received similar protection. It was hypothesized that the anti-inflammatory properties of quercetin may have contributed to the protective properties of this compound.

Despite the numerous suggestions of compounds and treatment regimes for T-2 toxicoses, to date there are no acceptable treatments.

Project Justification

Ames Plantation, the site of the National Championships for purity all-age bird dogs, supports a bobwhite quail population that is integral to the mission of the Plantation. Over the past 20 years quail populations have been fluctuating despite management practices aimed at improving habitat for quail. Recent analysis of soybeans left as late winter feed showed the presence of T-2 toxin. It was hypothesized that T-2 toxin may be a contributing factor to the decreasing quail population. However, mycotoxin invasion of agricultural grains is not a phenomenon limited to

Ames Plantation. Wherever agricultural grains or naturally occurring grains (e.g. lespedezas) compose a significant portion of quail diets and mycotoxin outbreaks are possible, then negative effects of toxicity may be periodically possible. The objectives of the following studies are: 1) to determine the acute lethal dose of T-2 toxin that elicits 50% mortality (LD_{50}) , 2) to perform a histological assessment of the effects of T-2 toxin on cellular integrity, morphology, complete blood chemistry and liver enzyme profiles, 3) to determine the effect of acute vs. chronic exposure to T-2 mycotoxin on Bobwhite quail reproductive parameters and peripheral white blood cell populations.

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PART II

DETERMINATION OF THE ACUTE 50% LETHAL DOSE (LD_{50}) OF T-2 TOXIN IN ADULT BOBWHITE QUAIL: ADDITIONAL STUDIES ON THE EFFECT OF T-2 MYCOTOXIN ON PERIPHERAL LEUKOCYTE POPULATIONS, BLOOD CHEMISTRY, AND THE MORPHOLOGY AND CELLULAR INTEGRITY OF INTERNAL ORGANS.

Abstract

Four experiments were conducted to assess mortality rate, hematopoietic, and histological changes associated with exposure to T-2 mycotoxin. In Experiment 1, adult bobwhite quail were orally dosed with T-2 toxin to determine the acute lethal dose that resulted in 50% mortality of the affected population. The acute one time lethal dosage of T-2 toxin that resulted in 50% mortality was determined to be 14.7 milligrams of T-2 toxin per kilogram of body weight (BW).

A second experiment was performed to compare the effects of intermittent vs. chronic exposure to T-2 toxin on peripheral blood leukocyte populations. Intermittent exposure to T-2 toxin results in highly significant ($P \leq .01$) increases in absolute number of heterophils among LD_{40} (40% of the acute 100% lethal dose) and LD_{60} (60% of the acute 100% lethal dose) birds as compared to control birds. In contrast, absolute number of monocytes and leukocytes were significantly ($P \leq .01$) decreased among LD_{40} and/or LD_{60} birds as compared to controls. Chronic exposure to T-2 toxin resulted in a highly significant ($P \leq .01$) increase in absolute number of heterophils as a result of any T-2 toxin treatment. In contrast, absolute number of monocytes and lymphocytes were less ($P \leq .01$) among hens receiving 16 or 20 mg toxin/kg feed as compared to hens receiving the control diet. Results indicate that T-2 toxin is a potent immunosuppressor of peripheral lymphocyte populations.

In a third study, the effects of 12-18 mg/kg BW T-2 toxin on blood chemistry and liver enzyme profiles was determined. Posttreatment plasma glucose, total protein, calcium, cholesterol and triglyceride levels numerically decreased as compared to pretreatment values. In contrast, post treatment potassium, uric acid, aspartate aminotransferase, lactic dehydrogenase, creatinine phosphokinase and gamma glutamyl transferase increased as compared to pretreatment values,

which is indicative of liver damage.

Histological analyses of spleen, liver, small intestine, kidney, and heart tissues were conducted on birds dosed in Experiment 3 and on birds fed T-2 toxin for 4 weeks in a 4th experiment. Marked lymphocyte necrosis and depletion throughout the spleen, thymus, bursa and gut associated lymphoid tissue in the small intestine was observed in birds dosed with 15 and 18 mg/kg BW T-2 toxin. Necrosis of liver and lipid accumulation as a result of malfunctioning hepatocytes was also observed. The most prevalent lesion in chronically fed birds was pronounced depletion of splenic lymphocytes. Heart and liver tissue of chronically treated birds showed little or no morphological changes.

The LD_{50} for adult bobwhite quail as found in this study is 2-3 times higher than that reported for other species of commercial poultry. Results from these data confirm previously reported studies on the immunusuppressive and cytotoxic effects of T-2 toxin in other mammalian and avian species. T-2 toxin may have a negative impact on the viability of wild quail populations.

Introduction

T-2 toxin is a trichothecene mycotoxin primarily produced by the fungi *Fusarium tricinctum* (Nakadate and Uneo, 1990), as a byproduct of secondary metabolism. This toxin is best known for it's immunosuppressive effects on hematopoietic and lymphoid tissues as well as it's acute toxic effects at relatively low dosages (La-Farge-Frayssinet *et al.*, 1981; Smith *et al.*, 1994; Faifer *et al.*, 1992; Chi *et al.*, 1977). The acute lethal dose of T-2 mycotoxin that results in 50% mortality of affected populations (LD_{50}) has been determined in laying hens and broiler chicks. However, little or no information is available on the LD_{50} dose of quail of any age, and/or the effects of T-2 mycotoxin in adult Bobwhite quail.

Chi *et al.* (1977) reported the single oral LD_{50} dose of T-2 toxin for one-day-old broiler chicks was 5 milligrams of toxin per kilogram (mg/kg) body weight (BW). In addition, it was determined that the LD_{50} for eight week old broiler chicks and 24 week old laying hens was 5 and 6.3 mg/kg BW respectively. A single report by Ruff *et al.* (1992) reported 20% and 22.5% mortality among bobwhite quail fed 8 or 16 milligrams T-2 toxin/kg feed respectively. This experiment was a feeding trial, and did not determine the acute LD_{50} for either juvenile, 1 week old, or adult bobwhite quail.

The immunosuppressive effects of T-2 toxin has been well documented in many species. T-2 toxin is a potent inhibitor of protein synthesis (McLaughlin *et al.*, 1977) and causes necrosis of rapidly dividing cells of the hematopoietic and lymphoid tissues of mice (LaFarge-Frayssinet *et al.*, 1981). Smith *et al.* (1994) observed profound thymic atrophy, significant decreases in the total number of B and T cells, and significant bone marrow hypocellularity in adult mice dosed with 0.75, 1.25, or 1.75 mg/kg body weight (BW) T-2 for 5 days; thus confirming hematopoetic compartments as targets of T-2 toxin. Other studies showed extremely small doses of T-2 toxin (0.30 mg/kg) produced a significant degree of bone marrow cytotoxicity in mice (Faifer *et al.*, 1992). Holladay *et al.* (1995) proposed that lymphocyte precursor cells may be highly sensitive targets of T-2 toxin exposure.

In poultry, T-2 toxin causes similar immunosuppressive effects as are seen in rodents. Richard *et al.* (1978) observed a significant decrease of cortical lymphocytes in the thymus glands of White turkey poults fed diets containing 10 mg T-2/kg food. Niger *et al* (1994) observed a moderate decrease of thymic cortical lymphocytes in 6 week old mallard ducklings fed diets containing 2 mg T-2 toxin/kg feed. To our knowledge, the immunosuppressive effects of T-2 toxin in Bobwhite quail of any age have not been studied.

Hoerr *et al* (1981) orally dosed seven day old male broiler chickens with 2.0 and 2.5 mg

T-2 toxin/kg BW. Birds were killed at 1, 6, 12, 18, 24, 72 and 168 hours after T-2 treatment for histopathological analysis. Necrosis of lymphoid tissue and bone marrow began 1 hour after treatment, followed by rapid cell depletion, however, cell repletion was rapid with recovery occurring within 24 hours. Hepatic lesions included necrosis and hemorrhage of bile ductiles. Necrosis of duodenal epithelium, villi and crypt epithelium of the small and large intestine and mucosal epithelium of the proventriculus and gizzard were observed. All tissue appeared normal by 72 hours after exposure.

The objectives of the following experiment were: 1) to determine the acute one time lethal dose of T-2 toxin that results in 50% mortality of the affected population, 2) to determine the effect of intermittent vs. chronic exposure to T-2 toxin on peripheral leukocyte populations, 3) to determine the effect of acute dosages of T-2 toxin on blood chemistry and liver enzyme profiles, and 4) to determine the effect of acute vs. chronic T-2 toxin exposure on histopathology of internal organs.

Materials and Methods

Experiment 1: Determination of LD_{50}

Three hundred and seventy four bobwhite quail (4 to 30 per treatment) were used to determine the acute lethal dose of T-2 toxin that results in 50% mortality (LD_{50}). Birds were housed in grower batteries (Petersime Co., Gettysburgh, OH; model number 515FU), and allowed *ad libitum* access to feed (Mazuri Brand Pheasant Grower. PMI Feeds Inc., St. Louis, MO) and water. After a one month acclimation period, birds were changed to Mazuri Pheasant Breeder and Developer. Birds (mixed sex) were randomly assigned to treatment groups and orally gavaged

with 0, 1, 3, 5, 7, 9, 10, 12, 15, 18, and 20 milligrams of purified T-2 toxin (Sigma, St. Louis, MO) per kilogram of body weight. T-2 toxin was dissolved in 100% ethanol, and diluted with corn oil as described by Holladay *et al.* (1993). Birds were monitored for mortality every hour for the first twenty four hours, and at four hour intervals for the next four days. Data were analyzed by the Probit procedure of SAS (The SAS Institute, 1989).

Experiment 2: Differential Leukocyte Counts

In a second study, the type of exposure, intermittent vs. chronic, was compared, and the effect on peripheral blood leukocyte populations was determined. For intermittent exposure, 180 bobwhite quail hens (45 hens/treatment) were randomly assigned to receive 0% (LD_0, control; 0 mg toxin/kg BW), 20% (LD_{20}; 12.4 mg toxin/kg BW), 40% (LD_{40}; 14.0 mg toxin/kg BW), or 60% (LD_{60}; 15.5 mg toxin/kg BW) of the acute 100% lethal dose T-2 toxin (20.0 mg toxin/kg BW) as determined in Experiment 1. Hens were housed in Petersime Grower batteries as in Experiment 1, and provided *ad libitum* access to feed and water (Mazuri Brand Pheasant Breeder, PMI Feeds Inc., St. Louis, MO). Prior to initiation of experiment, birds were delayed from puberty by feeding a grower diet and limiting light exposure to 8 hours per day. On day 1 of this experiment, hens were changed to the breeder diet and light exposure was raised to 16 hours a day to stimulate ovarian development. Purified crystalline T-2 toxin (Sigma, St. Louis, MO) was dissolved in 100% ethanol, and diluted to volume with corn oil. Control birds were dosed with a mixture of corn oil and 100% ethanol. Doses were administered by oral gavage over a four week period; 1/4th dose per week. Blood was collected immediately before initiation of the experiment, and 24 hours after the fourth and final treatment. Blood smears were made on microscope slides, dried and stained with Wright-Giemsa stain (Brown, 1993). Estimated total white blood cell populations

were counted using a 1 mm x 1mm micrometer square, fitted into the 10X ocular of the microscope. Five fields per slide were counted under 40X power to give an estimation of the total white blood cell population per 0.25 mm^2 (actual area measured as determined by calibration using a stage micrometer). Additionally, two fields of 100 total white blood (WBC) cells were counted under oil immersion (100X) per slide. Monocytes, lymphocytes and heterophils were counted and recorded as the relative percentage of 100 WBC. Absolute number of leukocytes were determined by multiplying the relative count (% of each leukocyte population) times the estimated total white blood cell population (in 0.25 mm^2).

For chronic exposure, 139 bobwhite quail hens were assigned to one of four treatment groups of 0, 12, 16, or 20 mg T-2 toxin/kg feed. Treatments were replicated four times with 8-11 birds per replicate, for a total of 32-44 birds per treatment. T-2 toxin was dissolved into 100% ethanol, diluted to volume in corn oil, and added to commercial breeder diets (Mazuri Pheasant Breeder, PMI Feeds Inc., St. Louis, MO). Hens were housed in Petersime Grower batteries as before, and provided feed and water *ab libitum*. T-2 toxin contaminated feed was fed for a total of four weeks. Grower batteries were contained in a Bioshield 350 (Airo Clean Inc., Exton, PA) positive pressure biological containment unit with light stimulation of 16 hours per day. Daily mortality was recorded. Blood samples were collected as in Experiment 1; immediately before and 24 hours after treatment. Blood smears were made on microscope slides, dried and stained with Wright-Giemsa stain. Total and differential white blood cell populations were determined as described above. Data were analyzed using the MIXED model procedure of SAS (1996).

Experiment 3: Blood Chemistry - Acute Dosage

In the acute study, ten (approximately 15 weeks old) bobwhite quail were orally dosed

with 0, 12, 15, or 18 mg T-2 toxin/kg BW (3 controls , 2 12 mg/kg birds, 3 15 mg/kg birds and 2 18 mg/kg birds) which is representative of 20-80% mortality as determined in Experiment 1. T-2 toxin was dissolved in 100% ethanol, diluted to volume with corn oil, and administered via oral gavage. Control birds were gavaged with a mixture of corn oil and 100% ethanol. However, one bird (15 mg/kg) died as a result of handling stress and was omitted from this analysis. Blood samples were drawn prior to dosing and again immediately prior to sacrifice (12 hours after treatment) in order to obtain blood chemistry and liver enzyme profiles. Blood samples were centrifuged to isolate plasma, and sent to Dr. Carolyn Cray, University of Miami, FL, School of Medicine, Comparative Pathology Laboratory for analysis. Complete blood chemistry values were measured and include glucose, sodium, potassium, CO_2 content, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, total protein, calcium, phosphorus, uric acid, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactic dehydrogenase, creatine phosphokinase, amylase, lipase, gamma glutamyl transferase (GGT), cholesterol, triglycerides, and total bile acids.

Experiment 4: Histopathology analysis

A fourth study was performed to compare the effect of acute vs. chronic exposure to T-2 toxin on morphological integrity of internal organs. In the acute study, the ten bobwhite quail as described in Experiment 3 and orally dosed with 0, 12, 15, and 18 mg T-2 toxin/kg BW, were used in histopatological studies. Birds were sacrificed 12 hours after dosing, and spleen, liver, intestine, kidney, thymus, and bursa of Fabricius were removed, fixed in 10% buffered formalin (Fischer Scientific, Pittsburgh, PA), embedded in paraffin, and evaluated for histopatological changes by Dr. Malcolm McCracken (University of Tennessee, Department of Pathology, College of

Veterinary Medicine).

In the chronic phase of this experiment, a total of nineteen birds (approximately 15 weeks old) were assigned to receive 0, 12, 16, or 20 mg T-2 toxin/kg feed. Birds were provided feed, water and lights as previously described in Experiment 2. Birds remained on feed for 4 weeks. After the 4 week treatment period, surviving birds were killed by cervical dislocation and samples of spleen, heart and liver and bone marrow were collected, fixed in 10% buffered formalin (Fischer Scientific, Pittsburgh, PA), embedded in paraffin, mounted on slides by a commercial laboratory. Tissue samples were evaluated by University pathologist, Dr. Malcolm McCracken.

Results

Experiment 1: Determination of LD_{50}

Using the Probit procedure of SAS (1989), the acute 50% lethal dose of T-2 toxin in adult bobwhite quail was determined to be 14.7 mg/kg BW (Table 2-1). No mortality was observed among birds dosed with 0 to 10 mg T-2/kg BW. Six of fifteen birds (40.00%) dosed with 12.0 mg toxin/kg BW died within 5 days after dosing. Seven of fifteen birds (46.67%) dosed with 15.0 mg toxin/kg BW died within 5 days. Thirteen of fifteen (86.67%) birds dosed with 18 mg toxin/kg BW died within 5 days. All four birds (100%) dosed with 20.0 mg toxin/kg BW died within 24 hours of dosing. The time interval to death was decreased in a dose dependent manner as dosage increased.

Experiment 2: Differential Leukocyte Counts

Intermittent Study

In the intermittent dose study, highly significant differences were found as a result of T-2 toxin treatment (Table 2-2). Absolute number of monocytes significantly ($P \leq .01$) decreased as a result of T-2 toxin treatment among LD_{40} and LD_{60} birds as compared to control birds (1.57 and 1.61 vs. 2.44 monocytes/0.25 mm^2 respectively). Likewise, absolute number of lymphocytes also decreased ($P \leq .01$) as a result of T-2 toxin treatment in LD_{60} birds as compared to control birds (4.44 vs. 5.93 lymphocytes/0.25 mm^2 respectively). However, absolute number of heterophils significantly ($P \leq .01$) increased among LD_{60} birds as compared to control birds (4.03 vs. 2.97 heterophils/ 0.25 mm^2 respectively).

Chronic Study

In the chronic study, highly significant differences ($P \leq .01$) were found as a result of T-2 toxin treatment (Table 2-3). Absolute number of monocytes were significantly ($P \leq .01$) less among hens receiving 20 mg toxin/kg feed as compared to hens receiving the control diet (1.29 vs. 2.24 monocytes/0.25 mm^2 respectively). Likewise, absolute number of lymphocytes were significantly ($P \leq .01$) less among hens receiving the 20 mg T-2 toxin/kg food diet as compared to control hens (2.71 vs. 4.02 lymphocytes/0.25 mm^2 respectively). However, absolute numbers of heterophils were significantly ($P \leq .01$) increased as a result of any T-2 toxin treatment as compared to control birds (4.11, 4.25 & 5.19 vs. 2.65 heterophils/0.25 mm^2 for 12, 16 & 20 mg/kg diets vs. control

respectively). Changes in leukocyte populations were more pronounced among chronic fed vs. acute dosed birds (Table 2-3 vs. 2-2).

Experiment 3: Blood Chemistry - Acute Study

Comparisons of blood chemistry values measured prior to, and 12 hours after treatment with 12 - 18 mg/kg BW T-2 toxin were made. Plasma glucose, sodium, potassium, total protein, calcium, uric acid, aspartate aminotransferase, lactic dehydrogenase, gamma glutamyl transferase, cholesterol and triglycerides were measured (Table 2-4). Due to the small sample size (<4 birds/treatment) no statistical differences ($P \geq .05$) were found between pre and post treatment blood chemistry values. T-2 treatment did not affect plasma sodium, calcium, CO_2, blood urea nitrogen (BUN), creatinine, BUN:creatinine ratio, phosphorus, alanine aminotransferase or lipase levels. However, post treatment levels of plasma glucose, total protein, calcium, cholesterol and triglycerides numerically decreased as compared to pre treatment levels (Table 2-4). Conversely, post treatment levels of potassium, uric acid, aspartate aminotransferase, lactic dehydrogenase, creatinine phosphokinase, and gamma glutamyl transferase numerically increased as compared to pretreatment levels. (Table 2-4). Post treatment aspartate aminotransferase levels were numerically higher (*pretrt*: 530, 507 & 660 vs. *posttrt*: 668, 950 & >1000 units/liter [U/L]) for control, 12 and 18 mg/kg BW respectively. Lactic dehydrogenase was almost double post treatment for 18 mg/kg BW birds (*pretrt*: 1087 vs. *posttrt*: >2150 U/L respectively). Post treatment gamma glutamyl transferase levels were highly elevated as compared to pre treatment levels (14.5 & 8 vs. 51 & 70 U/L) for 15 and 18 mg/kg BW birds.

Experiment 4: Histological Findings

Acute Study

Microscopic lesions were observed in the following tissues: spleen, liver, intestine, kidney, thymus, and bursa of Fabricius (Table 2-5). Significant lesions were not observed in spleen of control quail (Figure 2-1 A). The spleen from one of the 12 mg/kg BW T-2 toxin birds (672) exhibited moderate lymphocyte necrosis and depletion throughout germinal centers (not shown). Spleens from birds receiving 15 mg/kg BW T-2 toxin showed similar lymphocyte necrosis and depletion and moderate accumulation of granulocytes (Figure 2-1 B). Spleens from birds receiving the 18 mg/kg BW T-2 toxin exhibited marked necrosis and lymphocyte depletion characterized by pyknotic nuclei and karyorrhexis in the peri-arteriolar lymphoid sheath and mild to marked heterophil accumulation (Figure 2-1 C).

Liver tissue from control birds appeared normal (Figure 2-2 A). One 12 mg/kg BW T-2 toxin (672) had a few apoptotic hepatocytes but no lipid accumulation characteristic of malfunctioning hepatocytes. The other 12 mg/kg BW bird (654) only exhibited mild lipid accumulation (not shown). In contrast, livers of the 15 mg/kg BW T-2 toxin birds exhibited moderate lipid accumulation, moderate apoptosis of hepatocytes and a small number of lymphocytes (Figure 2-2 B). As compared to controls, liver samples from 18 mg/kg BW T-2 toxin birds showed focal areas of hepatic apoptosis characterized by pyknosis and karyorrhexis of nuclei. One 18 mg/kg T-2 toxin bird (644) had very mild necrosis accompanied by pronounced lipid accumulation (Figure 2-2 C).

Small intestine tissue from control birds appeared normal (Figure 2-3 A). Mild necrosis of lymphocytes in the small intestine was observed among 12 mg/kg BW treated birds (figure not

shown). Moderate to marked necrosis of lymphocytes in lymphoid aggregates in the lamina propria and necrosis of the crypt epithelial cells was observed in small intestines of 15 mg/kg BW treated birds (Figure 2-3 B). High doses of T-2 toxin caused marked necrosis of lymphocyte throughout the cecal tonsil of small intestine in 18 mg/kg BW birds (Figure 2-3 C). In these birds there were also focal areas of intestinal mucosa erosion accompanied by marked infiltration of heterophils and lesser numbers of lymphocytes and macrophages.

Very little necrosis and cellular changes were observed in kidney tissue (Table 2-4). Kidneys from 12 mg/kg BW (not shown) and control birds (Figure 2-4 A) appeared normal. In 15 mg/kg BW birds, very mild necrosis of individual cells of the kidney with minimal tubular necrosis was observed (Figure 2-4 B). In 18 mg/kg BW birds, very mild to mild necrosis characterized by pyknosis of nuclei in scattered tubules was observed (Figure 2-4 C).

Thymus and bursa tissues showed similar lymphocyte necrosis as spleen. However, the extent of the lesions were not as severe. Thymus from control birds appeared normal (figure 2-5 A). Significant lesions in thymus tissue were only observed in one 15 & one 18 mg/kg BW bird. The thymus in the 15 mg/kg BW bird had moderate to marked lymphocyte necrosis and moderate cortical lymphocyte depletion (Figure 2-5 B). The thymus from the 18 mg/kg BW bird had mild lymphocyte necrosis and marked loss of cortex cellularity (Figure 2-5 C).

The bursa of Fabricius from control birds appeared normal (Figure 2-6 A). Bursa of Fabricius was recovered from one 18 mg/kg BW bird and was characterized by lymphocyte necrosis with moderate heterophil infiltration in the necrotic germinal centers (Figure 2-6 B).

Significant lesions were not observed in cardiac tissue or bone marrow of acutely treated birds.

Chronic Study

Birds chronically fed T-2 toxin for a period of 4 weeks had fewer pathological changes in tissue which was generally limited to the spleen (Table 2-6). Only one control bird showed very mild amounts of lymphoid depletion but all had extensive numbers of lymphoid follicles (Figure 2-7 A). Two of five control birds had vague traces of hemosiderin, an iron carrying pigment present in red blood cells. Birds fed the 12 mg/kg T-2 toxin diet showed very mild to mild lymphocyte depletion with more periarteriolar lymphoid follicles than in birds fed higher concentrations of T-2 toxin (Figure 2-7 B). Hemosiderin was present in moderate amounts in three of five birds, slight amounts were present in one bird, and absent in another. Splenic tissue from birds fed the 16 mg/kg diet showed moderate lymphoid depletion with few lymphoid follicles, but more than were seen in 20 mg/kg birds (Figure 2-7 C). Moderate amounts of hemosiderin were present in three of four birds. Moderate lymphoid depletion, with an absence of lymphocytes from periarteriolar sheaths was observed in spleen tissue from birds fed the 20 mg/kg diet (Figure 2-7 D). Hemosiderin was present in moderate amounts, indicative of erythrocyte lysis.

Cardiac lesions were not found among any tissue collected from chronically T-2 fed birds (Figure 2-8 D).

Only mild hepatic damage from chronic ingestion of T-2 toxin were observed (Table 2-7). Of the livers from control birds, two showed moderate amounts of bile duct hyperplasia, but all others were normal (Figure 2-9 A). There were mild amounts of focal inflammatory cell infiltrates in the livers of control birds. Mild bile duct hyperplasia with little or no hemosiderin and no focal inflammatory cell infiltration was observed among the 12 mg/kg feed birds (Figure 2-9 B). Three of four samples among birds fed 16 mg/kg T-2 toxin diets showed mild-moderate bile duct hyperplasia (Figure 2-9 C). Hemosiderin was present in moderate amounts in one bird, present in

rare scattered Kuppfer cells in two birds, and not present in another. Mild bile duct hyperplasia and slight amounts of hemosiderin, indicative of erythrocyte lysis with mild focal inflammatory cell infiltration was seen among birds fed 20 mg T-2/kg diets (Figure 2-9 D).

Discussion

Experiment 1

The acute lethal dosage of T-2 toxin that resulted in 50% mortality has been determined for a variety of poultry species but not adult bobwhite quail. The discovery of T-2 mycotoxin on soybeans, a heavily utilized winter food source for bobwhite quail on Ames Plantation (University of Tennessee) has made the investigation of the effects of this toxin paramount. Results from this study indicate the adult bobwhite quail LD_{50} dose to be 14.7 mg purifiedT-2 toxin/kg BW. The acute LD_{50} for 24 week old hens and 8 week old broiler chicks has been reported as 5.0 and 6.5 mg T-2 toxin/kg BW respectively (Chi *et al.*, 1977). While no LD_{50} trials with Bobwhite quail of any age have been reported, feeding week old bobwhite quail diets containing 16 mg T-2 toxin/kg feed for a three week period resulted in 22.5% mortality (Ruff *et al.*, 1992). The LD_{50} dose of 14.7 mg T-2 toxin/kg BW as found in this study is considerably higher than that reported in chickens.

Experiment 2

Exposure of experimental animals to T-2 toxin has been associated with a variety of immunosuppressive effects, including altered parameters of humoral mediated immunity (Kubena *et al.*, 1995; Thurman *et al.*, 1988). Because of T-2 mycotoxin's ability to prevent protein and

DNA synthesis, the immune system is particularly sensitive to this toxin (Corrier and Ziprin, 1987). The effect of T-2 toxin on absolute leukocyte populations has not been previously studied in adult Bobwhite quail. Studies in mice have revealed significant decreases in the total number of B and T lymphocytes in mice exposed to T-2 toxin (Smith *et al.*, 1994). Similarly, Richard *et al* (1978) observed a significant depletion in cortical thymic lymphocytes in turkey poults fed 10 mg T-2/kg feed.

Results from Experiment 2 which compared intermittent vs. chronic exposure to T-2 toxin showed a significant ($P<.01$) decrease in monocyte and lymphocyte populations. In the intermittent study, numbers of monocytes in LD_{40} and LD_{60} birds were less ($P\leq.01$) as compared to control hens. Similarly, in the chronic exposure study we observed a significant ($P\leq.01$) reduction in the number of monocytes and leukocytes in the LD_{60} hens as compared to control hens. This decrease in circulating monocytes and lymphocytes may be interpreted as a measure of immunosuppression. It has been observed that T-2 induced immunosuppression is transient with recovery occurring within 24 hours after cessation of treatment (Hoerr *et al.*, 1981). In our studies, birds were bled 24 hours after cessation of treatment so the reduction in circulating lymphocytes persisted for at least that period of time. Observed increases in heterophils in both studies is an interesting phenomenon. Heterophils are elements of nonspecific immunity and represent the first line of defense against extracellular pathogens. The increased numbers of heterophils as seen in both studies may represent the immune system attempting to compensate for loss of lymphocytes and monocytes. Results shown support those reported previously, and show T-2 toxin a causative agent for peripheral leukocyte reduction.

Experiment 3

Since no reported information is available on normal blood chemistry values in Bobwhite quail, this study made comparisons between pre-treatment (pretrt) vs. post-treatment (posttrt) blood chemistry and liver enzyme values of birds intensely treated with T-2 toxin. Unfortunately, blood chemistry among treated birds were incomplete due to the difficulty in obtaining an adequate volume of blood from sick and dying birds. Post-treatment CO_2, BUN, Creatine, BUN/Creatine ratio, phosphorus, ALT, creatine phosphokinase, amylase, or lipase values were not measured in treated birds. However, comparisons between pre- and post- treatment values were made for glucose, sodium, potassium, total protein, calcium, uric acid, aspartate aminotransferase, lactic dehydrogenase, gamma glutamyl transferase, cholesterol and triglyceride levels. Post treatment values of glucose, total protein, calcium, cholesterol and triglycerides decreased as compared to pretreatment values. Decreased plasma glucose levels may be indicitive of hepatic dysfunction, impaired glucose production or excessive utilization (Ritchie *et al.*, 1994). Post-treatment increases were observed in potsasium, uric acid, aspartate aminotransferase, lactic dehydrogenase, creatinine phosphokinase, and gamma glutamyl transferase as compared to pre treatment values. Increased uric acid levels are indicative of nucleic acid release caused by severe tissue damage. Elevated aspartate aminotransferase levels are indicative of liver or muscle damage. Elevated lactic dehydrogenase levels are most commonly associated with hepatic disease in birds. Elevations in gamma glutamyl transferase levels have been described in association with liver disease (Ritchie *et al.*, 1994), and are consistent with the hepatic damage induced by T-2 toxin. It should be noted that correlation's between histopathological lesions and blood chemistries can not be easily made. Similarly, blood chemistries for quail have not been well quantified and exact interpretation of these results is difficult.

Experiment 4

Histological examination of lymphoid and intestinal tissues showed varying degrees of hypocellularity, necrosis and apoptosis. Cell death is defined as being the ultimate result of cell injury, and is one of the most crucial events in pathology (Cotran *et al.*, 1994). There are two morphologic patterns of cell death, necrosis and apoptosis. Necrosis refers to the spectrum of morphologic changes that follow cell death in living tissue, largely resulting from the progressive degredative action of enzymes on lethally injured cell. Characteristics of the necrotic cell are increased eosinophilia, nuclear shrinkage, increased nuclear basophilia (pyknosis) and nuclear fragmentation (karyorrhexis). Apoptosis is the process by which unwanted cell populations are eliminated and is characterized by chromatin condensation and fragmentation. The morphological features associated with apoptosis included cell shrinkage, chromatin condensation, formation of apoptotic bodies and phagocytosis of apoptotic cells or bodies by adjacent healthy cells. When cells die there can also be a characteristic accumulation of pigments which are notable as lesions. Hemosiderin is a hemoglobin-derived granular pigment in which iron is stored in cells. Under normal conditions small amounts of hemosiderin are observed in the mononuclear phagocytes of the spleen, bone marrow, and liver. However, when excessive hemosiderin is observed it is an indication of increased red blood cell breakdown or cellular injury (Cotran *et al.*, 1994).

Smith *et al.* (1994) observed profound thymic atrophy, bone marrow hypocellularity, and a significant decrease in the total number of both B and T lymphocytes in the spleen of mice dosed with T-2 toxin. It was concluded that T-2 toxin may selectively target multiple hematopoietic compartments involved in the production of lymphocytes. T-2 toxin has been documented to cause rapid necrosis of lymphoid, myeloid, alimentary and hepatic tissues in chickens (Hoerr *et al.*, 1981). The most significant lesion observed in both the acute and chronic studies was pronounced

lymphocyte necrosis and depletion particularly in splenic and thymic tissue (Figures 2-1, 2-5, & 2-7). Lymphoid nodules of the small intestine from acutely dosed birds were characterized by lymphocyte necrosis and depletion of lymphoid foci in the lamina propria. This is consistent with the lesions observed by Hoerr *et al* (1981). Splenic tissue from acutely dosed birds showed marked depletion of lymphocytes throughout with necrosis of germinal centers and peri-arteriolar lymphoid sheaths. In contrast, splenic tissue from birds chronically fed T-2 toxin exhibited mild to moderate amounts of lymphocyte depletion. The ability of T-2 toxin to produce dose related lymphoid depletion of bone marrow, thymus and spleen has been well documented (Holliday *et al.*, 1995; Corrier and Norman, 1988; Smith *et al.* 1994). Although the exact mechanisms of immunosuppression are not known, the results of this study are consistent with those seen in the literature.

The liver is the major site of toxin and drug clearance in poultry (Dalvi *et al.*, 1987) and therefore a sensitive target of T-2. Hoerr *et al.*(1981) observed multiple foci of coagulation necrosis, hemorrhage and hyperplasia of bile ductiles of chickens treated with T-2 toxin. Mild amounts of hepatic necrosis characterized by moderate apoptosis and individual hepatocyte necrosis were observed in acutely challenged birds in our study. Liver tissue of birds chronically fed T-2 toxin showed no lesions other than an increased occurrence of binucleated hepatocytes, a possible indication of increased mitotic events and tissue regeneration.

It is interesting to note that the lesions in birds acutely dosed with T-2 toxin were more severe than those seen in birds chronically fed T-2 infested feed for a four week period. Liver lesions in particular were more severe in acutely dosed birds vs. chronic fed birds. One possible explanation is that the birds chronically fed T-2 toxin were ingesting less toxin over a longer period of time than those dosed with 12, 15, or 18 mg/kg BW T-2 toxin in one dose. The longer duration

of the study coupled with the lower levels ingested may have allowed liver recovery, which has a huge capacity for regeneration.

Results from these studies may have implications for wild quail. If wild quail ingest agricultural grains infested with T-2 mycotoxin at levels sufficiently high enough to cause immunosuppression, altered blood parameters, and cellular breakdown of internal organs; this may account for the declining quail population throughout the southeastern United States. However, if wild quail are ingesting lower levels of T-2 toxin than were used in our studies, this may still lead to an overall decrease in health and fitness of the bird. While wild quail may not be ingesting an acute lethal dose of T-2 toxin, lower levels of T-2 toxin may cause physiological stress, potentially making them more susceptible to disease or predators. Results from these studies indicate that the presence of T-2 mycotoxin on agricultural grains left as late-winter food may lead to decreased health and fitness of the wild quail and contribute to declining populations.

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Appendix I: Tables

Table 2-1: Mortality over a 120 hour period of adult bobwhite quail dosed orally with 0 to 20 mg T-2 toxin/kg body weight (Experiment 1).

Dose	# dosed	0h	6h	12h	24h	36h	48h	60h	72h	84h	96h	120h	Totals	% of Total
0 mg/kg	95	--	--	--	--	--	--	--	--	--	--	--	--	0
1 mg/kg	30	--	--	--	--	--	--	--	--	--	--	--	--	0
3 mg/kg	30	--	--	--	--	--	--	--	--	--	--	--	--	0
5 mg/kg	74	--	--	--	--	--	--	--	--	--	--	--	--	0
7 mg/kg	80	--	--	--	--	--	--	--	--	--	--	--	--	0
10 mg/kg	10	--	--	--	--	--	--	--	--	--	--	--	--	0
12 mg/kg	15	--	--	--	--	1	--	--	--	2	1	2	6	40.00
15 mg/kg	15	--	--	--	--	--	1	2	--	1	--	3	7	46.67
18 mg/kg	15	--	--	--	1	5	2	3	1	--	--	--	13	86.67
20 mg/kg	4	--	--	3	1	--	--	--	--	--	--	--	4	100.00

Table 2-2. Average absolute leukocyte populations[1] from bobwhite quail hens intermittently treated with T-2 toxin (Experiment 2).

Treatment	Monocytes	Lymphocytes	Heterophils
LD_0 (0 mg T-2/kg BW)	2.44^{A}	5.93^{A}	2.97^{A}
LD_{20} (12.4 mg T-2/kg BW)	$1.99^{A,B}$	6.22^{A}	2.68^{A}
LD_{40} (14.0 mg T-2/kg BW)	1.58^{B}	$5.38^{A,B}$	3.12^{A}
LD_{60} (15.5 mg T-2/kg BW)	1.61^{B}	4.44^{B}	4.03^{B}

[1] numbers expressed are numbers of individual cells/0.25 mm^2 (% of WBC * total WBC/mm^2)

[A,B,C] values in the same column with different letters are significantly different ($P \leq .01$).

Table 2-3. Average absolute leukocytes populations[1] from bobwhite quail hens fed T-2 toxin for a 4 week period (Experiment 3).

Treatment	Monocytes	Lymphocytes	Heterophils
0 mg toxin/kg	2.24^{A}	4.02^{A}	2.65^{A}
12 mg toxin/kg	2.28^{A}	3.61^{A}	4.11^{B}
16 mg toxin/kg	1.90^{A}	$3.93^{A,B}$	4.25^{B}
20 mg toxin/kg	1.29^{B}	2.71^{C}	5.19^{C}

[1] numbers are expressed as number of individual cells/0.25 mm^2 (% of WBC * total WBC/mm^2)

^{A,B,C} values in the same column with different letters are significantly different ($P \leq .01$).

Table 2-4: Blood chemistry and liver enzyme profiles of bobwhite quail intensely treated with T-2 toxin (Experiment 3).

Parameter (n)	Pre Treatment Values* Control (3)	12 mg/kg (1)	15 mg/kg (2)	18 mg/kg (2)	Post Treatment Values* Control (3)	12 mg/kg (2)	15 mg/kg (2)	18 mg/kg (2)
Glucose (mg/dL)	437	384	456	376	357	260	21	286
Sodium (mmol/L)	154	152	153	156	155	159	153	153
Potassium (mmol/L)	3.3	1.9	4.2	2.7	3.9	2.2	11.0	5.4
CO_2 (mmol/L)	19.2	21.0	21.2	15.6	34.8	–	–	–
BUN (mg/dL)	3.0	3.0	4.5	3.0	3.0	–	–	–
Creatinine (mg/dL)	0.13	0.10	0.34	0.20	0.25	–	–	–
BUN/Creatine	17.0	25.3	12.5	13.0	12.1	–	–	–
Total Protein (g/dL)	4.6	4.4	4.9	4.5	4.1	2.5	2.2	1.9
Calcium (mg/dL)	11.4	11.4	18.0	11.5	11.2	9.8	>16.5	9.1
Phosphorus (mg/dL)	4.5	4.2	10.7	6.8	8.1	–	–	–
Uric Acid (mg/dL)	7.8	2.6	6.9	5.0	8.7	7.9	51.1	45.0
ALT (u/L)	19.6	<3	<3	<3	78.5	–	–	–
AST (u/L)	530	507	846	660	668	950	507	>1000
Lactic dehydrogenase (u/L)	841	882	1544	1087	864	901	>2150	>2150
Creatine phosphokinase (u/L)	1882	1482	2213	3776	>1600	>1600	142	>1600
Amylase (u/L)	422	782	186	462	367	–	–	–
Lipase (u/L)	43.3	33.0	30.5	29.0	70.0	–	–	–
GGT (u/L)	8.9	8.0	14.5	8.0	8.3	7.0	51.0	70.0
Cholesterol (mg/dL)	170	181	250	156	156	110	82	102
Triglyceride (mg/dL)	132	162	155	134	170	67	50	42
Bile Acids (umol/L)	35	32	10	56	79	5	71	16

* Values shown are pooled average values from all birds in a treatment group with outliers omitted from analysis.
– Data not available, insufficient sample to conduct analysis.

Table 2-5: Morphological changes observed in bobwhite quail orally dosed with T-2 toxin (Experiment 4).

Treatment/ Band #	Spleen Lymphoid Necrosis	Spleen Lymphoid Depletion	Liver Apoptosis	Liver Fatty Change	Thymus Necrosis	Bursa Necrosis	Intestinal Necrosis	Intestinal Erosion	Kidney Necrosis	Intestinal Crypt Necrosis
Control - 630	0	0	0	0	--	--	0	0	0	0
Control - 659	0	0	0	0	0	0	0	0	--	--
Control - 677	0	0	0	1	--	--	0	0	--	--
12 mg/kg - 654	1	0	0	3	0	2	0	0	0-1	0
12 mg/kg - 672	4	4	1	0	--	--	2	0	0	0
15 mg/kg - 632	4	3	3	2	4	--	4	0	1	0
15 mg/kg - 649	4	4	2-3	3	3	--	4	0	1	2
15 mg/kg - 655	4	2	3	1	--	--	4	2	2	2
18 mg T-2/kg BW	3	4	1	0	--	--	4	3	2	2
18 mg T-2/kg BW	4	4	+/-	3	0	4	4	0	1	2

Key: -- = tissue not isolated; lesions graded on a scale of 0-5: 0 = none, 1 = very mild, 2 = mild, 3 = moderate, 4 = marked, 5 = severe

Table 2-6: Spleen morphological changes observed in bobwhite quail chronically feed T-2 toxin for a 4 week period (Experiment 4).

Treatment - Band number	Lymphoid depletion	Lymphoid follicle #	Hemosiderin content
Control - 10	0-1	+++	0
Control - 13	0	+++	1
Control - 14	0	+++	0
Control - 11	0	+++	1
Control - 12	0	+++	0
12 mg/kg - 1	1	++	1
12 mg/kg - 3	1	+	5
12 mg/kg - 4	0	+	0
12 mg/kg - 501	3	+	4
12 mg/kg - 505	3	--	4
16 mg/kg - 6	2	++	4
16 mg/kg - 7	2	+	4
16 mg/kg - 512	2	+	1
16 mg/kg - 740	3	--	4
16 mg/kg - 879	0	--	1
20 mg/kg - 507	1	+	3
20 mg/kg - 508	5	+	1
20 mg/kg - 510	3	+	4
20 mg/kg - NB	3	+	4

Key: lesions graded on a scale of 0-5: 0 - none, 1 - very mild, 2 - mild, 3 - moderate, 4 - marked, 5 - severe; Lymphoid follicle #: -- none, + few, ++ several, +++ many

Table 2-7: Hepatic morphological changes observed in bobwhite quail chronically fed T-2 toxin for 4 weeks (Experiment 4).

Treatment - band number	bile duct hyperplasia	vacuolation	hemosiderin content	focal inflammatory cell infiltration
Control - 10	0	0	0	1
Control - 13	0	0	1	0
Control - 14	3	0	0	0
Control - 11	0	0	0	1
Control - 12	2	0	0	0
12 mg/kg - 1	0	0	0	0
12 mg/kg - 3	1	1	0	0
12 mg/kg - 4	1	0	0	0
12 mg/kg - 501	0	2	0	0
12 mg/kg - 505	0	0	2	0
16 mg/kg - 6	2	0	1	1
16 mg/kg - 7	1	2	1	1
16 mg/kg - 512	0	1	0	0
16 mg/kg - 740	1	1	2	0
16 mg/kg - 879	1	0	0	1
20 mg/kg - 507	2	1	0	1
20 mg/kg - 508	2	0	0	1
20 mg/kg - 510	1	1	1	1
20 mg/kg - NB	0	0	3	0

Key: lesions graded on a scale of 0-5: 0 - none, 1 - very mild, 2 - mild, 2 - moderate, 4 - marked, 5 - severe

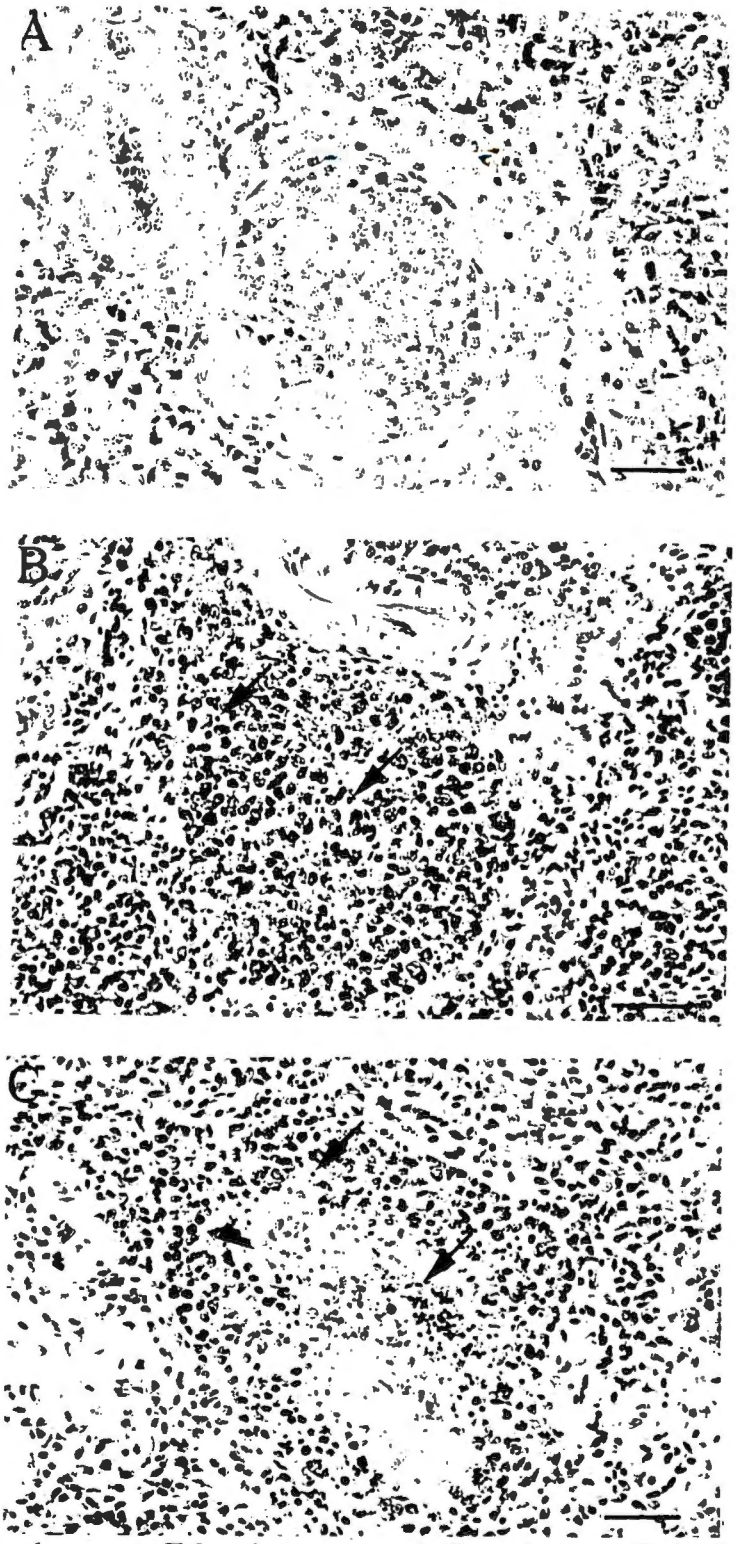


Figure 2-1: Quail spleen, acute T-2 toxin treatment. A. Control spleen. B. Moderate necrosis of periarteriolar lymphoid sheath with pyknotic lymphocyte nuclei (15 mg/kg T-2). C. Marked lymphocyte necrosis of periarteriolar lymphoid sheath characterized by pyknotic nuclei (18 mg/kg T-2). 1cm bar = 140 microns.

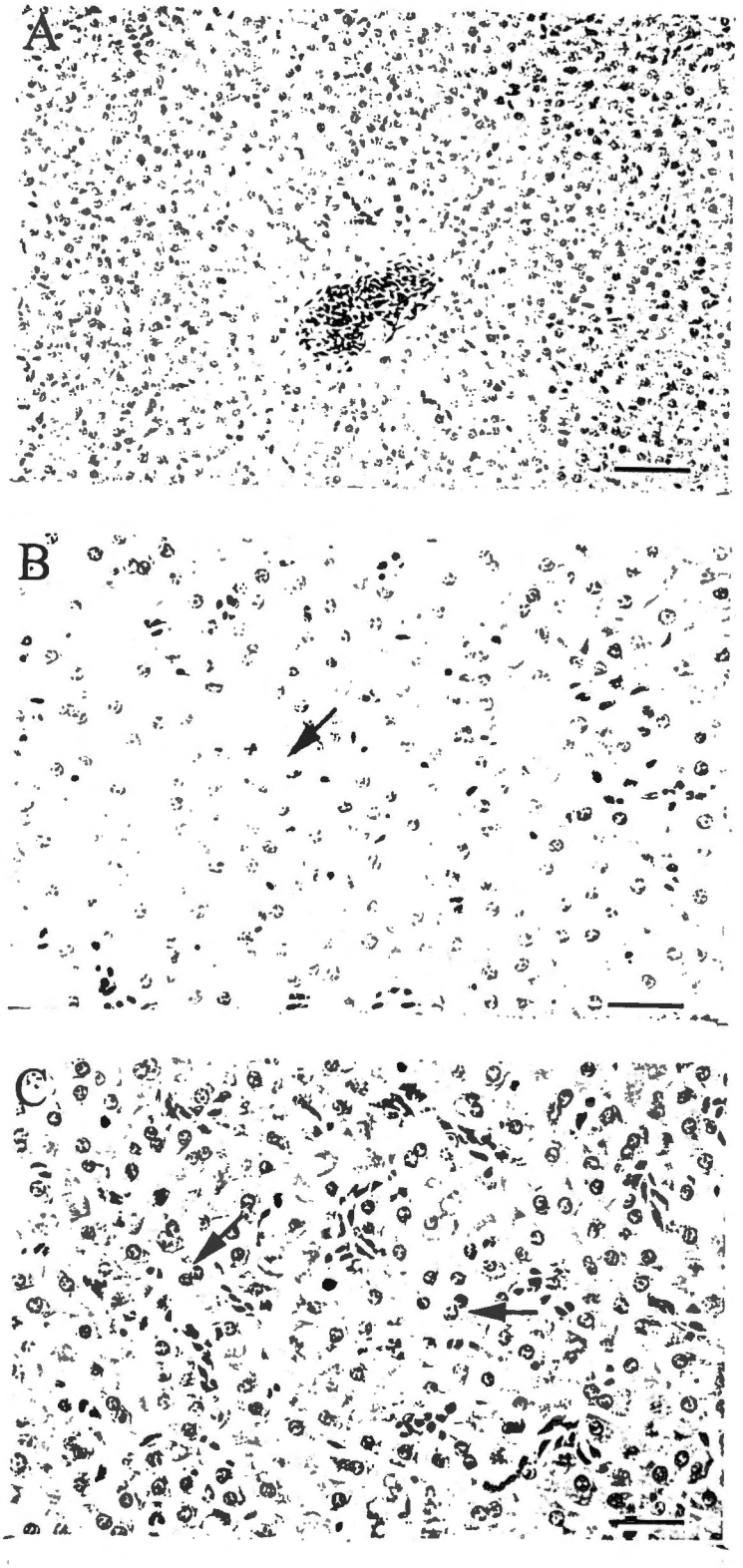


Figure 2-2: Quail liver, acute T-2 toxin treatment. A. Control liver. B. Fatty change associated with malfunctioning hepatocytes, binucleated hepatocyte (15 mg/kg T-2). C. Binucleated hepatocyte and more fatty change (18 mg/kg T-2). 1 cm bar = 140 microns.

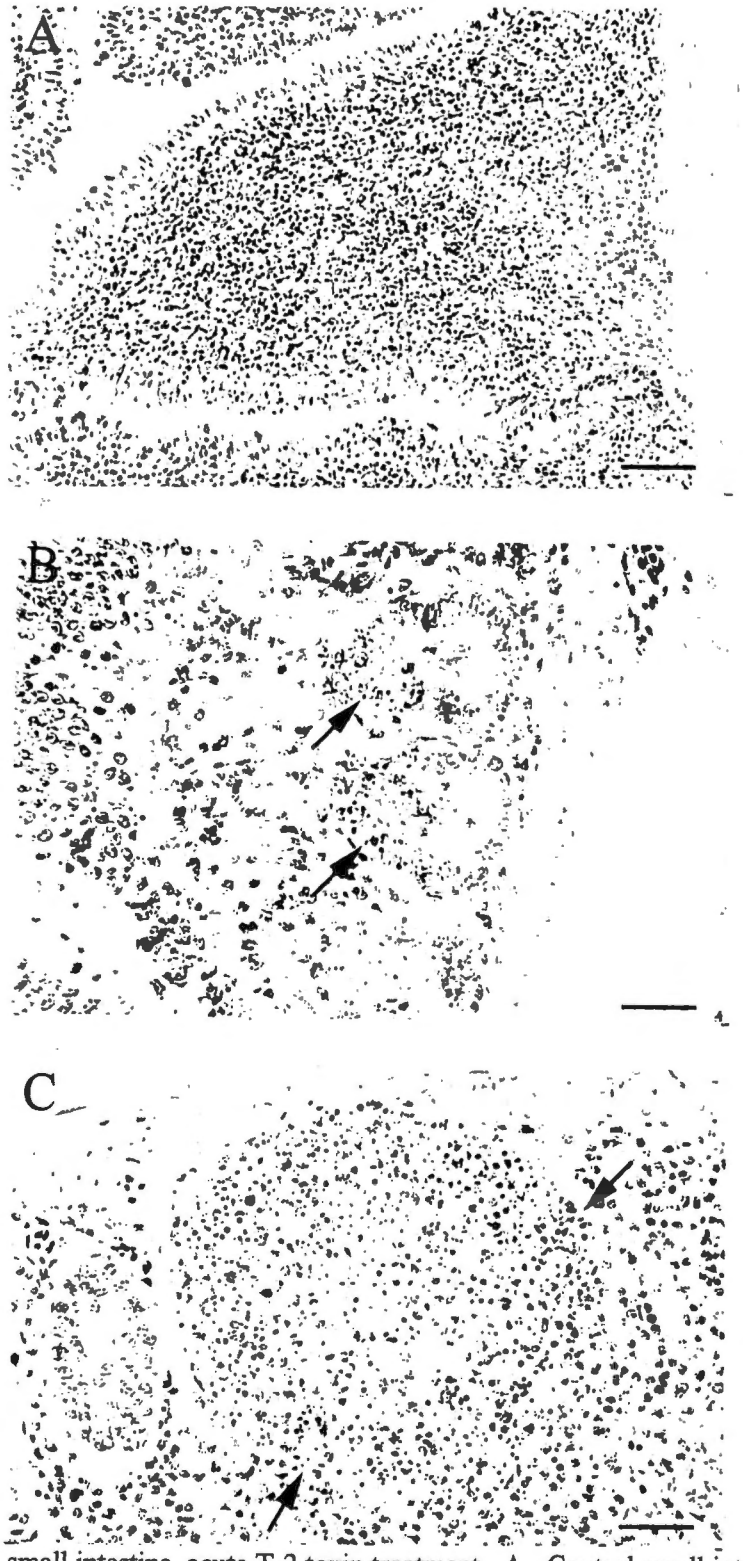


Figure 2-3: Quail small intestine, acute T-2 toxin treatment. A. Control small intestine. 1 cm bar = 70 microns. B. Mild crypt necrosis characterized by pyknotic nuclei of crypt epithelial cells (arrow; 15 mg/kg T-2) 1 cm bar = 140 microns. C. Extensive crypt necrosis characterized by pyknotic nuclei of crypt epithelial cells (18 mg/kg T-2). 1 cm bar = 70 microns.

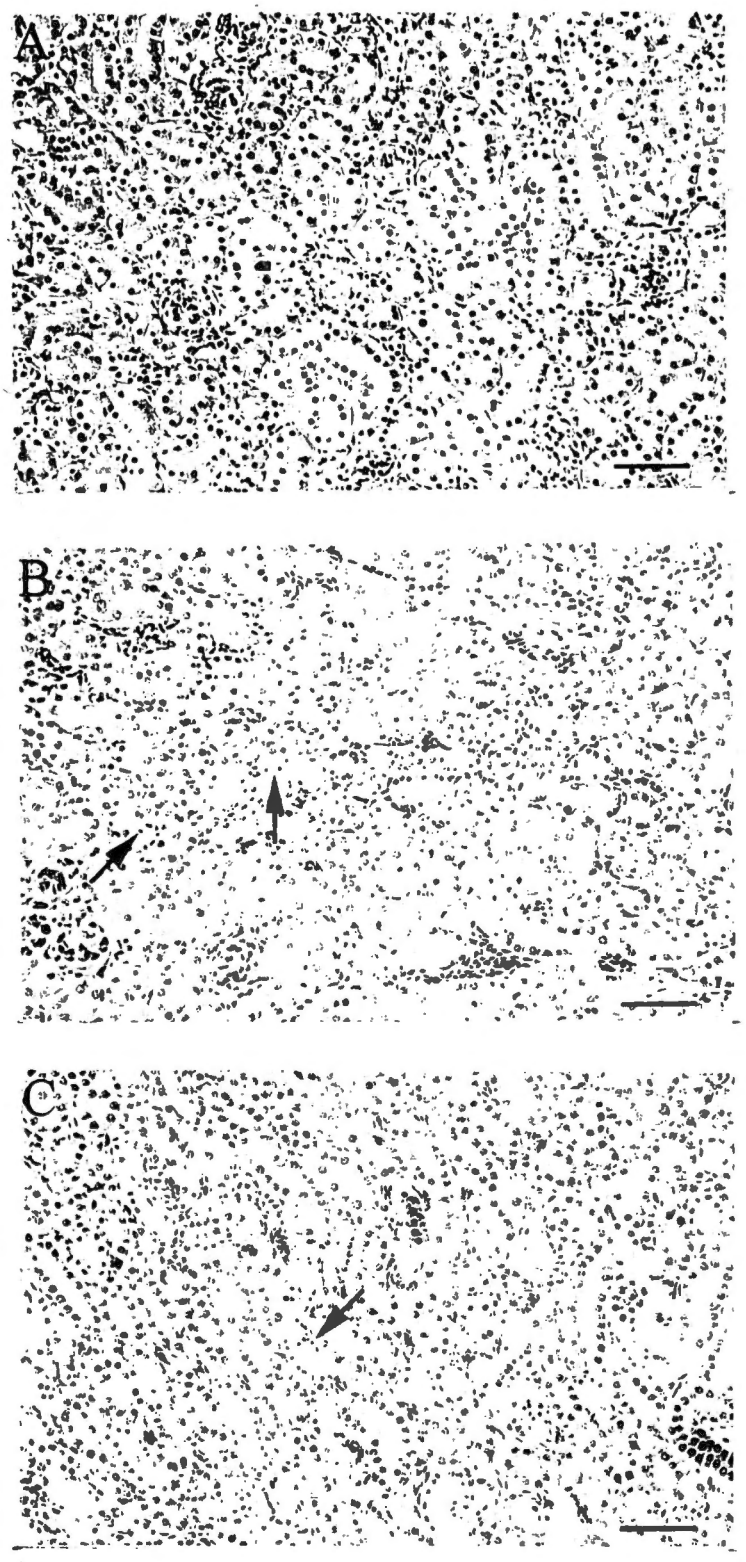


Figure 2-4: Quail kidneys, acute T-2 treatment. A. Control kidney. B. Pyknotic nuclei of tubular epithelial cells (15 mg/kg T-2). C. Dilated renal tubule and pyknotic nuclei of tubular epithelial cells (18 mg/kg T-2). 1 cm bar = 70 microns.

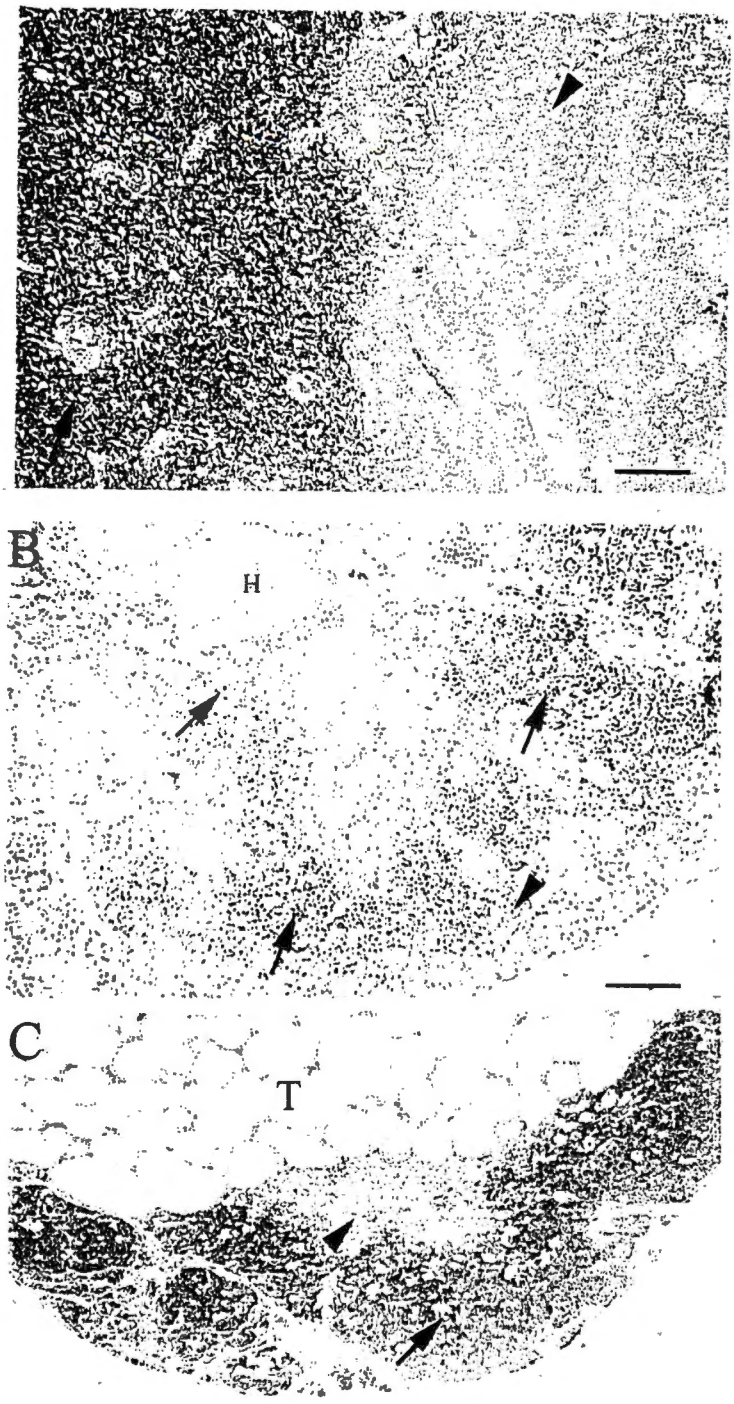


Figure 2-5: Quail thymus, acute T-2 treatment. A. Control thymus. arrow = cortex, arrowhead = medulla, white arrow = Hassel's corpuscle. B. Moderate thymic necrosis (15 mg/kg T-2). dark arrowhead = loss of cortical lymphcytes, arrowhead = cortex pyknotic lymphcyte nuclei, grey arrow = pyknotic nuclei. H = Hassel's corpuscle. C. Marked thymic necrosis (18 mg/kg T-2). T = thyroid, arrow = loss of cortex cellularity, arrow head = loss of medullary lymphocytes. 1 cm bar = 35 microns.



Figure 2-6: Quail bursa of Fabricius, acute T-2 treatment. A. Control bursa, extensive numbers of lymphocytes. B. Extensive loss of cellularity with pyknotic nuclei of lymphocytes (18 mg/kg T-2). 1 cm bar = 70 microns.

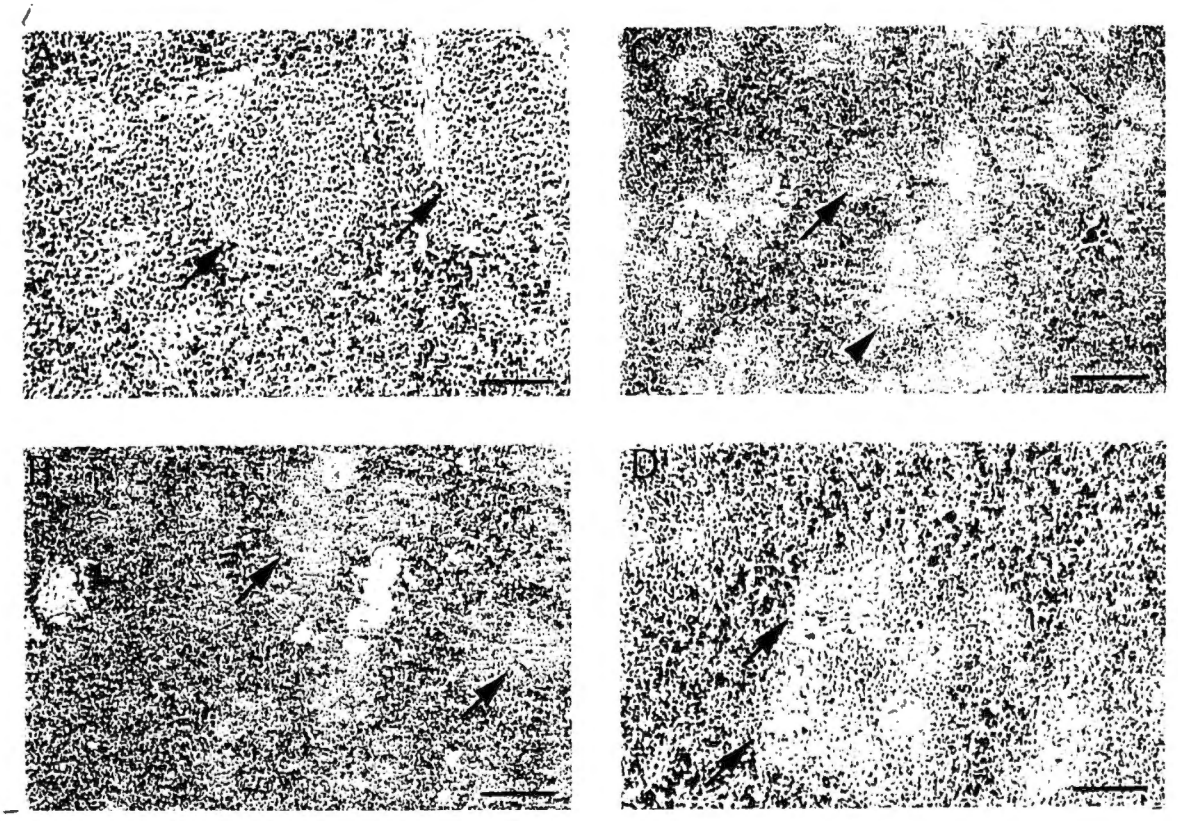


Figure 2-7: Quail spleen, chronic T-2 treatment. A. Control periarteriolar lymphoid sheaths. B. Mild lymphoid depletion of periarteriolar lymphoid sheath (12 mg T-2/kg feed). C. Moderate lymphoid depletion, loss of lymphocytes throughout (15 mg T-2/kg feed). D. Extensive loss of lymphocytes with pyknotic nuclei (18 mg T-2/kg feed). 1 cm bar = 70 microns.

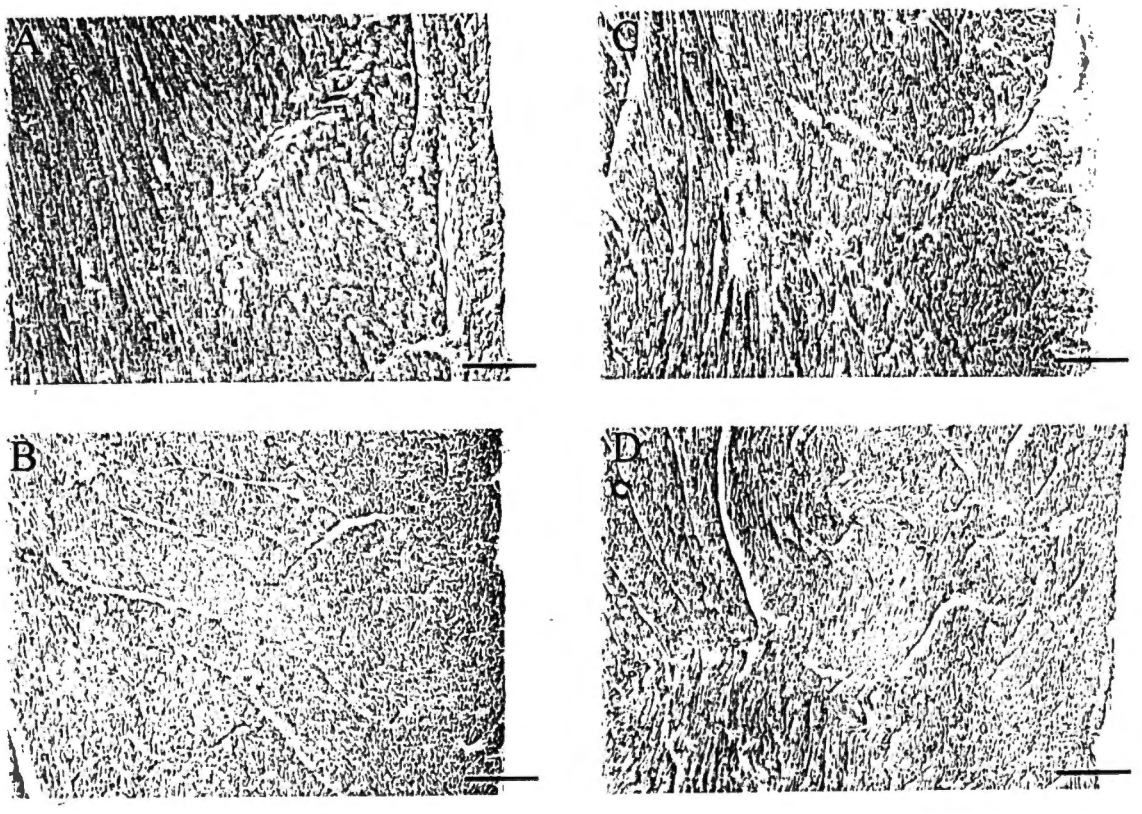


Figure 2-8: Quail heart, chronic T-2 treatment. A. Control - no significant lesions. B. 12 mg T-2/kg feed - no significant lesions. C. 16 mg T-2/kg feed no significant lesions. D. 20 mg T-2/kg feed - no significant lesions. 1 cm bar = 35 microns.

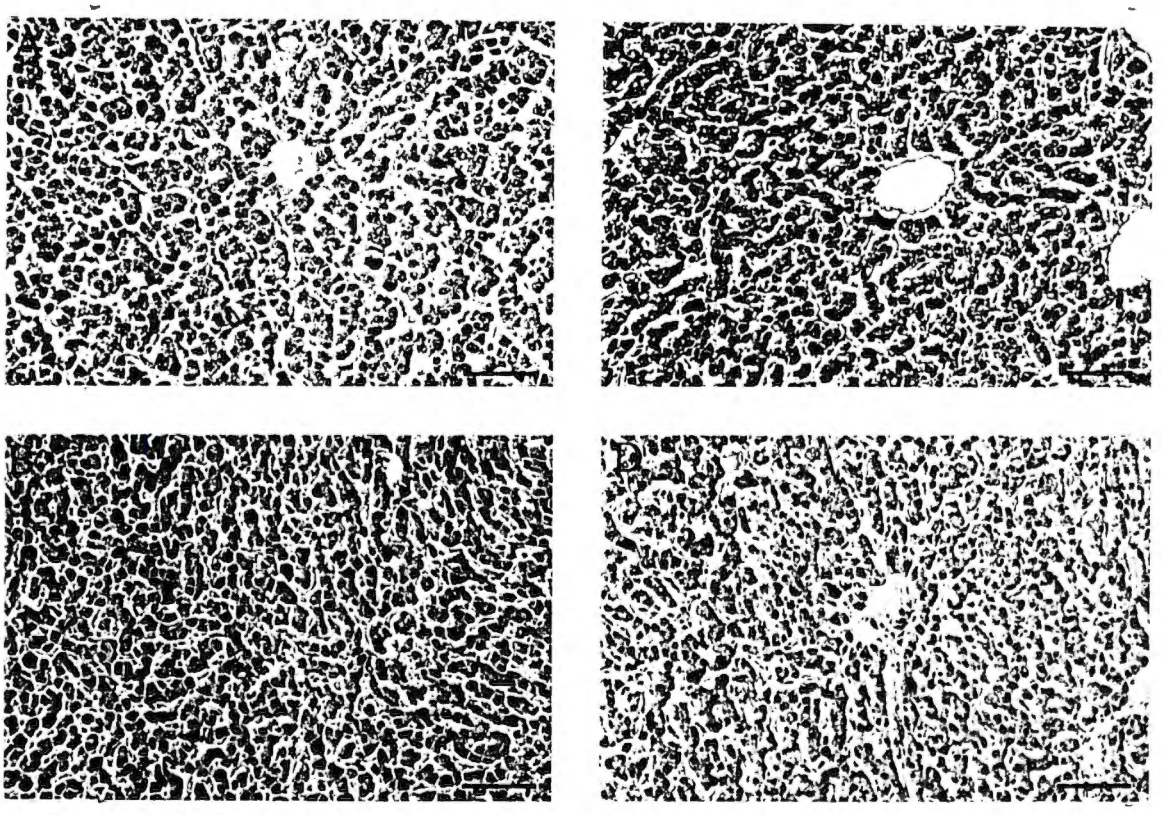


Figure 2-9: Quail liver, chronic T-2 treatment. A. Control - no significant lesions. B. 12 mg T-2/kg feed - no significant lesions. C. 16 mg T-2/kg feed - no significant lesions. D. 20 mg T-2/kg feed - no significant lesions. 1 cm bar = 70 microns.

PART III.

EFFECT OF CHRONIC VS. INTERMITTENT EXPOSURE TO T-2 TOXIN ON REPRODUCTIVE PERFORMANCE OF ADULT BOBWHITE QUAIL.

Abstract

A laboratory study was designed to determine the effect of chronic vs. intermittent exposure to T-2 toxin on reproductive performance in adult bobwhite quail (*Colinus virginianus)*. In Experiment 1, 180 hens were orally dosed with 0% (LD_0), 20% (LD_{20}), 40% (LD_{40}) or 60% (LD_{60}) of the acute 100% lethal dose of T-2 toxin as determined in a previous experiment. One quarter of the dosage was administered each week for 4 weeks. Date of puberty was delayed 5 days as a result of the highest dose of T-2 toxin. There were no significant ($P>.05$) differences in hen-day egg production or fertile hatchability as a result of intermittent exposure to T-2 toxin. Fertility and total hatchability of eggs collected from LD_{60} hens on days 1-7 following cessation of treatment were less ($P\leq.05$) than eggs from control hens.

In Experiment 2, 139 hens were provided *ad libitum* access to 0, 12 , 16, and 20 mg T-2 toxin/kg feed for a 4 week period. Puberty was delayed 11 days as a result of ingestion of feed with the highest amount of toxin. No differences ($P>.05$) in hen-day egg production or fertile hatchability were found. However, percent fertility and total hatchability were lower ($P<.005$) among hens receiving 20 mg T-2 toxin/kg feed as compared to control hens receiving no T-2 contaminated feed for the first 7 days following cessation of T-2 treatment. Chronic and intermittent exposure to T-2 toxin resulted in significant reductions in reproductive performance during the first weeks following exposure.

Introduction

T-2 toxin is a trichothecene mycotoxin primarily produced by the fungi *Fusarium tricinctum* (Nakadate and Uneo, 1990), and has been shown to be a potent inhibitor of protein and DNA synthesis *in vivo* and *in vitro* (Rosenstein and Lafarge-Frayssinet, 1983). The immunosuppressive effects of T-2 toxin include decreased levels of circulating antibodies, numbers of circulating B and T cells and increased susceptibility to extracellular pathogens (Mann *et al.*, 1983; Holladay *et al.*, 1995; Corrier and Ziprin, 1987). The suppressive effects of T-2 toxin on reproductive performance in species other than bobwhite quail (*Colinus virginianus)* have been reported (Speers *et al.*, 1977; Chi *et al.*, 1977b; Tobias *et al.*, 1992). However, little or no information is available on the effects of T-2 toxin in bobwhite quail.

In chickens, significant reductions in both body weight and egg production have been reported in laying hens. Speers *et al.* (1977) reported a significant ($P \leq .05$) reduction in body weight among laying hens 16 mg T-2 toxin/kg of feed vs. controls (1525 g vs. 1595 g respectively). Likewise, egg production was lower among hens fed 16 mg/kg T-2 toxin (3.46 eggs per day for 16 mg/kg diets vs. 6.13 eggs per day for control diets). In a similar study, Chi *et al.* (1977b) observed a significant ($P \leq 0.05$) decrease in feed consumption, egg production, and egg shell thickness among laying hens fed 8 mg T-2 toxin/kg feed. Lowered ($P \leq 0.05$) fertile hatchability was observed among hens fed 2 or 8 mg T-2 toxin/kg feed as compared to controls. Likewise, Tobias *et al.* (1992) observed a significant reduction in egg production among chickens fed 5 or 10 mg T-2 toxin/kg feed ($P \leq .05$ and $P \leq .01$ respectively). Significantly ($P \leq .05$) more infertile eggs were observed among treated birds than in control birds.

The effect of T-2 toxin on body weight gain in young growing animals has been

extensively studied in many poultry species. Ruff *et al.* (1992) fed young bobwhite and Japanese quail diets containing 4, 8 and 16 mg T-2/kg feed starting at day 1 of age. In bobwhite quail, body weight was significantly ($P \leq .05$) reduced by the 8 and 16 mg/kg diets by one week of age as compared to controls. In Japanese quail, the 16 mg/kg diet reduced body weight during the same time period. In a similar study, Huff *et al.* (1992) fed day old ring-neck pheasants diets containing 4, 8 and 16 mg T-2 toxin/kg feed. Body weight gain was less ($P \leq .05$) among birds fed 16 mg/kg diet when compared to controls. Kubena et al. (1995) observed a 26% decrease in weight gain among turkey poults fed 5 mg T-2 toxin/kg of feed as compared to controls. Similarly, body weight gain was less among laying hens fed 8 mg T-2/kg feed (Chi *et al.*, 1977a).

Ames Plantation, the site of the National Championships for purity all age bird dogs, supports a bobwhite quail population that is integral to the mission of the Plantation. Over the past 20 years quail populations have been fluctuating despite management practices aimed at improving habitat for quail. Recent analysis of soybeans left as late winter feed showed the presence of T-2 toxin. It was hypothesized that T-2 toxin may be a contributing factor to the decreasing quail population. However, mycotoxin invasion of agricultural grains is not a phenomenon limited to Ames Plantation. Wherever agricultural grains or naturally occurring grains (e.g. lespedezas) compose a significant portion of quail diets and mycotoxin outbreaks are possible, then negative effects of toxicity may be periodically possible. The objectives of the following study was to determine the effect of intermittent vs. chronic exposure to T-2 toxin on reproductive performance of adult pen-raised bobwhite quail.

Materials and Methods

Two experiments were conducted to determine the effect of intermittent (Experiment 1) vs. chronic (Experiment 2) exposure to dietary T-2 toxin on reproductive performance in bobwhite quail. In Experiment 1, 180 bobwhite quail hens (45 hens/treatment) were randomly assigned to receive 0% (LD_0, control; 0 mg toxin/kg body weight [BW]), 20% (LD_{20}; 12.4 mg toxin/kg BW), 40% (LD_{40}; 14.0 mg toxin/kg BW), or 60% (LD_{60}; 15.5 mg toxin/kg BW) of the acute 100% lethal dose T-2 toxin (20.0 mg toxin/kg BW) as previously determined (Kersten, 1998). Hens were housed in grower batteries (Petersime Company, Gettysburgh, OH; Model 515) and provided *ad libitum* access to feed and water (Mazuri Brand Pheasant Breeder, PMI Feeds Inc., St. Louis, MO). Purified crystalline T-2 toxin (Sigma, St. Louis, MO) was dissolved in 100% ethanol, and diluted to volume with corn oil. Doses were administered by oral gavage over a 4 week period; 1/4th dose per week. Only hens were dosed with T-2 toxin, although males were maintained with hens at a rate of one male per two females. Light stimulation (16 hours/day) was initiated at the beginning of the treatment period to stimulate egg production. Data were collected on body weight, date of puberty, egg weight, specific gravity of eggs, hen-day egg production (HDEP), percent fertility, fertile hatchability, and total hatchability of eggs. Eggs were collected for 17 weeks, while hatchability data was measured for 12 weeks. Hens began laying in April and the experiment ended in August. Body weights of chicks from hens intermittently exposed to T-2 toxin were determined at hatch, 4 and 8 weeks of age for a total of 9 hatches.

Date of puberty was determined to be the first day a hen from each treatment group laid its first egg. Calculations of HDEP data began when 15% of the hens from each treatment group were producing eggs.

Eggs were collected for 2 days each week for specific gravity and egg weight

measurements.

All eggs laid during the first 7 days following cessation of treatment were collected and set. Thereafter, eggs were set on a biweekly basis beginning on day 7 following cessation of treatment when hens reached 15% egg production. Eggs were candled 7 days after setting. Infertile, fertile but dead, and cracked eggs were removed. Percent fertility was calculated as total fertile + fertile but dead eggs divided by the total number of eggs set minus the number of cracked eggs times one hundred. Percent fertile hatchability was determined to be the number of live chicks divided by the total number of fertile eggs times one hundred. Total hatchability was determined to be the number of live chicks divided by the total number of eggs set minus the number of cracked eggs times one hundred.

In Experiment 2, 139 bobwhite quail hens were assigned to 1 of 4 treatment groups; 0, 12, 16, and 20 mg T-2 toxin/kg feed. Treatments were replicated four times with 8-11 birds per replicate, for a total of 32-44 birds per treatment. T-2 toxin was dissolved into 100% ethanol, diluted to volume in corn oil, and added to commercial breeder diets as in Experiment 1 (Mazuri Pheasant Breeder, PMI Feeds Inc., St. Louis, MO). Hens were housed in grower batteries (Petersime Company, Gettysburgh, OH; Model 515) and provided feed and water *ab libitum*. T-2 toxin contaminated feed was fed for a total of four weeks. Batteries were contained in a Bioshield 350 (Airo Clean Inc., Exton, PA) positive pressure biological containment unit. Body weight and feed consumption data were collected immediately before and at the end of the treatment period. Photo-stimulation, 16 hours/day, began 2 weeks after initiation of T-2 treatment to bring hens into egg production. Males were added to the cages the day after cessation of contaminated feed treatment (2 weeks after light stimulation was initiated). Thereafter all birds were fed the non-contaminated commercial pheasant breeder feed. Date of puberty, hen-day egg production, hatchability and mortality data were collected for 12 weeks. Eggs were set at weekly intervals for

a total of 12 hatches of 12 weeks egg production. Body weights of chicks from hens chronically exposed to T-2 toxin were determined at hatch, 4 and 8 weeks of age for all 12 hatches set. The started in January and ended in April. Specific gravity and egg weight were not measured in Experiment 2.

Data were analyzed using the PROC FREQ and PROC MIXED procedures of SAS (1996). Means were separated by Fisher's Least Significant Difference Test.

Results

Experiment 1

There was no affect of T-2 toxin on body weights of bobwhite hens when administered once a week for a four week period prior to puberty (Table 3-1). Light stimulation was accompanied by a pre-puberal weight gain so that all hens were significantly heavier ($P \leq .05$) 4 weeks after light stimulation exposure than at the beginning of the experiment. This weight change was not affected by T-2 treatment. There was no mortality among hens in Experiment 1.

Date of puberty was 22 days after initiation of light stimulation for control and LD_{40} hens, 26 days after initiation of light stimulation for LD_{20} hens, and 27 days after initiation of light stimulation for LD_{60} hens.

Average egg weight over a 12 week period increased ($P \leq .05$) in eggs from treated hens as compared to eggs from controls (Table 3-2).

While egg weights increased, twelve week average specific gravity of eggs was significantly lower ($P \leq .05$) among eggs from any T-2 toxin treated hen vs. controls (Table 3-3).

There were no differences (P>.05) in hen-day egg production due to intermittent T-2 toxin treatment, however, birds receiving the highest dose of T-2 toxin laid at a rate 7.3% less than untreated control hens (Table 3-4). Likewise, there were no overall differences (P>.05) in fertility, fertile hatchability or total hatchability due to intermittent T-2 toxin treatment (Table 3-4).

All eggs laid during the first 7 days immediately following cessation of T-2 toxin treatment were collected and set. However, only 38 eggs were laid which represented 3.37% egg production. This was considered an insufficient sample size, and the data was omitted from the analysis. On day 7 following cessation of T-2 toxin treatment, hens were laying at a rate of 14.33% and eggs were set for hatchery data. Thereafter, eggs were collected on a biweekly basis for hatchery data.

Significant reductions (P≤.05) in percent fertility of eggs were observed among eggs collected during week 2 following cessation of T-2 toxin treatment (Table 3-5). Percent fertility of eggs from the LD_{60} hens was significantly lower (P≤.05) as compared to control hens (85.71 vs. 100.00 respectively). Likewise, total hatchability of eggs from LD_{60} hens was significantly lower (P≤.05) as compared to eggs from control hens (64.84 vs. 84.21). No differences (P>.05) in fertile hatchabilty were observed during week 2 of egg production. Eggs collected during week 4 of egg production were not different in either fertility, fertile hatchability or total hatchability (Table 3-5; P>.05). Likewise, there were no differences in fertility, fertile hatchability or total hatchability of eggs for any of the remaining weeks of egg production (data not shown).

Significant (P≤.05) differences in body weights of chicks from hens intermittently treated with T-2 toxin were observed (Table 3-6). At hatch, chicks from LD_{20} hens were significantly (P≤.05) heavier than chicks from control, LD_{40} and LD_{60} hens. At 4 weeks of age, chicks from control and LD_{20} hens were significantly (P≤.05) heavier chicks from LD_{60} and LD_{40} hens. At 8 weeks of age, chicks from control hens were significantly (P≤.05) heavier than chicks from LD_{20}

and LD_{60} hens which were significantly (P≤.05) heavier than chicks from LD_{40} hens.

Experiment 2

Significant (P≤.005) reductions in body weight were observed as a result of chronic exposure to T-2 toxin (Table 3-7) and resulted in the death of 0, 2, 3 and 4 hens from consumption of 0, 12, 16, and 20 mg T-2/kg feed respectively. Feeding higher concentrations of T-2 toxin/kg feed resulted in an increase in body weight loss and increased mortality.

Significant reductions (P≤.05) in average daily feed consumption were observed as a result of chronic exposure to T-2 toxin (Table 3-8). Feeding higher concentrations of T-2 toxin/kg feed resulted in decreased feed consumption.

As with Experiment 1, date of puberty was delayed with T-2 toxin exposure. Date of puberty was 23, 29, 37 and 34 days after initiation of light exposure for 0, 12, 16 and 20 mg/kg feed treatment respectively which was a longer interval than in Experiment 1. There were no differences in HDEP due to consumption of T-2 toxin (P>.05; Table 3-9) during the pre-puberal period. Likewise, there was no overall differences (P>.05) in percent fertility, total hatchability, or fertile hatchability of eggs set at weekly intervals for 12 weeks following chronic exposure to T-2 toxin (Table 3-9).

In Experiment 2, eggs were set on a weekly basis as compared to a biweekly basis in Experiment 1. More eggs were laid during the first week following puberty and cessation of T-2 treatment than in Experiment 1. A total of 301 eggs (16.41% HDEP) were collected and set during the first weeks following puberty. During week 1 following puberty, fertility of eggs was significantly (P≤.005) less among hens fed 20 mg/kg T-2 toxin as compared to control hens

(81.23% vs. 100.00%; Table 3-10). Similarly, percent total hatchability was significantly ($P \leq .005$) less among hens fed the 20 mg/kg diet as compared to control hens (65.82% vs. 82.20% respectively). A numerical reduction in both fertility and total hatchability was seen among hens fed 16 mg T-2/kg feed. As in Experiment 1, no difference ($P > .05$) in fertile hatchability was observed due to chronic feeding of T-2 toxin.

During the second week following cessation of T-2 treatment no differences ($P > .05$) in fertility, fertile hatchability or total hatchability were observed (Table 3-10). However, a trend in fertility and total hatchability was observed. Hens fed the 16 or 20 mg T-2 diets during the pre-puberal period laid eggs that were 7-10% lower in fertility (84.98% and 86.59%) than control hens (95.41%). The difference between 16 mg and control birds approached significance ($P \leq .086$). Likewise, total hatchability was less among 16 and 20 mg/kg feed birds (76.85% & 76.07% for 16 and 20 mg T-2 diets respectively) vs. controls (83.74%).

Body weights of chicks from hens chronically treated with T-2 toxin were significantly ($P \leq .05$) different at hatch, 4 and 8 weeks of age (Table 3-11). However, while overall experiment-wise differences in chick body weights were observed due to parental T-2 treatment, data were highly variable and differences were not considered significant.

Discussion

This study was designed to compare the effects of intermittent vs. chronic exposure to T-2 toxin on reproductive performance of adult bobwhite quail. The intent was to mimic the type of mycotoxin exposure wild quail might experience if ingesting infested soybeans. If wild quail throughout the south eastern United States were continually ingesting mycotoxin infested soybeans,

the chronic study would closely duplicate this situation. If wild quail are ingesting mycotoxin infested soybeans on an irregular basis, the intermittent study would mirror this situation.

The negative effect of T-2 toxin on body weight gain has been well documented in many poultry species. Ruff *et al.* (1992) observed a significant ($P \leq .05$) reduction in body weight over a three week period in bobwhite and Japanese quail fed diets containing 4, 8 and 16 mg T-2/kg feed from day 1 of age. Kubena *et al.* (1995) observed a 26% decrease in weight gain in turkey poults fed 5 mg T-2 toxin/kg feed from hatch to day 21 of age as compared to controls. Likewise, they (Kubena *et al.*, 1989) observed significant ($P \leq .05$) reductions in body weight gain of broiler chicks fed T-2 toxin from day 1 to 3 weeks of age as compared to controls. Similarly, over a 30-day period, Chi *et al.* (1977a) observed a 43.7% decrease in weight gains of day old broiler chicks dosed with 6.5 mg T-2/kg BW. These data agree with the results from Experiment 2 where hens were chronically given T-2 toxin, but not in Experiment 1, where birds were dosed on a weekly basis and allowed to recover. In Experiment 2, overall hen weight decreased 18.65% (among treated birds) which is not consistent with the accumulation of yolk for egg production as was seen in Experiment 1 (Table 8 vs. Table 1). During the 4 week T-2 feeding period, hens lost weight due to T-2 toxin treatment. Experiment 2 hens were only exposed to long day lengths for 2 weeks at the end of the T-2 exposure period, as compared to 4 weeks in Experiment 1. Likewise, Experiment 2 hens were constantly exposed to T-2 toxin and were not allowed to recover as in Experiment 1. Onset of puberty in treated hens was delayed an average 8 days longer in Experiment 2 than in Experiment 1.

Significant decreases ($P \leq .05$) in feed consumption were observed in chicken hens fed 8 mg/kg T-2 toxin for a 8 weeks (Chi *et al.*, 1977b). This agrees with our results, ingestion of any dietary level of T-2 results in a significant ($P \leq .05$) decrease in feed consumption. As Chi *et al.*

concluded, decreases in feed consumption were a result of the necrotic oral lesions, making feeding painful.

Allen *et al.* (1983) observed a significant (P≤.05) increase in egg weight from Large white turkey females fed diets containing 5 mg/kg T-2. This agrees with our observed increase in egg weight from hens intermittently dosed with T-2 toxin as compared to controls. This may possibly be explained by the delayed onset of puberty caused by T-2 toxin treatment. Delayed puberty would allow for the deposition of more yolk, increased body weight and the increase in average egg weight from treated hens as compared to control hens.

Previous studies with chickens have reported decreased shell thickness of eggs from hens fed T-2 toxin (Chi *et al.*, 1977b). Similarly, in our studies, hens dosed with T-2 toxin on a weekly basis laid eggs of a lower (P≤.05) specific gravity and thus a thinner shell as compared to controls. Obviously, thin shelled eggs are a detriment to success of wild quail because they are more susceptible to physical damage than thick shelled eggs. Wild quail populations on Ames Plantation and throughout the southeastern United States have been fluctuating for the past 20 years (Dimmick, 1996). If wild quail are exposed to mycotoxin infested feeds, the resulting thin shelled eggs may be a contributing factor to this trend.

Laying hens fed diets containing 2 and 8 mg T-2 toxin/kg feed experienced no adverse affect on number of fertile eggs (Chi *et al.*, 1977b). However, hatchability of fertile eggs from these hens was significantly lower than that of control hens (P≤.05; Chi *et al.*, 1977b). Likewise, Tobias *et al* (1992) reported poor hatchability accompanied by reduced feed intake among laying hens fed diets containing 5 and 10 mg T-2 toxin/kg feed. These authors concluded that the total hatchability was a result of T-2 toxin induced infertility.

In Experiment 2, no overall (P>.05) differences in percent fertility, fertile hatchability, or

total hatchability were observed due to chronic exposure to T-2 toxin contaminated feed. However, among eggs collected for the first 7 days after T-2 treatment significant reductions ($P \leq .005$) in percent fertility were found. Chi *et al.* (1978a) and Tobias *et al.* (1992) reported that T-2 toxin and its metabolites are deposited into egg yolk for 11 days following cessation T-2 exposure. Therefore, the early decline in percent fertility may be a result of yolk T-2 content. After the first 2 weeks of egg production, the toxin was cleared from the body and no further experimental changes were observed (Chi *et al.*, 1978b).

Body weights of chicks from hens intermittently vs. chronically exposed to T-2 toxin have not been previously investigated in bobwhite quail. In both Experiment 1 and Experiment 2, differences in body weights of chicks from treated hens at hatch, 4 and 8 weeks of age were found, but the data were highly variable and not considered to be significant. In the intermittent study, at hatch chicks from control, LD_{40}, and LD_{60} hens were significantly ($P \leq .05$) lighter than chicks from LD_{20} hens. At 4 weeks of age chicks from LD_{40} hens were significantly ($P \leq .05$) lighter than chicks from all other treatments. At 8 weeks of age chicks from treated hens were significantly ($P \leq .05$) lighter than controls indicating a treatment effect. However, this result did not occur in the chronic study.

In Experiment 2, which was of a longer duration, chick weights at hatch, 4 and 8 weeks of age were lowest among first three hatches set. Average weights increased until hatch 7, and then declined slightly through hatches 8, 10, 11, and 12. The first hatch from Experiment 1 was set in April, the last in August. The first hatch from Experiment 2 was set in January, the last in April. Weights among chicks in the hatches set in winter may have initially been lower as a consequence of cold stress as batteries were in poorly heated buildings. Chicks from hatch 6 and hatch 9 died as a result of exposure during a power outage at the University. In Experiment 1, data from the hatch

1 was omitted due to insufficient sample size. Hatch 2 was omitted from the analysis because at hatch LD_{40} chicks crossed the dividers in the hatch tray and mixed with other treatments. Overall egg weight in the first several hatches may have been lower as a result hens not being in full egg production. Because chicks from control hens were not significantly heavier than chicks from treated birds, differences between treatment groups cannot be explained as a result of T-2 toxin treatment.

Based upon our results, we conclude that T-2 toxin may have similar effects on adult Bobwhite quail as have been reported in chickens. However, it appears that slightly higher levels of T-2 may be needed to elicit the same effects in pen-raised quail. Levels of 1, 3, 5, 8, and 10 mg T-2 toxin/kg feed resulted in body weight depression, growth retardation, reduced egg shell thickness and hatchability of fertile eggs in chickens (Wyatt *et al.*, 1975; Kubena *et al.*, 1989; Tobias *et al.*, 1992). The levels of T-2 toxin used in our study were twice these values. It has been documented that there are large differences in toxin metabolism within avian species (Pan and Fouts, 1979; Dalvi *et al.*, 1987). Biotransformation is the sum of processes by which a foreign chemical or xenobiotic is subjected to chemical change by living organisms. Hydrolysis of ester linkages seem to be the major pathway in the metabolism of T-2 toxin with hydrolysis of the ester at carbon 4 being the primary site of attack (Leeson *et al.*, 1995). Species differences in drug and toxin metabolism have been attributed to different levels and forms of liver enzymes (Dalvi *et al.*, 1987). The hepatic microsomal enzymes thought to participate in detoxifying reactions include cytochrome, benzphetamine N-demethylase, aniline hydroxylase, and glutathione S-transferase (Dalvi *et al.*, 1987). Since it takes more T-2 toxin in quail to obtain similar effects, one would assume that chickens have higher levels of detoxifying enzymes than quail. However, all available information indicates that quail have lower levels of hepatic microsomal enzymes than chickens (Dalvi *et al.*, 1987; Enkvetchakul *et al.*, 1995). Other avian species differences in T-2 toxin

susceptibility have not been attributed to differences in metabolism or elimination of T-2 toxin from the body (Giroir *et al.*, 1990). To date, we have no reasonable explanation as to why bobwhite can withstand higher levels than chickens. Exposure to T-2 toxin during the pre-puberal period may be a contributing factor to nest failure of wild quail.

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II.

Appendix: Tables

Table 3-1: Average body weight (BW) of bobwhite quail hens during intermittent T-2 toxin treatment period (Experiment 1).

Treatment	Week 0 (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
LD_0 (0 mg T-2/kg BW)	203^{a}	211^{b}	213^{b}	212^{b}	223^{c}
LD_{20} (12.4 mg T-2/kg BW)	207^{a}	213^{a}	209^{a}	209^{a}	225^{b}
LD_{40} (14.0 mg T-2/kg BW)	205^{a}	212^{ab}	209^{ab}	215^{b}	223^{c}
LD_{60} (15.5 mg T-2/kg BW)	208^{a}	215^{b}	213^{ab}	209^{ab}	222^{c}

^{a,b,c} values in the same row with different letters are significantly different ($P \leq .05$)

Table 3-2: Average weight (g) of eggs collected for 12 weeks following puberty from bobwhite quail hens dosed with T-2 toxin (Experiment 1).

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Total
LD_0 (0.0 mg T-2/kg BW)	10.29^{b}	10.35^{b}	10.35^{b}	10.60^{a}	10.65^{a}	10.46^{a}	10.56^{a}	10.38^{b}	10.66^{a}	10.53^{a}	10.63^{a}	10.45^{b}	10.49^{b}
LD_{20} (12.4 mg T-2/kg BW)	10.68^{a}	10.66^{a}	10.62^{ab}	10.73^{a}	10.80^{a}	10.69^{a}	10.63^{a}	10.53^{ab}	10.66^{a}	10.52^{a}	10.87^{a}	10.78^{a}	10.6
LD_{40} (14.0 mg T-2/kg BW)	10.50^{ab}	10.54^{ab}	10.71^{a}	10.76^{a}	10.80^{a}	10.71^{a}	10.54^{a}	10.36^{b}	10.73^{a}	10.27^{a}	10.65^{a}	10.68^{ab}	10.61^{a}
LD_{60} (15.5 mg T-2/kg BW)	10.51^{ab}	10.57^{a}	10.73^{a}	10.78^{a}	10.73^{a}	10.72^{a}	10.52^{a}	10.74^{a}	10.50^{a}	10.49^{a}	10.72^{a}	10.90^{a}	10.66^{a}

^{a,b} values in the same column with different letters are significantly different ($P \leq .05$).

Table 3-3: Average weekly specific gravity of eggs collected for 12 weeks after puberty from bobwhite quail hens dosed with T-2 toxin (Experiment 1).

Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 10	Week 11	Week 12	Totals
LD_0 (0.0 mg T-2/kg BW)	1.0705[a]	1.0732[ab]	1.0739[a]	1.0757[a]	1.0758[a]	1.0734[a]	1.0747[a]	1.0745[a]	1.0729[a]	1.0732[a]	1.0709[a]	1.0724[a]	1.0734[a]
LD_{20} (12.4 mg T-2/kg BW)	1.0703[a]	1.0718[b]	1.0736[a]	1.0753[a]	1.0752[ab]	1.0729[ab]	1.0738[a]	1.0752[a]	1.0717[ab]	1.0730[a]	1.0700[a]	1.0719[a]	1.0729[b]
LD_{40} (14.0 mg T-2/kg BW)	1.0702[a]	1.0737[a]	1.0732[a]	1.0745[a]	1.0744[ab]	1.0715[b]	1.0738[a]	1.0742[a]	1.0705[b]	1.0732[a]	1.0698[a]	1.0709[a]	1.0725[b]
LD_{60} (15.5 mg T-2/kg BW)	1.0703[a]	1.0734[ab]	1.0727[a]	1.0757[a]	1.0742[b]	1.0725[ab]	1.0737[a]	1.0752[a]	1.0728[a]	1.0724[a]	1.0703[a]	1.0711[a]	1.0728[b]

[a,b] values in the same column with different letters are significantly different ($P \leq .05$).

Table 3-4: Seventeen week average hen day egg production (HDEP), percent fertility, fertile hatchability, and total hatchability of eggs from bobwhite quail hens intermittently dosed with T-2 toxin (Experiment 1).

Treatment	HDEP	Fertility	Fertile Hatchability	Total Hatchability
	--	--	(%) --	--
LD_0 (0.0 mg T-2/kg BW)	67.19	92.14	85.23	78.63
LD_{20} (12.4 mg T-2/kg BW	65.41	94.34	88.95	83.98
LD_{40} (14.0 mg T-2/kg BW)	69.50	94.14	83.92	79.21
LD_{60} (15.5 mg T-2/kg BW)	59.89	88.50	87.76	77.97

Table 3-5: Percent fertility, fertile hatchability, and total hatchability of eggs laid by bobwhite quail hens during week 2 and 4 immediately following cessation of intermittent treatment with T-2 toxin (Experiment 1).

Treatment	Week 2			Week 4		
	Fertility	Fertile Hatchability	Total Hatchability	Fertility	Fertile Hatchability	Total Hatchability
	--	%	--	--	%	--
LD_0 (0.0 mg T-2/kg BW)	100.00[a]	89.47[ab]	89.47[ab]	100.00	90.00	90.00
LD_{20} (12.4 mg T-2/kg BW)	96.60[a]	92.00[a]	89.61[a]	97.37	90.54	88.16
LD_{40} (14.0 mg T-2/kg BW)	89.84[ab]	78.75[b]	71.59[b]	97.74	88.73	82.89
LD_{60} (15.5 mg T-2/kg BW)	85.71[b]	75.64[b]	64.86[c]	95.89	94.29	90.41

[a.b.c] values in the same column with different letters are significantly different ($P \leq .05$).

Table 3-6: Average weekly[A] hatch, 4, and 8 week weights of chicks from bobwhite quail hens intermittently treated with T-2 toxin (Experiment 1).

	Hatch Weights (g)				4 Week Weights (g)				8 Week Weights (g)			
	Treatment				Treatment				Treatment			
Hatch	LD_0	LD_{20}	LD_{40}	LD_{60}	LD_0	LD_{20}	LD_{40}	LD_{60}	LD_0	LD_{20}	LD_{40}	LD_{60}
3	7.53^{b}	7.81^{a}	7.65^{ab}	7.80^{a}	68.48^{a}	69.67^{a}	69.61^{a}	71.33^{a}	150.65^{a}	144.32^{b}	141.49^{b}	141.52^{b}
4	7.71^{a}	7.57^{ab}	7.47^{b}	$7.57^{a,b}$	74.79^{b}	84.76^{a}	78.57^{b}	78.47^{b}	143.55^{a}	147.60^{a}	145.69^{a}	147.13^{a}
5	7.88^{a}	7.41^{c}	7.80^{ab}	7.63^{b}	75.95^{a}	66.19^{b}	61.27^{c}	69.68^{b}	151.67^{a}	151.16^{a}	143.92^{b}	150.95^{a}
6	7.68^{b}	7.90^{a}	7.80^{ab}	7.64^{b}	75.75^{a}	66.08^{b}	61.08^{c}	69.68^{b}	151.74^{a}	136.94^{c}	143.52^{b}	149.57^{ab}
7	7.66^{b}	7.94^{a}	7.84^{ab}	7.87^{a}	62.84^{a}	62.09^{a}	55.15^{b}	65.09^{a}	152.53^{a}	142.94^{bc}	138.30^{c}	144.78^{b}
8	7.24^{c}	7.91^{a}	7.36^{bc}	7.55^{b}	58.62^{b}	65.50^{a}	44.70^{d}	51.86^{c}	157.79^{b}	165.06^{a}	146.78^{c}	156.39^{b}
9	7.30^{b}	7.66^{a}	7.64^{a}	7.57^{a}	56.80^{ab}	60.30^{a}	49.40^{c}	54.08^{ab}	---[A]	---[A]	---[A]	---[A]
Average	7.57^{b}	7.76^{a}	7.65^{b}	7.66^{b}	67.75^{a}	67.80^{a}	59.97^{c}	65.74^{b}	151.32^{a}	146.95^{b}	141.97^{c}	146.89^{b}

[a,b,c,d] different letters in the same row are significantly different ($P \leq .05$) within weigh periods.

[A] chicks were hatched from eggs set at biweekly intervals for 17 weeks following cessation of T-2 treatment. Chicks from hatch 1 were omitted from this analysis due to insufficient sample size. LD_{40} chicks from hatch 2 crossed the treatment dividers in the hatch tray, so hatch 2 is omitted from this analysis. All chicks from hatch 9 died due to a power failure at 5 weeks of age.

Table 3-7: Average body weights and body weight change of bobwhite quail hens chronically treated with T-2 toxin for a 4 week period (Experiment 2)

Treatment	Body wt. (g) Day 0	Body wt. (g) Day 28	Wt. change (g)	% change	Mortality
0 mg T-2 toxin/kg	214.59	210.06[a]	- 4.53[a]	- 2.11%[a]	0
12 mg T-2 toxin/kg	213.37	183.47[b]	- 29.9[b]	- 14.01%[b]	2
16 mg T-2 toxin/kg	213.41	171.83[bc]	- 41.6[bc]	- 19.49%[bc]	3
20 mg T-2 toxin/kg	211.45	163.97[c]	- 47.5[c]	- 22.46%[c]	4

[a,b,c] values in the same column with different letters are significantly different (P≤.005).

Table 3-8: Average daily feed consumption (grams of feed/bird) of bobwhite quail hens chronically treated with T-2 toxin for a 4 week period (Experiment 2)

Treatment	Week 1	Week 2	Week 3	Week 4	Total
0 mg T-2 toxin/kg	14.90[a]	15.91[a]	16.68[a,b]	22.14[a]	17.40[a]
12 mg T-2 toxin/kg	13.21[a]	11.84[b]	17.68[a]	17.20[b]	14.98[b]
16 mg T-2 toxin/kg	12.23[a]	11.29[b]	16.25[b]	15.97[b]	13.93[b]
20 mg T-2 toxin/kg	12.01[a]	10.56[b]	15.02[b]	16.93[b]	13.63[b]

[a,b] values in the same column with different letters are significantly different ($P \leq .05$).

Table 3-9: Twelve week average hen-day egg production, percent fertility, fertile hatchability, and total hatchability of eggs collected from bobwhite quail hens chronically treated with T-2 toxin for a 4 week period (Experiment 2).

Treatment	HDEP	Fertility	Fertile Hatchability	Total Hatchability
	--	-- (%)	--	--
0 mg T-2/kg	51.93	96.29	88.68	85.50
12 mg T-2/kg	54.53	96.76	89.20	86.23
16 mg T-2/kg	53.58	90.45	87.56	79.16
20 mg T-2/kg	55.50	89.14	89.39	80.07

Table 3-10: Percent fertility, fertile hatchability, and total hatchability of eggs laid by bobwhite quail hens during week 1 & week 2 immediately following cessation of 4 week feed exposure to T-2 toxin (Experiment 2).

	Week 1			Week 2		
Treatment	Fertility	Fertile Hatchability	Total Hatchability	Fertility	Fertile Hatchability	Total Hatchability
	--	%	--	--	%	--
0 mg T-2/kg	100.00[a]	82.20	82.20[a]	95.41	87.78	83.74
12 mg T-2/kg	96.75[a]	84.86	82.08[a]	96.39	91.45	88.17
16 mg T-2/kg	89.44[ab]	86.61	77.84[ab]	84.98	90.38	76.85
20 mg T-2/kg	81.23[b]	79.19	65.82[b]	86.59	86.53	76.07

[a.b] values in the same column with different letters are significantly different (P≤.05).

Table 3-11: Average weekly[A] hatch, 4, and 8 week weights of chicks from bobwhite quail hens chronically exposed to T-2 toxin for a 4 week period (Experiment 2).

	Hatch Weights (g)				4 Week Weights (g)				8 Week Weights (g)			
	Treatment				Treatment				Treatment			
Hatch	0mg	12 mg	16mg	20mg	0mg	12mg	16mg	20mg	0 mg	12mg	16mg	20mg
1	7.25^{a}	7.30^{a}	7.29^{a}	7.28^{a}	55.09^{a}	55.88^{a}	55.00^{a}	50.20^{a}	131.19^{a}	123.86^{a}	132.56^{a}	125.49^{a}
2	7.36^{a}	7.40^{a}	7.48^{a}	7.37^{a}	$51.29^{a,b}$	50.28^{b}	48.58^{b}	54.84^{a}	143.65^{a}	146.92^{a}	146.69^{a}	142.49^{a}
3	7.45^{ab}	7.30^{b}	7.45^{ab}	7.53^{a}	46.26^{a}	46.80^{a}	40.96^{b}	39.48^{b}	141.30^{b}	149.58^{a}	151.77^{a}	155.16^{a}
4	7.61^{bc}	7.44^{c}	7.81^{a}	7.67^{ab}	41.20^{b}	39.94^{b}	50.74^{a}	38.59^{b}	151.77^{b}	147.98^{b}	168.14^{a}	148.99^{b}
5	7.65^{a}	7.50^{a}	7.59^{a}	7.49^{a}	81.99^{b}	90.78^{a}	92.85^{a}	82.11^{b}	142.89^{b}	150.25^{a}	152.24^{a}	140.12^{b}
7	7.78^{a}	7.73^{a}	7.85^{a}	7.88^{a}	$75.15^{b,c}$	80.59^{ab}	73.20^{c}	81.50^{a}	148.59^{a}	153.07^{a}	143.08^{b}	147.23^{ab}
8	7.72^{a}	7.51^{b}	7.67^{ab}	7.55^{b}	$92.31^{b,c}$	88.40^{b}	107.72^{a}	94.47^{b}	154.30^{b}	157.68^{ab}	161.89^{a}	154.19^{b}
10	7.76^{a}	7.45^{b}	$7.65^{a,b}$	7.70^{a}	$95.23^{b,c}$	93.70^{c}	104.79^{a}	100.46^{ab}	140.45^{b}	137.56^{b}	151.15^{a}	143.69^{b}
11	7.79^{a}	7.64^{a}	7.77^{a}	7.64^{a}	77.08^{a}	73.32^{ab}	74.67^{a}	68.85^{b}	134.91^{a}	135.43^{a}	134.93^{a}	130.18^{a}
12	7.59^{a}	7.54^{a}	7.76^{a}	7.59^{a}	80.11^{a}	78.38^{ab}	79.96^{a}	74.67^{b}	148.58^{a}	145.69^{a}	145.52^{a}	145.33^{a}
Average	7.60^{a}	7.48^{b}	7.63^{a}	7.57^{a}	69.57^{b}	69.81^{b}	72.85^{a}	68.52^{b}	143.76^{b}	144.80^{b}	148.80^{a}	143.29^{b}

^{a,b,c} values in the same row with different letters are significantly different ($P \leq .05$) within weigh periods.

[A] chicks were hatched from eggs set at biweekly intervals for 12 weeks following cessation of chronic T-2 toxin treatment. Chicks from hatch 6 and hatch 9 died due to a power outage at Cherokee Poultry Farm and are omitted from this analysis.

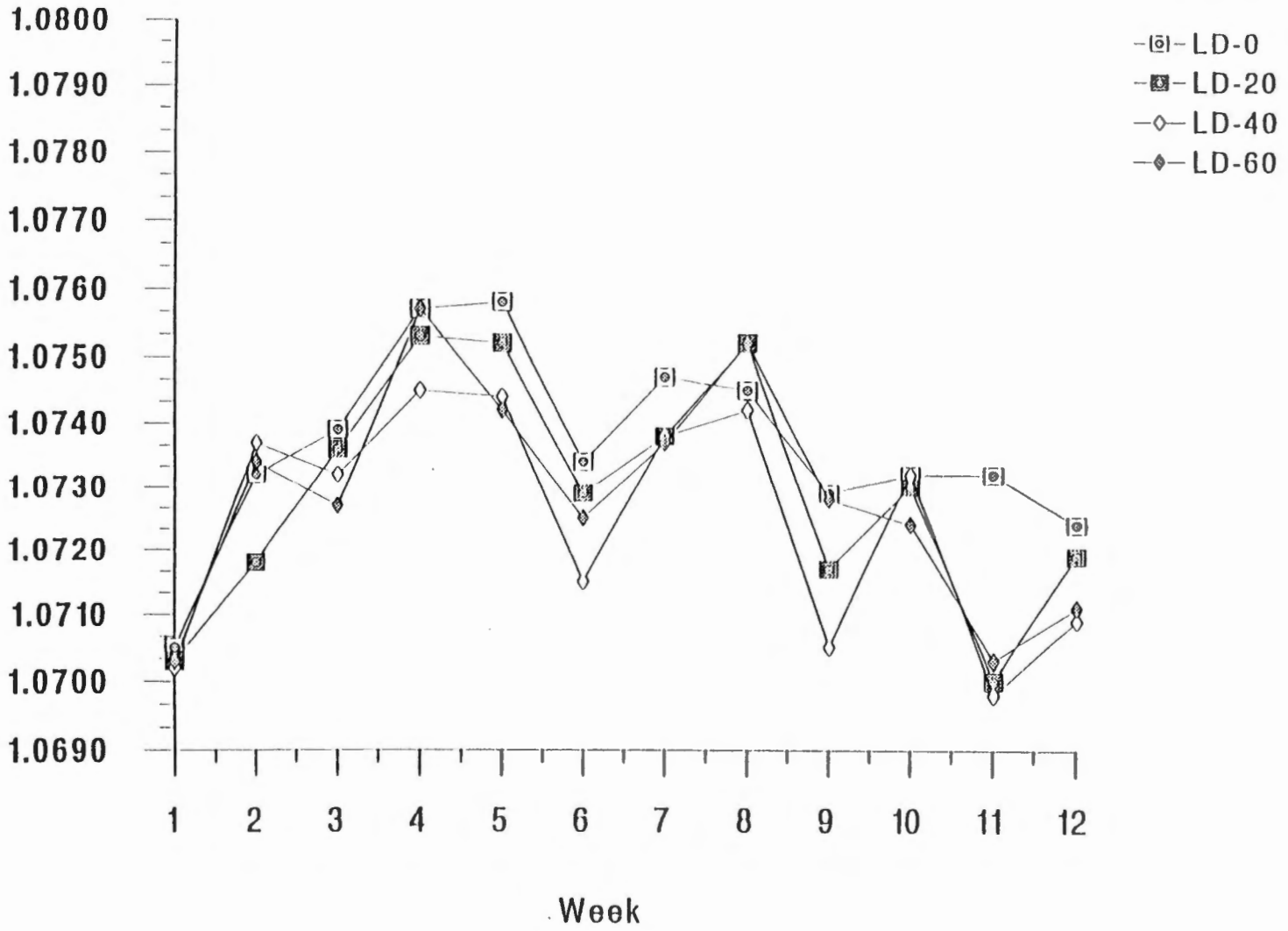


Figure 3-1: Specific gravity of eggs from bobwhite quail hens intermittently treated with T-2 toxin (Experiment 1).

Part 4

Conclusion

The goals of the study presented herein were to provide information on the effect of chronic vs. intermittent exposure to T-2 toxin on reproductive performance, immune function and overall health and fitness of adult pen-raised bobwhite quail. Ames Plantation, the site of the National Championships for purity all-age bird dogs, supports a bobwhite Quail population that is integral to the mission of the Plantation. Over the past 20 years Bobwhite quail populations at Ames have been fluctuating despite management practices aimed at improving quail habitat (Dimmick, 1996). Recent analysis of soybeans left as late winter feed showed the presence of T-2 toxin. Since soybeans comprise up to 80% of late winter feed of wild quail, it was hypothesized that T-2 toxin may be a contributing factor to fluctuating quail populations (Houston, 1996).

T-2 toxin is produced primarily by the fungi *Fusarium tricinctum* which is often found growing on cereal grains (Nakadate and Uneo, 1990). T-2 has a variety of biologic effects including toxicity to plants, animals and humans, and is a potent inhibitor of protein synthesis in eukaryotic cells (Uneo, 1979). Exposure to T-2 toxin will induce single strand breaks in the DNA of lymphoid cells (LaFarge-Frayssinet *et al.*, 1981). T-2 toxin is extremely cytotoxic and induces cellular damage to tissues characterized by the destruction of actively dividing cells in the thymus, testis, intestine, spleen, liver, muscle and heart (Uneo 1979; Thompson and Wannemacher, 1990). Ingestion of T-2 toxin in laying chickens results in increased numbers of infertile eggs, and decreased total hatchability (Tobias *et al.*, 1992).

Experiments herein were designed to mimic the type of exposure wild quail might have to T-2 toxin. If wild quail were sporadically eating T-2 infested soybeans, the intermittent study would mimic this situation. If wild quail were to be constantly ingesting T-2 infested soybeans, the chronic study would mimic this situation. To our knowledge, this is the first study to compare the effect of intermittent vs. chronic exposure to T-2 toxin on reproductive performance and blood parameters in adult bobwhite quail.

Data from these experiments indicate that both chronic and acute exposure to T-2 toxin may have a detrimental effect on reproductive performance in adult bobwhite quail. Ingestion of T-2 mycotoxin may be characterized by decreases in fertility and total hatchability of eggs produced within the first two weeks following exposure. Chi *et al.* (1978) reported that T-2 toxin and its metabolites are deposited into egg yolk for eleven days following cessation of exposure. Our data support these conclusions. Decreases in specific gravity of eggs, as observed in our study may also have implications for wild quail populations. Thin shelled eggs may be a detriment to reproductive success of wild quail due to their increased susceptibility to physical damage. Our observed decreases in absolute numbers of monocytes and lymphocytes in both the intermittent and chronic study may be interpreted as a measure of immunosuppression. As a result of this immunosuppression, wild quail ingesting mycotoxin infested soybeans may be more susceptible to extracellular pathogens and disease, which may play a role in mortality rates and fluctuating population numbers.

The implications of this study may not be limited to Ames Plantation and mycotoxin invasion of soybeans. Mycotoxin infestation of agricultural grains (including corn, barley, peanuts, oats, and animal feeds) has been a problem throughout the world for many years (Nakadate and Uneo, 1990). The severity of mycotoxin contamination is determined by major environmental factors such as excessive moisture in the field and in storage, temperature extremes, humidity, drought, variations in harvesting practices and insect infestation (Coulumbe, 1993). The practice of providing agricultural grains to wild or released pen-raised quail is increasing in popularity. Guidelines are available for the level of aflatoxin and other highly toxic mycotoxin found commonly in food grains, but not T-2 toxin. However, no numbers have been determined for the acceptable mycotoxin level in agric-grains intended for quail consumption. The current action level for aflatoxin in poultry feed is 20 parts per billion and for mature poultry is 300 parts

per billion (Meronuk and Concibido, 1998). Any grains found with this level of aflatoxin should be considered unusable for feeding to poultry. The reader is refered to this publication for discussion of other livestock. Another factor to consider is multiple types of mycotoxins found on the same agricultural grain. There have been reports of synergistic toxicity between T-2 toxin and aflatoxin. Huff *et al* (1988) concluded that T-2 and aflatoxin can interact synergistically, and that this synergism is a threat to poultry production due to the prevalence of these mycotoxins and the severity of their toxicity. Therefore, when considering contamination levels of agricultural grains, synergism probably should be considered.

The bulk of quail populations are replaced each year, with most quail lost after the first but before the second nesting season (Rossne, 1969). Often increases in the population numbers do not result from the first clutch of eggs laid, but rather, from the second clutch laid late in the season (Houston, 1998). If quail throughout the southeastern United States are ingesting mycotoxin infested agric-grains the impact on population dynamics may be severe. Even if birds are not ingesting mycotoxins at levels used in this study, any negative effect on the health and/or fitness of the bird may influence population numbers. If wild quail are immunosuppressed as a result of mycotoxin ingestion, an increase in disease related mortality may be observed. Likewise, a decrease in health may lead to increased susceptibility to predation. Similarly, reduced numbers of fertile eggs, and hatched chicks may contribute to fluctuating quail populations.

In summation, the negative impacts of mycotoxin ingestion may be reflected at several points throughout the life cycle of wild quail. Slight depressions in immune function, fertility, and hatchability of eggs may be contributing factors to fluctuations in population numbers. Future research should be directed at how to reduce toxic effects of ingested T-2 toxin. Reports of the immunoprotective effects of bioflavonoids are encouraging and may be an avenue of inquiry worth pursuing (Markham *et al.*, 1987).

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