EFFECTS OF CONTINOUS ADMINISTRATION OF LOW-DOSE OF *Escherichia coli* LIPOPOLYSACCHARIDE IN CHICKS AND POULTS FED NON TOXIC DOSES OF AFLATOXIN B1 AND T-2 TOXIN

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by

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EFFECTS OF CONTINOUS ADMINISTRATION OF LOW-DOSE OF *Escherichia coli* LIPOPOLYSACCHARIDE ADMINISTRATION IN CHICKS AND POULTS FED NON TOXIC DOSES OF AFLATOXIN B1 AND T-2 TOXIN

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EFFECTS OF CONTINOUS ADMINISTRATION OF LOW-DOSE ENDOTOXIC LIPOPOLYSACCHARIDE IN CHICKS AND POULTS FED NON TOXIC DOSES OF AFLATOXIN B1 AND T-2 TOXIN

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ABSTRACT

Six studies (three with broilers and three with turkeys) were conducted to determine if *E. coli* lipopolysaccharide (LPS) would enhance the effects of aflatoxin B1 (AFB1) and the T-2 toxin (T-2) in chicks and poults fed from hatch to 21 days of age. In mortality rate, there was a toxic synergy between AFB1 and LPS in chicks and poults. In birds exposed to LPS and T-2 alone or in combination, LPS did not enhance the effects of T-2. In poults, a decrease in performance observed in birds at 2 mg/kg T-2 alone was atypical. In birds exposed to AFB1, T-2, and LPS singly or in combination, LPS did not enhance the effects T-2 and AFB1 on performance. However, LPS did enhance the effects of T-2 on mortality rate and oral lesion in poults and acute mortality rate in broiler chicks. In chicks and poults, LPS-treated groups had lower feed intake immediately after treatment when compared to saline groups. Little or any interactions were detected which may be due to the atypical responses observed when toxins were fed alone.

CHAPTER I

INTRODUCTION

Animals are exposed simultaneously to a myriad of stressors resulting in poor performance which can lead to high mortality. Stressors like the presence of low doses of mycotoxins in the feed and exposure to unsanitary environments can stimulate the immune system to release cytokines and other mediators necessary to overcome the problem. The body enters a catabolic state and shifts nutrients toward recovery of the animal. At this point, the priority is host recovery. In some cases, antibiotics therapy is an efficient alternative to diminish the damaging effects of this constant exposure. However, due to the unfavorable public opinion towards the use of antibiotics, other alternatives should be found to prevent inefficient production. Chronic exposure to low doses of mycotoxins in the diet does not necessarily increase or cause mortality. However, exposure to low doses can render the animal more sensitive to pathogens presents in the environment further decreasing performance and increasing mortality rate. These effects can increase the cost of production and decrease production efficiency. The objective of this research was to clarify the impact that common environmental stressors like *E. coli* endotoxin, aflatoxin B1, and T-2 toxin have on performance of chicks and poults fed from hatch to day 21.

CHAPTER II

LITERATURE REVIEW

AFLATOXIN B1

Aflatoxins were first identified as the causative agent of "Turkey X" disease, which killed more than 100,000 turkey poults in England in 1960 (Lancaster et al., 1961). The two fungi that are major producers of aflatoxin are *Aspergillus flavus* and *A. parasiticus*, and are found virtually everywhere in the world. The predominant and most toxic aflatoxin produced by *A. flavus* is aflatoxin B1 (AFB1). *Aspergillus spp*. are mainly storage fungi which generally do not contaminate grains prior to harvest. However, conditions such as drought stress and insect damage may allow infection by *Aspergilli* and production of aflatoxins in the field before crop harvest (Anderson *et al*., 1975; Fennel *et al*., 1975; Lillejoh et al., 1976). For instance, drought stress in the 3 to 4 weeks following silking has been correlated with high levels of aflatoxins in corn in the USA (Schmitt and Hurburg, 1989; Payne *et al.*, 1986). In nuts, aflatoxin content is related to insect damage (Schatzki and Ong, 2001). Several publications have suggested that almonds free of such damage are not contaminated by the toxin (Schade *et al.*, 1975; Fuller *et al.*, 1977; Schatzki, 1996). *Aspergillus* species are able to grow on many different substrates and most foods and feeds are susceptible to invasion at any stage of production, processing, transportation, and/or storage. Aflatoxins are extremely stable in grains and may persist long after mold growth has stopped. For example, the groundnut

meal associated with the outbreak of "Turkey X" disease was sterile, although it contained large amounts of aflatoxins (Austick and Ayerst, 1963).

Occurrence of aflatoxins, in foods and feeds worldwide has been documented (Jelinek *et al*., 1989; Wood, 1992). Survey results, from the Contamination Monitoring Program for mycotoxins conducted by the Food and Agriculture Organization, World Health Organization, and United Nations Environmental Program from 1976 to 1983, showed that much of the monitored grain contained aflatoxins above 5 to 20 μg/kg, the regulatory levels for food in most countries, or 20 to 50 μg/kg, the regulatory limits in feeds in most countries (Jelinek *et al.,* 1989). The mean levels for aflatoxins in corn and corn products ranged from < 0.1 to 321 μg/kg (Jelinek *et al.,* 1989; Chamberlain *et al*., 1993). Aflatoxin B1 has been shown to be prevalent in samples of almonds and peanuts at levels of 95 μg/kg and < 10 μg/kg, respectively (Jimenéz et al., 1991). Other reports have shown higher levels ranging from 7 to 11,600 μg AFB1/ kg (Jindal *et al*., 1993; Shetty *et al*., 1987; Hegazy *et al.,* 1991).

The knowledge that mycotoxins can have serious effects on humans and animals has led many countries to establish regulations on mycotoxins in food and feed in the last four decades (FAO, 2003). The first limits for mycotoxins were set in the late 1960s for the aflatoxins. By the end of 2003, approximately 100 countries had developed specific limits for mycotoxins in foodstuffs, and the number continues to grow (FAO, 2003).

On a worldwide basis, at least 99 countries had mycotoxin regulations for food and/or feed in 2003, an increase of approximately 30% compared to 1995 (FAO, 2003).

The current maximum levels set by the European Commission are 2 μg/kg for AFB1, 4 μg/kg total aflatoxins for groundnuts, nuts, dried fruits and cereals, and 0.05

µg/kg for aflatoxin M1 in milk (Stroka and Anklam, 2002). New limits are being established for AFB1 in baby food (most probably 0.1 µg/kg) and animal feeds (in discussion at 1 µg/kg) (Stroka and Anklam, 2002). Another 21 countries from Africa, Asia/Oceania, Latin America, and some countries in Europe have set their limits at 5 µg/kg. The United States and Canada do not have a single limit for aflatoxin (FAO, 2003).

Scudamore and Patel (2000) examined 140 samples of imported whole corn into the United Kingdom and determined that 92.1% of the samples fell below the 2 µg/kg of AFB1. This could indicate a determined attempt by suppliers to meet the regulated level for AFB1 (Scudamore and Patel, 2000). For feed for dairy cattle, the limit set by 29 countries in the European Union (EU) and European Free Trade Association (EFTA) is 5 µg/kg (FAO, 2003). A study conducted by Fayokun and Adegoke (2000) found that, in Nigeria, AFB1 contamination in poultry feeds far exceeded the 20 ppb EEC limits for feedstuffs (Coker *et al.*, 1984) with results ranging from 31.7 to 66.7 ppb of AFB1. In contrast, Oliveira *et al.* (1997) analyzed 60 samples of broiler mashed feed from several farms in Brazil for the presence of AFB1. From the 60 samples, AFB_1 at a level of 28 ppb, was confirmed in just one sample (1.66%). Gunsen and Yaroglu (2002), determined the aflatoxin levels in 18 dog and 30 horse feed samples collected from different farms from June 2000 to June 2001, in Turkey and found that the minimum and maximum levels of total aflatoxins were < 1.75 to 20 µg/kg and < 1.75 to 14 µg/kg, respectively. Three out of 18 dog feed samples (16.7%) and two out of 20 (10%) horse feed samples exceeded the Turkish tolerance limit of 10 µg/kg for aflatoxins (Gunsen and Yaroglu, 2002).

Aflatoxins can severely damage animal health. Aflatoxin B1, the most toxic of the aflatoxins, causes a variety of adverse effects in different animal species, including chickens. In poultry, these include liver damage, impaired productivity and reproductive efficiency, decreased egg production in hens, inferior egg shell quality, inferior carcass quality, and increased susceptibility to disease (Wyatt, 1991). Dietary AFB1 tends to infiltrate most of the soft tissues and fat depots of the chicken (Trucksess *et al.*, 1983; Sudhakar, 1992). Furthermore, AFB1 and its metabolites can also be transferred to eggs in laying hens (Jacobson and Wiseman, 1974; Sudhakar, 1992; Qureshi et al., 1998; Oliveira et al., 2000). Eggs laid soon after AF feeding contained aflatoxicol, the most toxic of the known AFB1 metabolites (Trucksess *et al.*, 1983). Results of previous studies (Hamilton, 1982; Trucksess et al.,1983; Qureshi et al., 1998; Oliveira et al., 2000) have demonstrated that the transmission ratio of AFB1 from layer food into the egg is 1/2000 to1/2500. Jacobson and Wiseman (1974) have reported that the eggs from hens fed 0.1, 0.2 and 0.4 mg/kg AFB1 contaminated feeds contained measurable amounts of AFB1. They found 9 ng AFB1/egg after 10 days of receiving diets containing 0.l mg/kg dietary AFB1, whereas Oliveira et al.(2000) detected 6 ng AFB1/egg in the eggs of layers fed diets containing 0.5 mg/kg AFB1 for 8 weeks. However, Sudhakar (1992) found 5 ng AFB1/egg in the eggs of layers fed a diet containing 0.6 mg/kg AFB1 for 8 weeks. The legal upper limits in Turkey for total AF and AFB1 in layer feed are 20 ppb (20 mg/kg food) and 10 ppb (10 mg/kg food), respectively (Ministry of Agriculture of Turkey, 1997). However, results from surveys have shown that the legal limits were frequently exceeded (Kaya, 1982; Tuncer, 1987).

In the southern United States, artificial feeding of white-tailed deer (*Odocoileus*

virginianus), wild turkeys (*Meleagris gallopova silvestris*), and other wildlife species has created an opportunity for wildlife to be exposed to aflatoxins (AF), particularly via contaminated corn. Aflatoxin levels in 51% of 39 submitted samples of shelled corn picked up at deer bait piles in North and South Carolina (USA) (Fischer et al.,1995) ranged from < 10 to 750 µg AF/kg feed (parts per billion). More recently, analysis of corn offered by retailers in Georgia for use as wildlife feed revealed AF in three of 31 bags; one bag had 385 µg AF/kg feed (C. Quist, unpubl. data). Corn that is deliberately left unharvested for a wildlife food source is another source of AF. Aflatoxin levels found in standing corn during a 3-year study on a bobwhite quail plantation ranged from 42 to 1,210 µg AF/kg feed (Stewart, 1985). Among wildlife species, the effects of AF have only been studied in white-tailed deer and bob-white quail. Subclinical liver damage was detected in both species (Quist et al., 1997; Stewart, 1985).

The effect of AF on domestic turkeys has been well documented. Extensive mortality was produced in young domestic turkeys that were given 400 µg aflatoxin/kg feed or more of dietary aflatoxin (Giambrone et al., 1985b). Lower levels of aflatoxin cause blood-clotting abnormalities, immune dysfunction, and decreased feed conversion (Witlock and Wyatt, 1981; Giambrone et al., 1985a). In four-month-old wild turkeys, 100 µg AF/kg of diet for 2 weeks decreased feed consumption and weight gains as compared with control poults. Decreased liver-to-body weight ratios, liver enzyme alterations, slightly altered blood coagulation patterns, and mild histological changes indicated low-level liver damage. Compromise of cell-mediated immunity was indicated by decreased lymphoblast transformation. The authors suggest that exposure of wild

turkeys to feeds containing AF levels of 100 µg AF/kg of feed or more should be avoided (Quist *et al.*, 2000).

In adult ruminants, exposure to AF can depress feed efficiency, immunocompetence, and reproductive performance, as shown by studies with dairy cattle (Diekman and Green, 1992). The effects on feed efficiency presumably arise from impaired ruminal function, including reduced cellulose digestion, volatile fatty acid production, and motility (Diekman and Green, 1992). In dairy cattle, another problem arises from the transformation of AFB_1 to a related metabolite, aflatoxin M_1 (AFM_1) which is secreted in the milk. Calves fed aflatoxin contaminated diets show clinical signs of aflatoxicosis at 2 to 14 days post-exposure (Barringer and Doster, 2001). The toxicity of AFM1 is about one order of magnitude less than that of AFB1 (Creppy, 2002).

In humans, aflatoxins are now incriminated in neonatal jaundice and there is circumstancial evidence that they cause perinatal death and reduced birth weight (Hendrickse, 1997). Aflatoxins have also been implicated in episodes of food poisoning that have been associated with serious morbidity and mortality, particularly among young children (Hendrickse, 1997).

In pigs, acute toxicity caused by the consumption of high doses of aflatoxins is characterized by feed refusal, reduced weight gain, changes in hematological and biochemical parameters, and liver and kidney lesions (Southern and Clawson, 1979; Miller *et al.*, 1981; Harvey *et al.*, 1988). Marin *et al.* (2002) suggest that, even when present at low doses, aflatoxins alter the immune response, which may predispose pigs to infectious disease. It also alters animal performance (Marin *et al.*, 2002). Van Heugten *et al.* (1994) reported a 31% decrease in growth of piglets fed a diet contaminated with

280 ppb of aflatoxin. For swine, the maximum tolerable level for aflatoxin is 200 ppb (FAO, 1997).

Liggett *et al.* (1986) reported that cornmeal in the ration of dogs presenting with icterus, anorexia and listlessness contained 511 µg/kg AFB1 and AFB2.

Aside from health risks, mycotoxin contamination can also reduce the price paid for crops (Wu, 2004). Losses from mycotoxins in the U. S. and other industrial nations are typically associated with market losses as opposed to illnesses or deaths from the effects of the toxins (Wu, 2004). Vardon *et al.* (2003) calculated the total mycotoxin-related losses to agriculture in the United States to be as high as $1.4 billion annually ($630 million to $2.5 billion). In particular years and regions, aflatoxin can contaminate crops so severely that farmers are forced to dispose of more than half of their total corn and peanut crop (Robens and Cardwell, 2003). In addition, there is an increase in operational costs from screening for the presence of mycotoxins. Salay and Mercadante (2002) reported that in Brazil the costs for implementing a mycotoxin control program were US$ 55,900 capital cost to buy the equipment to screen the grains, and the operational costs to be between US$ 0.02 and 0.06/test/month.

Several strategies for the reduction or inactivation of aflatoxins have been previously evaluated and include diverse physical, chemical, and biological methods (Phillips *et al.*, 1994, 2002; Phillips, 1999). One of the strategies of current interest is the inclusion of nonnutritive enteroabsorbents in contaminated feed for the inactivation of aflatoxins (Pimpukdee *et al.*, 2004).

The concentration of AFB1 in feed can be reduced by good manufacturing practices and good storage practices. If preventive measures fail, however, AFB1 can be

reduced in feed by blending – a method that is not acceptable in the United States –or by physical or chemical treatment. The physical treatments include heat, microwaves, gamma-rays, X-rays, ultra-violet light, and adsorption (Creppy, 2002). Adsorption of aflatoxin onto hydrated sodium calcium aluminosilicates and other inert materials can be used in the animal feed industry to reduce aflatoxins in the feed. The most successful chemical procedure for degrading aflatoxins in animal feed is ammoniation, which leads to decomposition of 95 to 98 % of the AFB1, and this procedure is used in various countries (Creppy, 2002).

TOXIC EFFECTS

The classical assessment of toxicity of compounds centers on the determination of LD_{50} values which is the medium lethal dose (D'Mello and MacDonald, 1997). An LD_{50} value of 6.5 to 16.5 mg AFB1/kg was reported for one-day-old chicks by Patterson (1973). Hamilton *et al*., (1972) reported the LD_{50} for 3-week-old poults to be 1 mg AFB1/kg. D'Mello and MacDonald (1997) reported the LD_{50} values for laboratory animals of 1.0 to 17.9 mg/kg BW, and 0.5 mg/kg BW for one-day-old ducklings.

The metabolic effects of aflatoxins include: inhibition of DNA, RNA and protein synthesis, reduction in activities of miscellaneous enzymes; depression of glucose metabolism; inhibition of lipid synthesis, including that of phospholipids, free fatty acids, triglycerides and cholesterol and its esters; and depression of clotting-factor synthesis (Busby and Wogan, 1981). Aflatoxins tend to block steroid binding sites in the tissues. They are also potent immunosuppressants, as shown by (1) reduced host resistance to a wide range of bacteria, viruses and fungi; (2) depressed complement activity; and (3)

impaired cell mediated immunity (Richard *et al.*, 1978a, 1978b). The liver is the main target for toxicity, and pathological changes include fatty infiltration, biliary duct proliferation and toxic necrosis in acute severe poisoning. The Krebs cycle and phosphorylation of substrates also are inhibited via alterations in mitochondrial functions (Cheeke and Shull, 1985).

Baker and Green (1987) observed decreased synthesis of clotting factors which explains homeostasis deficiency and the presence of disseminated intravascular coagulation (DIC) in animals with aflatoxicosis. Harvey *et al.* (1988) described disturbances in the bone marrow, coagulation and homeostasis, caused by the inhibition of protein synthesis. The increase in prothrombin time is caused by alterations in the coagulation factors and also in the depletion of platelets which may inhibit aggregation to form the clot and inhibition of the clot enzymes (Parent-Massin and Parchment, 1998).

Aflatoxins cause a variety of effects in poultry, including retarded growth rate, impaired feed conversion, increased liver weights, immunosuppression, negative effects on serum chemistry and hematological parameters, and histopathological lesions. Huff *et al.* (1986) showed that the most sensitive indicators of aflatoxicosis in young broiler chickens are the reduction in serum albumin and total protein levels. Rastogi *et al.* (2001) reported that administration of 2 mg/kg of AFB_1 in rats caused significant increase (15 to 75%) in the activites of γ-glutamyl transpeptidase, 5'-nucleotidase, acid phosphatase and acid ribonuclease in liver, indicating hepatic injury. In addition, there were significant depletions in the contents of reduced glutathione (27%) and glycogen (32%) (Rastogi *et al.*, 2001). The authors also reported a decrease (13 to 27%, respectively) in the activities of succinate dehydrogenase and glucose-6-phosphatase,

increased levels of lipid peroxides by 87%, and decline in the activities of enzymatic antioxidants levels viz. catalase (22%), superoxide dismutase (35%), glutathione-S-transferase (27%), glutathione reductase (20%), and glutathione peroxidase (16%) suggesting that initial changes induced by aflatoxin B1 are due to formation of lipid peroxides and toxicity is mediated through antioxidant enzymes as well as glutathione metabolism (Rastogi *et al.*, 2001). As aflatoxicosis progresses, hepatomegaly becomes apparent due to lipid accumulation in the liver (Huff *et al*., 1986).

Aflatoxin appears to be toxic at < 1 mg/kg for poultry with the liver considered to be the target organ, because compared with other organs the relative weight of the liver is altered by low levels of aflatoxin (Huff et al., 1986). Researchers have attributed an increase in the relative weight of the liver to an accumulation of lipid in the liver, which produces the characteristic, enlarged, friable, fatty liver associated with aflatoxicosis in broilers (Smith and Hamilton, 1970).

MECHANISM

Carvalho (1995) classifies aflatoxicosis in three clinical stages. First, primary acute disease observed usually after the ingestion of moderate quantities of AFB1. Animals show liver, central nervous system, and kidney lesions. Second, primary chronic disease, after consuming diets contaminated with moderate to low levels of AFB1. Animals show no classical lesions, but there is reduction in growth, in carcass quality, and, in cows, in milk production. Finally, secondary chronic disease at which

animals ingest very low levels of AFB1. These very low levels cause no signs of intoxication, but render the host susceptible to infectious diseases.

Aflatoxins are very liposoluble compounds and are readily absorbed from the gastrointestinal tract into the bloodstream. Aflatoxins tend to infiltrate most of the soft tissues and fat deposits of the chicken; however, the majority of the accumulation occurs in organs involved in the biotransformation of the mycotoxins such as liver and kidney (Lesson *et al.*, 1995). The major reactions in AFB_1 metabolism are relatively well established. Aflatoxin B1 *exo*-8,9-epoxide hydrolyzes rapidly to the dihydrodiol (Johnson *et al.*, 1996). Only the *exo* epoxide reacts efficiently with DNA, and the kinetics of this process have been analyzed in detail (Iyer *et al.*, 1994; Johnson and Guengerich, 1997). The *endo* epoxide (Raney *et al.*, 1992a) is a product of some P450 enzyme systems [e.g., P450 1A2 (Ueng *et al.*, 1995)] but does not bind to DNA because of intercalation of the coumarin ring system into DNA (Raney *et al.*, 1990; 1992b). The dihydrodiol/dialdehyde equilibrium is complex (p*K*a 8.2) and both forms are present at physiological pH (Johnson *et al.*, 1996). Aflatoxin B1 aldehyde reductase (AFAR) enzymes reduce the dialdehyde (and not the dihydrodiol) (Guengerich *et al.*, 2001). Only the epoxide (and not the dialdehyde) reacts with DNA, as documented by the nearly exclusive reaction of the *exo* epoxide (Iyer *et al.*, 1994). The epoxide is also a substrate for conjugation by several GSH transferase (GST) enzymes (Raney *et al.*, 1992a; Johnson *et al.*, 1997). Aflatoxin B1 dialdehyde is considered to be the major AFB1 derivative responsible for the formation of protein adducts (Sabbioni *et al.*, 1987).

Many effects of aflatoxin are a result of its ability to impair protein synthesis (Smith, 1963). Aflatoxin B1 itself is a relatively innocuous molecule but P450 enzymes

(and other oxygenases, to a lesser extent) oxidize it to the 8,9-epoxide, which has a central role in all succeeding reactions (Baertschi *et al.*, 1988; Johnson *et al.*, 1996; Guengerich *et al.*, 1998; Guengerich and Johnson, 1999). Aflatoxin B1 requires metabolic conversion to its *exo*-8,9-epoxide (Busby and Wogan, 1984; Miller, 1978) in order to damage DNA (Miller, 1978). The AFB1 epoxide reacts with guanine to form a number of adducts (Essigmann *et al.*, 1983), and one reasonable model is that these adducts, or secondary DNA lesions derived from them, lead to heritable genetic changes that help a cell along the pathway toward malignant transformation (Smela *et al.*, 2001). The oxidized form can bind to DNA, forming AFB1-guanine adducts, and to protein, forming AFB1-albumin adducts (Eaton and Gallagher, 1994). It may also conjugate to glutathione (GSH) by glutathione S-transferase (GST) (Wild *et al.*, 1996) which is an important route for detoxification (Hayes *et al.*, 1991).

The hepatocyte is the major target cell affected by AFB1. Aflatoxin B1 induces DNA adduct formation, binding mainly to guanine residues in guanine + cytosine-rich regions, and induces guanine to thymine substitutions almost exclusively (Kuiper-`Goodman, 1994). This characteristic has been extensively demonstrated in several species, including fish, birds, rodents, carnivores, and primates. In these animals, AFB1 causes hepatocellular carcinomas (HCC), even when ingesting low levels of the toxin causing it to be considered one of the most potent hepatocarcinogens (Oliveira and Germano, 1997). Even though the liver is the target organ, pancreatic and intestinal tumors have been reported in animals fed diets contaminated with aflatoxins (Busby and Wogan, 1984). Aflatoxin's toxicity is expressed principally through its disruption of

transcriptional events, mediated by the metabolism of aflatoxin to metabolites that bind DNA (Huff *et al.*, 1986).

T-2 TOXIN

The tricothecene mycotoxins (TCT) comprise a vast group of over 100 fungal metabolites with the same basic structure. Several fungal genera are capable of producing TCT; however, most of them have been isolated from *Fusarium spp.* All tricothecenes contain an epoxide at the $C_{12,13}$ position, which is responsible for their toxicological activity. Based on their chemical structure, TCT can be divided into two groups: macrocyclic and non-macrocyclic.

The tricothecene mycotoxins occur worldwide in grains and other commodities. Toxin production is greatest with high humidity and temperatures of 6 to 24˚C. Natural occurrence of TCT has been reported in Asia, Africa, South America, Europe, and North America (Scott, 1989). Tricothecenes have been detected in corn, wheat, barley, oats, rice, rye, vegetables, and other crops (Buck and Côté, 1991).

Macrocyclic tricothecenes are subdivided into type C and D. Type C tricothecenes (crotocin, baccharin) posses an additional epoxide group at the $C_{7,8}$ or $C_{9,10}$ position. Type D tricothecenes (satratoxin, roridin) contain a macrocyclic ring between the $C_{4,15}$ positions (Sudakin, 2003). Macrocyclic TCT have not been studied in poultry species; therefore, further discussion here is unnecessary.

On the other hand, non-macrocyclic TCT are common contaminants of poultry feeds and feedstuffs and their adverse effects on poultry health and productivity have been studied extensively (Leeson *et al.*, 1995). Non-macrocyclic TCT are subdivided into type A and B, A being more toxic for poultry species than type B (Leeson *et al.*, 1995) due to the influence of the different radicals on their hydrophilic and lipophylic

moieties (Leal and Mejía, 1997). Examples of type A TCT include T-2 toxin (T-2) and HT-2 toxin (HT-2), and diacetoxyscirpenol (DAS). Fusarenone-X (FUX), deoxynivalenol (DON), and nivalenol (NIV) are some of the common naturally occurring type B TCT. Type A and B tricothecenes are distinguished by the presence or absence of a carbonyl group at the C_8 position, respectively.

Fusarium species reported as major producers of type A TCT are *Fusarium sporotrichioides*, *F. sambucinum*, *F. venenatum*, *F. acuminatum*, and *F. compactum* (Chelkowski, 1989; Chu, 1991, Bosch and Mirocha, 1992; Miller and Trenholm, 1994) whereas synthesis of type B trichothecenes occurs principally by *F. culmorum* and *F. graminearum* (Placinta *et al.*, 1999). *F. sporotrichioides* is widespread on plants and in soil throughout the cold and cool temperate regions of the world (Visconti *et al.*, 1985). In Europe, the most frequently encountered *Fusarium* mycotoxins (associated with F*usarium* head blight) are DON, and ZEA produced by *F. graminearum* and *F. culmorum*, with the former more common in southern (warmer) and the latter in northern (colder) European areas (Bottalico and Perrone, 2002). Nivalenol was usually found associated with DON and its derivatives (mono-acetyldeoxynivalenols), along with FUX, which were produced by *F. graminearum, F. cerealis, F. culmorum* in the southern areas and in northern areas, by *F. poae*. Moreover, from central to northern European countries, moniliform has been consistently reported, as a consequence of the widespread distribution of *F. avenaceum*, whereas the occurrence of T-2 derivatives, such as T-2 and HT-2 , and DAS have been recorded in conjunction with sporadic epidemics of *F. sporotrichioides* and *F. poae* (Botallico and Perrone, 2002).

Epidemiological surveys have demonstrated that tricothecenes are distributed worldwide in cereals as natural contaminants, and a number of outbreaks of intoxication in humans and animals have been associated with the consumption of foods or feeds contaminated by these mycotoxins (WHO, 1990; Beardall and Miller, 1994). In China, 53 outbreaks of human food poisoning associated with scabby and moldy cereals took place during the period 1960 to 1991 (Luo, 1992). Huang (1992) reported a 1991 outbreak in Anhui - a province in China, in which the consumption of foods made from wheat and barley harvested during a very wet and cold season intoxicated more than 130,000 people. They were affected by gastrointestinal disorders, including abdominal pain and fullness, nausea, vomiting, fatigue and fever (Huang, 1992). Another mycotoxicosis outbreak, related to the consumption of corn gruel from moldy corn powder, occurred in Guangxi province (China) in April 1989 (He, 1990). Of 10 consumers, all were affected and one died (He, 1990). Deoxynivalenol was found in the causative cereals implicated in the intoxications in Anhui and Guangxi provinces (China). However, in 1999, Li *et al.* analyzed the leftover samples of wheat, barley, and corn for the presence of mycotoxins and found that, although DON was the predominant toxin, the grains also contained NIV and ZEA (Li *et al.*, 1999). The T-2 toxin was negative in all samples analyzed (Li *et al.*, 1999).

At least two serious cases of mycotoxicoses have been associated with the ingestion of fusariotoxin contaminated food and feed (Mateo *et al.*, 2002). The first is alimentary toxic aleukia (ATA) which occurred in Russia during World War II (Yagen and Joffe, 1976) when a shortage of manpower for harvesting caused the grains to overwinter in the field (Moss, 2002); furthermore, after an acute shortage of food, these

cereals were gathered and eaten, leading to the deaths of many thousands of people (Moss, 2002). The other is the bean-hull poisoning of horses in Japan (Ueno *et al.*, 1972). In both cases, isolates of F. *sporotrichioides* isolated from grains and beans were found to produce T-2 toxin and its derivatives (Moss, 2002).

The T-2 toxin is one of the most acutely poisonous of the fusarial toxins and is produced by *Fusarium acuminatum* (Ell. and Kellerm) and *F. equiseti* (Corda) Sacc. In addition, of particular importance are *F. poae* (Peck) Wollenw. and *F. sporotrichioides* Sherb which were possibly associated with the devastating outbreaks of alimentary toxic aleukia (ATA) in the former Soviet Union during, and immediately after World War II (Moss, 2002). In 1994, "powdery *F. poae*" was the most abundant potential producer of HT-2 and T-2 toxins in Norwegian cereals (Kosiak *et al.*, 2003).

A recent evaluation of T-2 in the European diet by JECFA (Joint FAO/WHO Expert Committee on Food Additives, 2002) suggested that there could be an incidence of contamination as high as 11% in European grain samples, albeit at very low levels (Moss, 2002). Using a lowest observed effect level (LOEL) of 0.029 mg/kg body weight per day for a sensitive biological test, and a safety factor of 500, the Committee derived a permitted maximum total daily intake of 60 ng/kg body weight which is about eight times the estimated mean exposure level in Europe (Moss, 2002).

Kawamura *et al.* (1990) carried out a survey of T-2 toxin in cereals randomly sampled from 11 countries. Among 540 samples, 72 (13%) contained over 10 ng/g of T-2 toxin with an average of 91.5 ng/g. The content of T-2 toxin was highest (500 ng/g) in oat samples from Western Germany, and a relatively high content was observed in

barley, wheat, oat, and rye produced in Finland, Norway, Western Germany, and the USSR.

Park *et al.* (1996) tested 98 corn samples received in late fall and winter of 1992 and 1993 in Wisconsin after an unusually cold summer (Pratt, 1993) for the presence of fusarium mycotoxins. The authors found frequent contamination with toxic fusaria as well as with various fusarium mycotoxins in the 1992 corn. In contrast to the usual case in nature in which *F. graminearum* is more prevalent and its metabolites, such as DON and related toxins, are more common than T-2 toxin (Hagler *et al.*, 1984; Jelinek *et al.*, 1989; Muller and Schwadorf, 1993; Park and Chu, 1993; Scott *et al.*, 1980; Vesonder and Hesseltine, 1981) mycological analysis of the 1992 Wisconsin moldy corn revealed that a large population of *F. sporotrichioides* was also present (Park *et al.*, 1996). *F. sporotrichioides* has a low optimal temperature (6 to 12˚) for T-2 toxin production (Cullen *et al.*, 1982; Mirocha, 1984; Park and Chu, 1993) and produces mycotoxin during overwintering under snow cover in the field and/or during storage (Park *et al.*, 1996). Poor storage of grains contaminated with this mold at mild temperatures (around 20˚C) usually during summer and the beginning of autumn in many cereal-producing areas might cause an increase in the T-2 content (Mateo *et al.*, 2002). The problem is not as important in countries with hot summers (average temperature around or higher than 30˚C (Mateo *et al.*, 2002). Low moisture levels are associated with storage of grains in these countries, which decreases the possibility of T-2 toxin production (Mateo *et al.*, 2002). In Wisconsin, Park (1993) specifically analyzed twenty two samples of maize, wheat, and rice for T-2 toxin (Park, 1995) and found that T-2 toxin accounted for about 30% (range, 9.5 to 76.7%) of the total type A TCTs (Park *et al.*, 1996). Haubruge *et al.*

(2003) analyzed barley samples from 25 farms located in Tibet and detect the presence of T-2 in 76% of the samples with a range of 1 to 163 mg/kg.

In 1992, in Poland, Bocarov-Stancic *et al.* (1995) examined samples of diets and feeds that included barley grain and determined that T-2 toxin occurred at 100 to 3750 µg/kg. Furthermore, Perkowski and Basinski (2002) determined T-2 and HT-2 concentrations in 99 naturally contaminated oat grain samples from 12 plant breeding stations in different parts of Poland. The HT-2 toxin was found in 24% of the samples (average 21 µg/kg; highest level 47 µg/kg) whereas T-2 was found in 15% of the samples (average 60 µg/kg; highest level 703 µg/kg) (Perkowski and Basinski, 2002).

The 1998 grain growing season in Denmark was colder than normal and there were regular and heavy rainfalls from the beginning of July throughout the summer (Rasmussen *et al.*, 2003). The harvest season was generally late and lodging was widespread, especially in winter wheat (Landsudvalget for Planteavl, 1999). The weather conditions were considered as being highly favorable for infection with *Fusarium* spp. Rasmussen *et al.* (2003) investigated the presence of T-2 along with other TCT in flour of common wheat, durum wheat, and rye on samples collected during this season and until 2000 from both mills and the retail market. The article reports that the incidence of T-2 toxin was lower in common wheat flour than in durum flour and rye flour (Rasmussen *et al.,* 2003). Most samples of common wheat flour showed levels below the detection limit of the method (10 µg/kg) (Rasmussen *et al.,* 2003). On the other hand, rye flour showed T-2 in over 65% of samples collected from the harvest years 1998 and 1999 (Rasmussen *et al.,* 2003). The mean concentration of T-2 toxin in rye samples was 68 µg/kg, with five of eighteen samples containing more than 100 µg/kg (Rasmussen *et al.,* 2003). Both

the incidence and concentration of T-2 in rye flour may be considered high when compared with the T-2 content in other cereals and cereal products reported at the meeting of the Joint FAO/WHO Expert Committee on Food Additives in Geneva (2001) (Rasmussen *et al.,* 2003). The T-2 toxin could not be detected in samples harvested in 2000 indicating annual variation for the presence of T-2 in rye flour (Rasmussen *et al.,* 2003). For durum wheat, the results form this survey indicate a frequent and relative high content of T-2 toxin (Rasmussen *et al.,* 2003). The T-2 toxin was found in 90% of the durum wheat samples (Rasmussen *et al.,* 2003). The mean T-2 concentration in these samples was 90 µg/kg and five of the samples contained more than 100 µg/kg (Rasmussen *et al.,* 2003). In Saudi Arabia, analysis of 843 commercial animal feed and foodstuff samples from the years 1997 to 2000 found levels of up to 18.75 µg/kg of HT-2 and 6.25 µg/kg of T-2 (Al-Julaifi and Al-Falih, 2001).

Despite the established toxicity of the fusariotoxins, statutory regulations do not exist for any of the *Fusarium* mycotoxins (D'Mello *et al.*, 1999). In contrast, stringent directives are in place for the *Aspergillus*-derived aflatoxins (D'Mello *et al.*, 1999). However, a selection of advisory and tolerance limits for the *Fusarium* mycotoxins are available in the literature (Table 1.4) (D'Mello *et al.*, 1999). Countries like Canada, Russia, and the USA have established tolerance limits for DON of 500 to 2000 µg/kg for food for human consumption and up to 4000 µg/kg for feed for animal consumption (Leal and Mejía, 1997). In Canada, wheat grain to be used for manufacturing infant food is temporarily permitted to contain up to 1000 µg/kg of DON (Egmond, 1989).

TOXIC EFFECTS

The adverse effects of a mycotoxicosis result from interactions of the original (or metabolically modified) mycotoxin with subcellular organelles in the animal cell (Leeson *et al.*, 1995). The toxicity of the tricothecenes is largely due to their ability to inhibit protein synthesis (Ueno, 1983). The immunotoxicity of A-tricothecenes especially that of T-2, was found to be less than that of the B-tricothecenes (Sharma *et al.*, 1983). Generally, T-2 toxin and HT-2 toxin exhibit the strongest acute toxicity effects, closely followed by NIV. Deoxynivalenol (DON) is considered to be about 10 times less toxic than T-2 and HT-2 toxins (Eriksen and Alexander, 1998). The illness associated with the consumption of cereals contaminated with T-2 includes nausea, vomiting, necrotic lesions in the mouth and throat (making it difficult to eat), severe hemorrhagic diarrhea, and hemorrhaging in many of the body organs (Moss, 2002).

The T-2 toxin is also a potent immunosuppressant, causing irreversible damage to the bone marrow, leading to a characteristic reduction in white blood cells (aleukia) (Moss, 2002). Animal studies have consistently demonstrated immunomodulatory effects, ranging from suppression to stimulation of the immune system (Bondy and Pestka, 2000). The T-2 toxin has been shown to cause necrosis and lymphoid depletion in the thymus, spleen, and lymph nodes of laboratory animals (Ueno, 1977). Necrosis and depletion of lymphocytes in the thymus, bursa of Fabricius, and spleen have been reported in T-2-toxin treated chickens and turkey poults (Wyatt *et al.*, 1973; Boonchuvit *et al.*, 1975; Richard *et al.*, 1978a, 1978b; Hoerr *et al.*, 1981). Tricothecenes have been associated with alterations of serum proteins and immunoglobulin profiles, reduced

antibody formation, thymic aplasia, reduced cell mediated immunity but with enhanced delayed cutaneous hypersensivity, and impairment of bacterial clearance and acquired immunity (Pier and McLaughlin, 1985).

The immunosuppressive effects of the tricothecenes on both cellular and antibody-mediated immunity decrease host resistance to infectious agents. Mortality caused by paratyphoid infection was reported to be increased in chickens exposed to T-2 toxin (Boonchuvit *et al.*, 1975; Ziprin and Elissalde, 1990). Antibody responses to sheep red blood cells were decreased in mice exposed to T-2 (Lafarge-Frayssinet *et al.*, 1979; Rosenstein *et al.*, 1981; Holt and DeLoach, 1988), and in swine vaccinated with necrotic enteritis vaccine (Rafai and Tuboly, 1982). In addition to immunossuppression, tricothecenes may induce immunomodulating effects including decreased spontaneous antibody producing cells in the spleen, increased IgA production in isolated splenocytes, enhanced delayed hypersensitivity, and blastogenic responses of T and B lymphocytes (Corrier, 1991).

The LD_{50} values of the tricothecenes vary with the particular toxin and the type and age of the animal. For male broiler chicks, Hoerr *et al.* (1981) reported that for T-2, the 72-h single oral LD_{50} was 4.0 mg/kg. In addition, T-2 administered as 14 daily oral doses had an LD_{50} of 2.90 mg/kg. In contrast, according to Nemanič (1992), a quantity of 0.5 mg/kg T-2 toxin in food over 2 weeks' exposure can cause pathological changes in the thymus, bursa of Fabricius, and spleen. However, it is hard to pinpoint the LD_{50} for these toxins under natural conditions (Konjević *et al.*, 2004). For instance, Konjević *et al.* (2004) reported necrosis and depletion of lymphocytes in the bursa of Fabricius as well as severe multifocal necrosis in the stomach and gut mucous in Brahma poultry

eating a diet contaminated with 0.70 mg/kg T-2 plus 0.50 mg/kg DAS. The chronicity factors (ratio of the acute to chronic LD_{50} doses) for T-2 is 0.73. Chronicity factors near to one are indicative of compounds that are rapidly eliminated and tend not to accumulate (Osweiler *et al.*, 1984).

In vivo experiments with different animal species indicate that orally or parenterally administered T-2 does not bioaccumulate. Short half-lives (less than 30 minutes) have been reported for T-2 in swine, cattle, and dogs (Yagen and Bialer, 1993). Major metabolic pathways for tricothecenes include oxidation, de-epoxidation, hydrolysis, and glucuronide conjugation (Yagen and Bialer, 1993). Enterohepatic re-circulation of T-2 and its metabolites have been reported in the rat (Coddington *et al.*, 1989). In vitro studies of human blood suggest that carboxylases may be important in the hydrolysis of T-2 to more polar and less toxic metabolites (Johnsen *et al.*, 1988).

In pigs, T-2 has also been implicated in both feed refusal and anorexia (Rafai *et al.*, 1995), reduced weight gain, diarrhea, hemorrhaging, skin lesions, and immunosuppresion (Rasmussen *et al.*, 2003). The authors observed dose-related depressions in feed intake within one-week of feeding diets contaminated with T-2. By the end of the three-week study, pigs fed T-2 toxin at 3 mg/kg had feed intakes, which were only 0.59 of control values (D'Mello *et al.*, 1999). Furthermore, in sows, T-2 may cause infertility, and after parenteral administration during the last trimester of gestation, is able to precipitate abortion within 48 h (Weaver *et al.*, 1986). The T-2 toxin also induces oral lesions in pigs, specifically on the mucosa of the pars oesophageal region, the incidence being dose related (Rafai *et al.*, 1995). In addition, T-2 can cause dermatitis of the snout, nose and buccal comissures in the pig (Rafai *et al.*, 1995).

In poultry, NIV, T-2, and DAS induce gizzard erosions and oral lesions (Kubena *et al.*, 1995; Kubena *et al.*, 1997; Neiger *et al.*, 1994; Vanyi *et al.*, 1994a; and Vanyi *et al.*, 1994b), longer prothrombin time and suppression of factor VIII and fibrinogen activities, as well as depletion and necrosis in the lymphopoetic organs and multifocal necrosis in the intestinal epithelium (Terao *et al.*, 1978; Bitay *et al.*, 1981; Hoerr *et al.*, 1982; Čonková *et al.*, 2003). Chicken macrophage viability may be reduced by exposure to T-2 tetraol, a derivative of T-2 (Kidd *et al.*, 1997). According to Mézes *et al.* (1999), the most sensitive poultry species to T-2 toxin are geese, followed by ducks and chickens. Dvorska and Surai (2001) reported the effect of T-2 on the antioxidant systems of the liver of growing quail fed 8.1 mg T-2/kg for 30 days. The authors reported that inclusion of T-2 in the quail diet was associated with a decrease in concentrations of the antioxidants alpha- and gamma-tocopherols, ascorbic acid, retinol, and retinyl esters. In addition, they reported that liver susceptibility to lipid peroxidation was increased (Dvorska and Surai, 2001).

In a murine model, oral doses of 10 mg/kg of T-2 resulted in lymphocyte apoptosis in thymus tissue, mesenteric lymph nodes, and Peyer's patches (Nagata *et al.*, 2001). The lowest-observable effect level for changes in immune parameters was 0.029 mg/kg body weight per day for T-2, based upon a 3-week dietary study in pigs (Rafai *et al.*, 1995).

In pregnant mice, oral or intraperitoneal administration of T-2 produces maternal toxicity, fetal death, and fetal malformations (Hood *et al.*, 1978; Rousseaux *et al.*, 1985, 1987; Stanford *et al.*, 1975). Likewise, Lafarge-Frayssinet *et al.* (1990) reported that T-2 given to dams by oral, intraperitoneal or intravenous route readily crossed the placenta

and was distributed to the fetal spleen, thymus and liver, resulting in inhibition of T- and B- cells in the spleen and thymus. Thymic atrophy (Holladay *et al.*, 1993), a decrease in the viability of hematopoietic progenitor cells in the liver, and a decrease in the number of B cells were also reported in fetuses after the dams were exposed to T-2 (Holladay *et al.*, 1995). Ishigami *et al.* (1999) also reported that the damage in mouse embryos obtained from dams exposed to T-2 at 11 days of gestation might be produced by apoptosis. Furthermore, Ishigami *et al.* (2001) studied apoptosis in mouse fetuses from dams exposed to T-2 at different days of gestation and concluded that T-2 toxin-induced apoptosis in the developing mouse fetuses might be a direct effect of T-2 on fetuses.

In human tissues, cytotoxic effects of tricothecenes on megakaryocyte progenitor cells, as well as on red and white blood cell progenitors, have been reported in vitro (Froquet *et al.*, 2001; Lautraite *et al.*, 1997; Rio *et al.*, 1997).

In rabbits, ingestion of 0.5 mg of T-2 toxin/kg/day for 21 days reduced packed cell volume, total white blood cells, leukocyte and neutrophil counts and serum alkaline phosphatase activity (Aziz *et al.*, 1995). Moreover, residues of T-2 toxin were found in the liver, kidney, and adrenal glands of animals that ingested food contaminated with T-2 (Aziz *et al.*, 1995). The authors also reported degenerative changes and fibrosis of the liver, nephrosis of the kidneys, hemorrhages in the heart and lymphocyte depletion in the spleen (Aziz *et al.*, 1995). Likewise, Guerre *et al.* (2000) reported damaging effects from ingestion of 0.5 mg T-2/kg/day for five days on body weight gain and mortality of New Zealand white rabbits. The authors also reported that a weak decrease in body weight gain and moderate signs of toxicity occurred in rabbits receiving 0.25 mg/kg/day. Guerre *et al.* (2000) also reported that toxin administration became difficult in all experimentally

intoxicated rabbits because of feed refusal, whereas it was easier in the animals from the control group which is indicative of feed refusal seen in other species. Furthermore, the authors described the signs of T-2 mycotoxicosis observed in these animals, which included reduced spontaneous movements, excessive salivation, and peribucal necrosis. In addition, the fur around the mouth, the nose, and on the foreleg was wet and tan-stained (Guerre *et al.*, 2000).

In young, growing rainbow trout, feeding 5 mg/kg or more of dietary T-2 significantly decreases growth rate and feed efficiency (Poston *et al.*, 1982). However, the observed anorexia or refusal of feed by fish continuously fed the mycotoxin at a concentration of 5 mg/kg or greater suggests that these were caused by inanition rather than by inefficient use of nutrients ingested (Poston *et al.*, 1982). In addition, feeding of graded levels of T-2 resulted in depressed hematocrit and blood hemoglobin concentration and feeding of 15 mg/kg T-2 toxin to adult trout caused hemorrhaging in the intestines and regurgitation of subsequently intubated feed regardless of T-2 toxin content (Poston *et al.*, 1982). Likewise, feeding fingerling rainbow trout a single acute dose (6.5 mg/kg body wt) caused extensive shedding of intestinal mucosa, severe edema with fluid in the body cavities (including those behind the eyes), and eventual death (Marasas *et al.*, 1969).

In juvenile channel catfish *Ictalurus punctatus*, feeding 1.25, 2.5, and 5.0 mg T-2/kg diet for 8 weeks adversely affected growth and hematocrit values. Survivability was adversely affected when levels of 2.5 and 5.0 mg/kg were fed. Significantly poorer feed conversion was found only for the highest level of T-2. In addition, histopathological

studies reviewed anomalies of stomach, head, and trunk kidneys when fish received the three highest levels of T-2 (Manning *et al.*, 2003).

MECHANISM

The T-2 toxin affects synthesis of proteins resulting in immunossuppression. In addition to immunosuppression, it has immunomodulatory effects and may induce lipid perroxidation.

Toxicodynamic studies have demonstrated that the tricothecenes function as inhibitors of eukaryotic protein synthesis (Sudakin, 2003). In eukaryotic cells, initiation of protein synthesis, elongation, and termination of the polypeptide chain takes place on ribosomes and requires peptidyltransferase (Corrier, 1991). Tricothecenes bind to the 60S ribosomal subunit and interact with the enzyme peptidyltranferase which is involved in elongation and termination steps of protein synthesis (Leeson *et al.*, 1995). The $C_{12, 13}$ epoxide group common to tricothecene is necessary for protein synthesis inhibition, while certain substitutions of the ring structure at the C_3 and C_4 positions lead to additional effects (Feinberg and Mc Laughlin, 1989). This interaction leads to varying degrees of inhibition of peptide bond formation depending upon the chemical structure of the specific tricothecene (Cundliffe and Davies, 1977). The more potent tricothecenes - including T-2 – may inhibit the initiation of protein synthesis as well as the elongation and termination (Murthy *et al.*, 1985). Inhibition of protein synthesis also acts to inhibit RNA synthesis as an indirect secondary effect. In addition, cells require newly synthesized protein for DNA synthesis and to complete mitosis (Mitchison, 1971).

Therefore, the cytotoxic-radiomimetric-like effects of T-2 toxin on rapidly dividing cells are caused by impaired DNA and RNA synthesis resulting indirectly from inhibition of peptidyltransferase and protein synthesis (Corrier, 1991). A comparison of the molecular-cellular basis of protein synthesis inhibition by aflatoxin, ochratoxin and the tricothecene toxins indicates that while the site and primary event may differ, the immunosuppressive effects are ultimately caused by a common mechanism resulting directly or indirectly from inhibition of protein synthesis (Corrier, 1991). Cells most susceptible to the action of tricothecenes are those having a high rate of regeneration such as lymphoid, erythroid, intestinal cryptal cells, and cells of parenchymal organs like liver, kidney, and pancreas (Leeson *et al.*, 1995). This implies that leukocytes and the immune system are the primary targets for T-2 (Bondy and Pestka, 2000).

The mechanism of action by which T-2 appears to modulate the immune system may be due to the fact that T-2 causes depletion of T-lymphocytes and the failure of sensitized T cells and T-cell dependent immune-activated macrophages to accumulate at the centers of infection (Corrier and Ziprin, 1986b) to fight off the bacteria. Masuko *et al.* (1977) and later on Otokawa *et al.* (1979) reported that T-2 interferes with at least one subset of T cells, T suppressor cells (Ts), which suppress immune responses (Roitt *et al.*, 1989) causing cell-mediated resistance against infectious agents. To illustrate, Corrier and Ziprin (1986a, 1986b) evaluated the immunotoxic effects of T-2 and cyclosphamide (Cy) - a well known cytotoxic drug - on cell-mediated resistance in mice exposed to *Listeria monocytes* infection. The authors found that necrosis and depletion of lymphoid tissue, a condition called lymphopenia, significantly decreased the influx and number of lymphocytes and macrophages occurring in *Listeria*-elicited peritoneal exudates and at

sites of infection in the liver and spleen of the toxin- and cyclophosphamide-treated mice. Furthermore, the authors concluded that the immunotoxic effects of T-2 and Cy were comparable and attributed primarily to the depletion of T lymphocytes and the subsequent failure of surviving immunologically committed T cells and T-cell dependent immune-activated macrophages to clear the host of bacteria (Corrier and Ziprin, 1986a, 1986b).

The sequence by which this mechanism occurs is: 1) bacteria are removed by T-lymphocyte dependent immune-activated macrophages (Cheers and McKenzie, 1978; Mackaness, 1961; North, 1973; Koster *et al.*, 1971); 2) Phagocytes may be recruited from circulating monocytes and neutrophils (North, 1970; Czuprynski *et al.*, 1984) or from granulocyte/macrophage precursor cells (Luster *et al.*, 1981; Bennett and Baker, 1977). Functional expression of resistance is further dependent on the accumulation of sensitized T cells at sites of *Listeria* infection where they promote the influx and activation of macrophages (Koster *et al*., 1971; McGregor and Logie, 1973, 74; North, 1974). Peritoneal exudates elicited by intraperitoneal inoculation of *Listeria* are especially rich in sensitized T lymphoblasts (Koster *et al.*, 1971; McGregor and Logie, 1974; North, 1974), which will trigger the immune response via signaling other immune cells (macrophages) - via secretion of macrophage-activating factor (interleukin-2) and other lymphokines - to migrate to the sites of infection to fight off the bacteria, which is characterized by the influx and accumulation of lymphocytes and macrophages in the peritoneal cavity and at centers of infection in the liver and spleen. When the immune response was negatively altered, the influx of mononuclear cells markedly decreased and the cells failed to populate the sites of infection; therefore, there was failure to clear the

host of infection, which explains why Corrier and Ziprin (1986a, 1986b) reported rapid growth of *Listeria* in the spleen, increased numbers of *Listeria* colonies at the foci of infection in the liver and spleen, and ultimately in the higher mortality that occurred in the T-2- and Cy-treated mice.

Second, the tricothecenes may induce other immunomodulating effects in addition to immunosuppression (Corrier, 1991). Oral gavage of mice with T-2 toxin was observed to increase spontaneous antibody producing cells in the spleen (Pestka and Bondy, 1990). The same researchers reported increased IgA production in splenocytes isolated from vomitoxin-treated mice (Corrier, 1991). T-2 toxin was also reported to enhance the delayed hypersensitivity response to sheep erythrocytes in mice (Masuko *et al.*, 1977; Otokawa *et al.*, 1979). The authors suggested that T-2 toxin might have depleted or interfered with suppressor cells that may regulate the cell-mediated response. Low dosages of T-2 were reported to enhance the blastogenic responses of T and B-lymphocytes (LaFarge-Frayssinet *et al.*, 1979; Holt *et al.*, 1988a, 1988b). In addition, superinduction of cellular proteins such as IL-1 and IL-2, that regulate intercellular communications in the immune system, have been reported using tricothecenes (Miller and Atkinson, 1986; Holt *et al.*, 1988a, 1988b). Proposed mechanisms that may account for these effects include interference with high turnover proteins that limit translation of mRNA for interleukin synthesis or impaired degradation of intracellular interleukins (Miller and Arkinson, 1986).

The T-2 toxin has been observed to suppress or enhance macrophage activity (Corrier, 1991). However, this activity was dependent on time of toxin treatment and the immune challenge (Corrier, 1991). For instance, post treatment of mice with *Listeria*

challenge suppressed resistance to listeriosis (Corrier and Ziprin, 1986b; Corrier *et al.*, 1987). In contrast, pretreatment with T-2 toxin prior to challenge markedly enhanced resistance to listeriosis (Corrier and Ziprin, 1986a). Enhanced resistance was associated with increased migration of macrophages and enhanced phagocytic activity (Corrier *et al.*, 1987). Enhanced resistance was also accompanied by necrosis and depletion of lymphocytes in the thymus and spleen, leukocytopenia, and lymphopenia. Increased resistance to listeriosis in thymus-deprived mice (Cheers and Waller, 1975; Newborg and North, 1980) and in mice treated with the cytotoxic drug cyclophosphamide (Tripathy and Mackaness, 1969a, 1969b; Luster *et al.*, 1981) was attributed to the removal of a radiosensitive population of regulatory T-cells. Supressor T cell are short lived lymphocytes that are more susceptible to cytotoxic agents than are other subpopulations of lymphocytes (Mitsuoka *et al.*, 1976; Chan *et al.*, 1977). Enhanced host resistance in mice exposed to T-2 prior to *Listeria* challenge may have been caused by the depletion or impaired function of regulatory T suppressor cell population and subsequent enhancement of macrophage activity (Corrier, 1991). The results of in vivo studies on T-2 toxin modulation of host resistance to listeriosis further demonstrate that the immunomodulating effects of the mycotoxins on host resistance may be influenced by numerous contributing factors that may not be present or detected during in vitro studies (Corrier, 1991). Likewise, the well-demonstrated inflammatory and irritant action of T-2 toward the gastrointestinal tract (Marasas *et al.,* 1969; Wyatt *et al.*, 1973) suggests that the natural barriers to invasion by the intestinal pathogen *Salmonella* (Perry *et al.*, 1972) may be more readily breached during T-2 toxicosis. The resulting bacteremia would normally be combated by the phagocytic cells, but the smaller spleen caused by T-2 and

the negative interaction on spleen size by T-2 suggest that the phagocytic cells of the reticuloendothelial system are impaired (Boonchuvit *et al.,* 1975).

To conclude, inhibition of synthesis of proteins will lead to immunosuppression because secretion of macrophage-activating factor (interleukin-2) and other lymphokynes by sensitized T lymphoblasts requires the induction of specific messenger RNA and new protein synthesis (Granelli-Piperno *et al*., 1984). The T-2 toxin inhibits peptidyltransferase activity necessary for new protein synthesis (Schindler, 1974). Although speculative, a mechanism may exist whereby T-2 may cause functional impairment of surviving lymphocytes by inhibiting the synthesis of lymphokine proteins that activate macrophages (Corrier and Ziprin, 1986a,1986b). For animal production, the implication of a suppressed immune system is the inhibition of vaccine efficacy and of resistance to different agents (Palyusik and Bitay, 1992) resulting in increased mortality (Boonchuvit *et al.*, 1975).

In addition, T-2 may also stimulate lipid peroxidation. It has been concluded that T-2 primarily causes damage to the membrane systems of liver cells, and that peroxidation of the membrane lipids is secondarily stimulated (Karppanen *et al.*, 1989). The T-2 toxin can be incorporated into the lipid or protein components of the cell membranes due to its amphipathic character (Leeson *et al.*, 1995). Several reports indicate that T-2 is able to induce a dose-dependent stimulation of lipid peroxidation in rat and mice liver (Tsuchida *et al.*, 1984; Ahmed and Ram, 1986; Chang and Mar, 1988; Karppanen *et al.*, 1989; Suneja *et al.*, 1989). Lipid peroxidation is one of the factors responsible for the damage and necrosis of liver induced by chemicals and mycotoxins (Plaa and Witschi, 1976). Lipid peroxidation is believed to be caused by depletion of

hepatic gluthanione (GSH) and/or production of free radicals (Leeson *et al.*, 1995). Guerre *et al.* (2000) demonstrated that total liver microsomal cytochrome P450 content and monooxygenase activities were decreased when 0.25 mg T-2/kg/day was given to rabbits. At this dose, the authors reported that T-2 exposure also produced microsomal oxidative damages (Guerre *et al.*, 2000) which impaired the P450 microsomal environment, leading to increased protein denaturation and degradation (Serbinova *et al.*, 1989; Kitada *et al.*, 1989; Mori *et al.*, 1991).

Karppanen *et al.* (1989) suggests that when toxic agents stimulate lipid peroxidation, the peroxidation reaction results in disturbances in the functioning and structure of the cell membranes. The nonenzymatic auto oxidation of the polyunsaturated fatty acids produces a complex mixture of products that have the same biological reactivities of the tricothecenes such as a strong inhibition of various enzyme reactions (4-hydroxyoctenal) and a strong inhibitory effect on protein synthesis (4-hydroxypentenal). When the tricotechenes trigger lipid peroxidation, their toxic effect is enhanced and continue even after the toxins have been metabolized and excreted (Karppanen *et al.*, 1989).

The epoxy group of T-2 competes with the substrates for gluthaniones-s-transferases (Ueno, 1977). This may result in a decreased content of GSH. Prevention of lipid peroxidation is primarily connected with GSH metabolism and with the ability of hepatic glutathione peroxidase to remove hydrogen peroxides (H_2O_2) and organic peroxides in the compartments where catalase is absent (Sies *et al.*, 1972). It is possible that T-2 reduces the level of GSH which is required for the elimination of peroxide radicals. The level of GSH is affected by the activities of the GSH-shuttle enzymes –

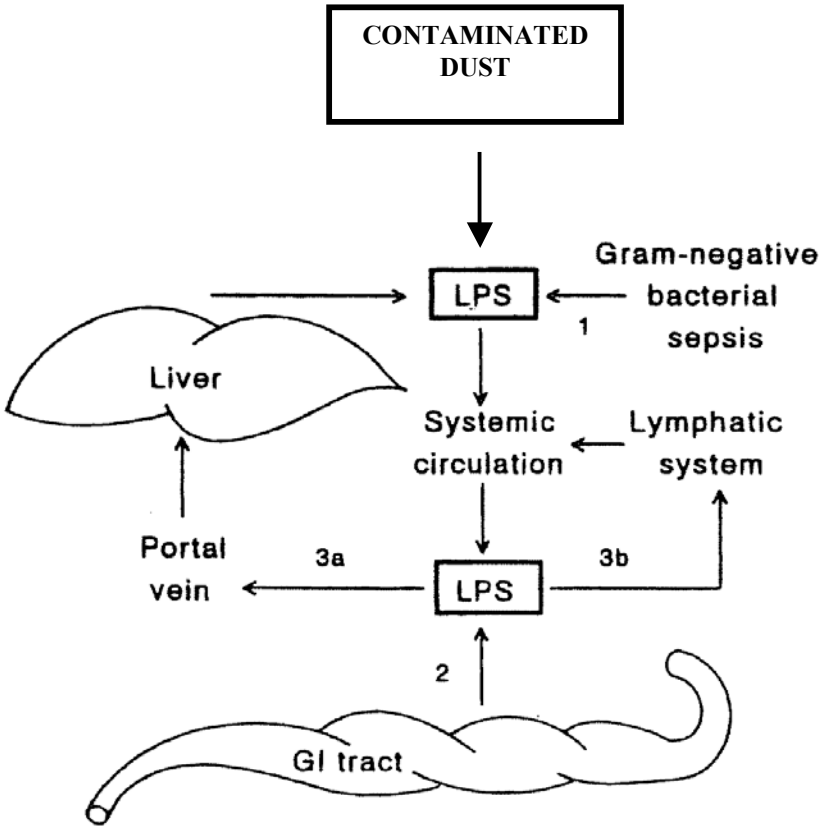
glutathione peroxidase, glutathione reductase, and glucose-6-phosphate - (Plaa and Witschi, 1976; Giri *et al.*, 1983). Agents that cause lung damage by generating reactive oxygen species stimulate a protective antioxidant cellular defense mechanism for detoxifying O_2 and lipid peroxides (Giri *et al.*, 1983) by enhancing lung lipid peroxidation and simultaneously raising the activities of glutathione-shuttle enzymes and catalase. Suneja *et al.* (1989) reported an increase in the activities of liver GSH-shuttle enzymes after short term T-2 feeding (2.0 mg/kg) and long term T-2 feeding (0.75 mg/kg) in rats and concluded that T-2 may be generating reactive oxygen radicals. In addition, it has been shown that T-2 administered to mice cause a significant increase in respiratory burst of macrophages, which lead to release of reactive oxygen species into the blood stream (Cooray and Jonsson, 1990).

In conclusion, antioxidants are of primary value biologically in restricting the damage that reactive free radicals can cause to cells and cellular components. The body's antioxidant defense mechanisms normally keep free radicals in check, protecting the body from oxidative damage. However, during exposure to high concentrations of toxicants, free radicals may overwhelm the cellular defense mechanism, causing excessive oxidative stress with consequent cell- and tissue damage (Rizzo *et al*., 1994). To illustrate, when Rizzo *et al.* (1994) fed male rats a diet deficient in vitamins C, E, and selenium, after administration of T-2 (3.6 mg/kg BW), there was an increase in lipid peroxidation by 307% as detected by analysis of thiobarbituric acid-reactive substances in liver samples when compared to the animals fed diets sufficient in these antioxidants.

E. coli lipopolysaccharide(LPS)

Bacterial endotoxin or lipopolysaccharide (LPS) is a major component of the cell wall of Gram-negative bacteria and is one of the features that distinguish these bacteria from Gram-positive bacteria (Hewett and Roth, 1993). Examples of gram negative bacteria include *Escherichia coli* and *Salmonella typhimurium*. The LPS molecule can be divided into three parts: the lipid moiety, the R-core, and the O-polysaccharide (Elin and Wolff, 1976). The lipid moiety consists of an insoluble fraction called lipid A and a soluble fraction which contains polysaccharides. Lipid A refers to all chemical compounds that become insoluble after acid hydrolysis of LPS whereas the R-core is the middle part of the LPS molecule linking the lipid moiety to the O-polysaccharide moiety. Finally, the O-polysaccharide portion is made of a regular sequence of either linear or branched sugars (Hellerqvist *et al.*, 1969). The O-polysaccharides determines the O-antigenic specificity of the bacterium (Hewett and Roth, 1993).

Exposure to LPS may occur as a result of infection by gram negative bacteria or in the absence of bacterial infection (Figure 2.1). First, as an integral part of Gram negative bacteria, LPS is not normally released into the extracellular fluid in a free form; however, it can be liberated from dividing or dead bacteria during infection by Gram negative bacteria (Hewett and Roth, 1993). LPS exposure can also occur through increased absorption of LPS from the gastrointestinal tract (GIT), and in the absence of bacterial infection via the respiratory tract by breathing of dust contaminated with endotoxin (Figure 2.1).



LPS is released from dividing and dead bacteria during infection by Gram-negative bacteria (1); LPS derived from endogenous Gram-negative bacterial flora is absorbed from the gastrointestinal tract (GI, 2); by breathing endotoxin-contaminated dust (4); and is transported to the liver or to the systemic circulation by the portal vein (3a) or lymphatic system (3b), respectively (Hewett and Roth, 1993).

Figure 2.1. Means of exposure to LPS (Adapted from Hewett and Roth, 1993)

The GIT, particularly the colon, normally contains a large concentration of LPS that is derived from endogenous Gram-negative flora of the gut (Ditter *et al.*, 1983). Under normal conditions, the intestinal wall acts as a formidable barrier to the passage of LPS from the GIT into the blood stream. However, disruption of this barrier during certain pathophysiological conditions, such as T-2 exposure, can lead to systemic endotoxaemia (Hewett and Roth, 1976) by increasing LPS absorption from the GIT. To illustrate, increased absorption of LPS has been associated with chemically induced liver injury (Grun *et al.*, 1976), partial hepatectomy (Cornell, 1985; Van Leeuwen *et al.*, 1991) and intestinal ischemia/reperfusion (Gathiram *et al.*, 1986; Caty *et al.*, 1990).

Lipopolysaccharide exposure can also occur in the absence of bacterial infection. For instance, endotoxin can be present in the house dust (Michel *et al.*, 1991; Peterson *et al.*, 1964). In humans, the level of this exposure is an important determinant of asthma severity (Michel *et al.,* 1996). Endotoxin may increase asthma severity by extending airway inflammation since inhalation of endotoxin contaminated dust increases the neutrophil number in bronchoalveolar lavage fluid (Sandstrom *et al.*, 1992). In horses, inhaled endotoxin and organic dust particulates have synergistic proinflammatory effects in equine heaves (a condition called organic dust-induced asthma). This condition is characterized by airway neutrophilia, mucus hypersecretion and obstructive lung dysfunction (Pirie *et al.*, 2003).

When a challenge occurs, the normal metabolism is altered and its priority is shifted to mechanisms that support the immune system. In rodent models, the physiological response includes a depression in peripheral tissue glucose uptake and insulin sensitivity (Ling *et al.*, 1994) and an increase in circulating triglycerides and

cholesterol (Grunfeld and Feingold, 1992). During sepsis and infection, cytokines are released to ensure adequate nutrient supply to the tissues with a large number of inflammatory cells resulting in an increase in muscle protein breakdown (Flores *et al.*, 1989), an increase in hepatic glucose output (Westfall and Sayeed, 1988; Filkins *et al.*, 1989), and a redistribution of infused glucose away from skeletal muscle (Ling *et al.*, 1994). Thus, this cytokine-regulated process is both essential for normal host defense, and detrimental to bodily functions (Wenzel *et al.*, 1996).

Nolan and co-workers noted a relationship between exposure to endotoxin and modification of liver injury (Nolan, 1981; Nolan and Camara, 1989; Nolan 1989) in that exposure of LPS augments the hepatoxicity of xenobiotic agents. The first studies were conducted with carbon tetrachloride (CCl_4) (Roth *et al.*, 1997). The hepatotoxic effects of CCl_4 are reduced in animal in which LPS exposure is prevented – e.g. by the use of the antibiotic polymyxin B – and in animals rendered insensitive to LPS (Leach and Forbes, 1941; Nolan and Ali, 1973). Finally, Formal *et al.* (1960) co-administered LPS with CCl_4 and concluded that LPS increases the toxicity of CCl_4 (Roth *et al.*, 1997).

In addition to CCl_4, concurrent exposure to LPS markedly increases the liver injury and/or lethality produced by a number of hepatotoxicants. Co-administration of LPS markedly increases the severity of ethanol hepatitis (Bhagwandeen *et al.*, 1987), and it potentiates liver injury from T-2 toxin (Tai and Pestka, 1988) as well as from halothane exposure in hypoxic rats (Lind *et al.*, 1984). The hepatotoxic effects of allyl alcohol are magnified by LPS co administration, and this occur at LPS doses that are three to four orders of magnitude smaller than those which produce liver injury when LPS is given alone. Roth *et al.* (1997) reported that treatment of rats with modest doses of LPS results

in liver injury upon co-administration of chlorpromazine, ethionine, deoxyvalenol, monocrotaline, or AFB_1 at doses that are otherwise not hepatotoxic.

It is also possible that hepatotoxicity from some chemicals arise indirectly from primary damage to the GIT which increases translocation of LPS into portal venous circulation (Roth *et al.*, 1997). Under these conditions, the liver is exposed to injurious amounts of this bacterial product, and the hepatotoxicity resembles liver injury seen in response to relatively large doses of LPS (Roth *et al.*, 1997). For example, both cadmium and T-2 damage the GIT. As a result, there is an increase in concentration of LPS in portal venous blood and the genesis of a liver lesion which resembles that produced by large noninjurious doses of LPS (Roth *et al.*, 1997).

Roth *et al.* (1997) proposed a hypothesis of how LPS exposure may be an important determinant of susceptibility to intoxication from certain xenobiotic agents. Hepatotoxic agents initiate tissue injury through a variety of mechanisms: lipid peroxidation, covalent binding of reactive metabolites to cellular macromolecules, DNA damage, and altered gene expression. At large xenobiotic doses, these mechanisms are so pronounced that they are sufficient to cause overt tissue injury. At smaller doses, the same mechanisms may initiate an alteration in cellular homeostasis that by itself is insufficient to lead to injury. However, the presence of additional host derived factors may force progression of such cellular alterations to tissue damage. These host factors can arise from the inflammatory response invoked by LPS exposure, caused either by action of the xenobiotic agent itself on the GIT or by independent events, such as locus of infection or a concurrent disease or dietary alteration that increases GI translocation of LPS. Diseases that impair hepatic clearance of LPS may further enhance its effects.

When activated by LPS, inflammatory cells such as Kupffer cells and neutrophils release numerous soluble mediators that may act on parenchymal cells stressed by xenobiotic exposure, leading to overt hepatocellular injury (Roth *et al.*, 1997).

Animals are naturally exposed to high levels of LPS, with poultry operations being the most contaminated, followed by swine operations (Simpson *et al.,* 1999). Endotoxin is ubiquitous and from a commercial stand-point, this means that performance may be affected. For instance, commercial pork producers are only able to achieve approximately 65% of the pig's genetic potential for growth rate as compared to the performance at a genetic nucleus farm. There is no doubt disease challenge is one of the factors contributing tremendously to this reduction in performance (Frank, 2003).

Besides exposure to high levels of endotoxin, animals are sometimes exposed to continuous low levels of mycotoxins in feed. Mycotoxins, such as aflatoxin B1 (AFB1) and the T-2 toxin (T-2) are immunosuppressive and hepatotoxic, and in combination with endotoxin can render the animal more susceptible to infections resulting in lower feed intake, lower body weight gain, and poor feed conversion. In fact, the possible interactions between mycotoxins and infectious disease have been the subject of concern in animal husbandry and veterinary medicine ever since reports of aflatoxicosis by Siller and Ostler (1961) described the isolation of Salmonella from the internal organs of turkeys during field outbreaks of the disease (Cooray and Jonsson, 1990).

Barton *et al.* (2001) reported that exposure to a non-toxic dose of bacterial endotoxin potentiates the hepatotoxicity of AFB1 in rats. Exposure to smaller doses of LPS initiates a more modest and noninjurious inflammatory response. However, exposure to such doses can render the liver more sensitive to injury from hepatotoxic

chemicals, including AFB1. A small dose of LPS can convert an otherwise nontoxic dose of AFB1 into one that is markedly hepatotoxic (Barton *et al.*, 2001).

In 1988, Tai and Pestka investigated the synergistic interaction between the tricothecene T-2 toxin and *S. typhimurium* LPS in both LPS-susceptible (C3H/HeN) and LPS-resistant (C3H/HeJ) mice. They found that the LD_{50} values for LPS decreased by 14-fold and 4.5-fold upon co-administration with T-2 at a level of 1 mg/kg in both C3H/HeN and C3H/HeJ mice, respectively and increased mortality due to impaired splenic function in the C3H/HeN mice.

Inhibition of protein synthesis by tricothecenes may increase susceptibility to microbial infections, because host defense is ultimately dependent upon the production of specific proteins in sufficient quantities to combact an infectious process as well as to repair cellular damage (Beisel, 1977).

TOXICITY

A number of factors influence the response to LPS in vivo, including species differences (Kuida *et al.*, 1961; McCuskey *et al.*, 1984), routes of exposure (Jacobson and Farrar, 1963), dosing protocols (e.g. bolus injection or infusion) (D'Orio *et al.*, 1987), timing of exposure (Elliott *et al.*, 1991), and age of the animal (Durham *et al.*, 1990). In addition, LPS from different strains and species of gram-negative bacteria expresses large differences in potencies (Sanchez-Cantu *et al.*, 1991). Although the outcomes of LPS exposure may vary under different conditions, it is clear that many of the adverse effects of LPS are dependent on endogenous mediators generated by the host in response to LPS

(Morrison and Ulevitch, 1979). Some of the mediators are lipid mediators such as arachidonic acid metabolites and platelet activating factor (PAF), cytokines such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN- γ), interleukins (ILs), and reactive oxygen metabolites, particularly superoxide, nitric oxide (NO), and their derivatives (Hewett and Roth, 1993). Factors derived from the coagulation and complement systems may contribute to tissue injury under certain circumstances (Hewett and Roth, 1993).

Lipopolysaccharide is cytotoxic to cells in vitro under certain conditions. For example, marked degenerative morphological changes occurred after exposure of cultured vascular endothelial cells to LPS (Gartner *et al.*, 1988), and were accompanied by increased cell detachment, leakage of cytoplasmatic contents and decreased DNA, RNA, and protein synthesis. Moreover, injury to cultured endothelial cells was both dose (> 0.1 μg/ml) and duration of LPS exposure (> 4 h) dependent and was enhanced by the presence of serum, suggesting that it could be modified by the presence of lipopolysaccharide binding proteins (LBP) (Harlan *et al.*, 1983; Meyrick, 1986; Patrick *et al.*, 1992).

Oxygen radical production and conjugated diene formation were associated with LPS-induced endothelial cell cytotoxicity, suggesting that injury to endothelial cells in vitro is mediated by oxygen radical-dependent lipid peroxidation (Brigham *et al.*, 1987). Moreover, the cytotoxicity was attenuated by oxygen radical scavengers (Hewett and Roth, 1993). Results from recent study suggest that a similar mechanism may contribute to LPS-induced injury to vascular endothelial cells in vivo (Matsuda *et al.*, 1991).

In an in vitro study, and at very high concentrations (Hewett and Roth, 1993), LPS was cytotoxic to other cells as well, such as cultured macrophages (Glode *et al.*,

1977) and fibroblasts (Ryan and McAdam, 1977). However, the involvement of reactive oxygen metabolites in these instances has not been reported. In contrast, at a low concentration of LPS, Jean and Roth (1993) determined that LPS was minimally cytotoxic to isolated hepatocytes. It is yet to be determined how important this cytotoxic effect of LPS is in vivo. It is likely that the most toxic effect of LPS in vivo is mediated indirectly by host-derived soluble mediators and cells such as PMNs (neutrophils).

In addition to its cytotoxic effects, LPS stimulates the release of a variety of inflammatory mediators from cultured cells. These potent substances are implicated in the pathophysiological alterations associated with LPS exposure in vivo.

Hewett and Roth (1993) reviewed LPS-induced injury on extrahepatic tissues, including the cardiovascular system, kidneys, gastrointestinal tract, and lungs as well as the hepatic changes. LPS-induced injury on the cardiovascular system is characterized by a dramatic decrease in cardiac output within 1 h after a bolus intravenous dose of LPS (Hinshaw, 1979; Somani and Sairi, 1981, Olson, 1987; D'Orio *et al.*, 1987; Milligan *et al.*, 1988) and is associated with a decrease in venous return (Hinshaw, 1979) . In addition to alterations in cardiac output, there is a decrease in mean arterial blood pressure (D'Orio *et al.*, 1987; Olson, 1987; Wakabayashi *et al.*, 1987) followed by a decrease in vascular contractility (Hewett and Roth, 1993). As a result, blood flow is altered to most tissues, but the most affected organs are heart, kidney, pancreas, and spleen (Ferguson *et al.*, 1978; Somani and Sairi, 1981; Breslow *et al.*, 1987).

In addition, in vitro experiments have demonstrated that LPS activates the coagulation system in both the extrinsic and intrinsic pathways, through the release of tissue factor from vascular cells (Rivers *et al.*, 1975; Colucci *et al.*, 1983), and promotes

disseminated intravascular coagulation (DIC) by antagonizing fibrinolysis (Hewett and Roth, 1993). Disseminated intravascular coagulation is characterized by a reduction in circulating platelet numbers (Emerson *et al.*, 1987; Smith *et al.*, 1988; Sandset *et al.*, 1991), clotting factors, and fibrinogen (Emerson *et al.*, 1987; Sandset *et al.*, 1991), and by the deposition of fibrin in the microvasculature of various tissues, including the liver, kidney, and lung contributing to the LPS pathogenesis of these organs. These changes are accompanied by increases in both prothrombin and partial prothrombin times (Lipinski *et al.*, 1976; Emerson *et al.*, 1987; Yoshikawa *et al.*, 1981).

Kidneys are also affected by LPS exposure. Lipopolysaccharide exposure causes renal tubular necrosis by a DIC-dependent pathway (Raij *et al.*, 1977). In addition, and via a number of lipid mediators, LPS exposure indirectly causes marked decreases in renal blood flow (RBF), glomerular filtration rate (GFT), sodium reabsorption, and renal tissue potassium concentration (Badr *et al.*, 1986; Cohen *et al.*, 1990; Badr, 1992). This is mediated by a number of lipid mediators (certain metabolites of arachidonic acid) (Hewett and Roth, 1993).

Furthermore, in the gastrointestinal tract (GIT) severe endotoxemia causes detachment of epithelial cells from the mucosal basement membrane, inflammatory cell infiltration, vascular congestion, and an increase in vascular permeability (Wallace *et al.*, 1987). These changes accentuate the leak of further endotoxin, causing a loss of circulatory volume into interstitial tissue, promoting hypotension and increasing tissue ischemia (Ghosh *et al.*, 1993).

In addition, pulmonary dysfunction is a hallmark of circulatory shock and sepsis (Ghosh *et al.*, 1993). The changes observed in acute respiratory distress characterized by

the adult respiratory distress syndrome (ARDS), frequently accompanies gram-negative infection (Fein *et al.*, 1983) and the symptoms are similar to those seen in experimental endotoxemia (Esbenshade *et al.*, 1982; Brigham and Meyrick, 1986): increased mean pulmonary arterial pressure, pulmonar vascular resistance, and alveolar-capillary membrane permeability together with decreased dynamic lung compliance and functional residual capacity leading to pulmonary edema, ventilation/perfusion mismatch, and hypoxia by impairment of gas exchange (Ghosh *et al.*, 1993).

Particular attention should be given to the liver as liver dysfunction may trigger multiorgan failure because of overt failure of metabolic, synthetic, and phagocytic functions of the liver (Hewett and Roth, 1993). The liver performs a number of important physiological functions such as carbohydrate metabolism, fat and cholesterol metabolism, and synthesis and excretion of bile constituents and plasma proteins. It also plays an important role in the elimination and detoxification of xenobiotics agents (Hewett and Roth, 1993). These functions are performed by hepatic parenchymal cells which comprise approximately 80% of the hepatic cells, and a heterogeneous population – the nonparenchymal cell fraction – consisting of fenestrated vascular endothelial cells, resident vascular macrophages (Kupffer cells), fat storage cells (Ito cells), and bile duct epithelial cells (Hewett and Roth, 1993).

The basic structural unit of the liver, in which hepatic cells come in close contact with the blood, is the liver lobule. The lobule consists of cords of parenchymal cells radiating from a central vein through which blood exits the lobule and is transported to the inferior vena cava. Endothelial cells and Kupffer cells comprise the bulk of the sinusoid lining (Nolan, 1981). Endothelial cells form the lining of the sinusoids and form

a sleeve-like plate that allows particles below a certain size to reach the hepatocytes. These cells ingest particles, such as endotoxin, by pinocitosis or absorptive endocytosis with vesicle formation and fluid incorporation (Nolan, 1981). Kupffer cells are scattered throughout the sinusoids. These cells remove dead blood cells as well as cell debris, and foreign substances, such as LPS, from the circulation. Therefore, they are critical to the detoxification and action of LPS.

During exposure to LPS, morphological and functional alterations occur in the liver during septicemia (Levy *et al.*, 1967; Utili *et al.*, 1977; Hirata *et al.*, 1980). The earliest changes in morphological alterations are characterized by dilation of Kupffer cell endoplasmatic reticulum, and damage to the plasma and nuclear membranes. This leads to injury to endothelial cells and appearance of fibrin clumps and platelet thrombin in the sinusoids, to swelling of the microvilli on the sinusoidal border, and dilation of bile canaliculi. These alterations will culminate with hepatocellular degeneration and necrosis. Lesions are multifocal, frequently involving parenchymal cells in the midzonal region of the liver lobule and are often infiltrated with PMNs. In addition, there are increases in plasma activities of liver enzymes, such as aspartate and alanine aminotransferase, suggesting membrane cell damage to parenchymal cells (Sleeman and Hardaway, 1967; Shibayama, 1987; Hewett *et al.*, 1992).

Among the functional changes in the liver after LPS exposure are changes in carbohydrate, lipid, and protein metabolism, cholesteasis, and circulatory alterations such as portal hypertension, and a disruption of hepatic blood flow (Hewett and Roth, 1993).

MECHANISM OF ACTION

- ***Cell activation:***

Bacterial endotoxins initiate the syndrome of sepsis associated with gram-negative rod infection (Wenzel *et al.*, 1996). This response is mediated by two biochemical elements known to recognize LPS (Figure 1.17): an LPS-binding protein (LPB), an acute phase reactant, and a membrane or soluble receptor known as CD14 (Wenzel *et al.*, 1996).

CD14 is anchored in the cell membrane of monocytes and macrophages as a glycerophosphatidylinositol (GPI)-tailed protein. Once LPS binds to it, the complex LPS-CD14 triggers an interaction of some portion of the CD14 protein with another cell membrane component (Tobias *et al.*, 1986; Ulevitch and Tobias, 1995). Both GPI-anchored CD14 and soluble CD14 bind LPS resulting in cell activation to new gene expression. In both cases, LPS-CD14 interactions are markedly facilitated by LBP; which acts as a transfer protein (Tobias *et al.*, 1986; Han *et al.*, 1994). Binding of LPS to GPI-anchored CD14 also results in rapid translocation of LPS from the cell-membrane to intracellular locations (Gallay *et al.*, 1993). The treatment of macrophages with LPS resulted in increased protein tyrosine phosphorylation (Han *et al.*, 1993; Weinstein *et al.*, 1991) targeting a new member of the mitogen activated protein (MAP) kinase family (Han *et al.*, 1994): protein p38 which activates the biosynthesis of cytokines like IL-1 and TNF (Wenzel *et al.*, 1996). Besides signaling, CD14 functions as a surface protein mediating internalization of LPS (Hack *et al.*, 1989) via a transmembrane protein which has been postulated but not yet identified (Ulevitch and Tobias, 1995).

- ***Cardiovascular system:***

Lipopolysaccharide reacts with leukocytes, platelets, the complement system, and other serum proteins to increase blood levels of proteolytic enzymes and of certain vasoactive substances, such as histamine, kinins, and serotonin (Hinshaw *et al.*, 1965) resulting in pooling of blood, primarily in the pulmonary and splanchnic vasculature (Lillehei *et al.*, 1965). As a result, there is an increased peripheral vasoconstriction resulting in poor tissue perfusion.

Cardiac output is diminished by the direct myocardial depressing action of LPS and by a decrease in venous return secondary to the pooling of blood (Cho, 1972). As a consequence, a series of events is initiated beginning with a drop in blood pressure and peripheral perfusion. Endogenous catecholamines are liberated to compensate for the falling blood pressure which will then lead to tissue acidosis and anoxia (Rosenberg and Rush, 1966). This initiates capillary congestion and dilatation which accentuates the decreased venous return and cardiac output. The cellular hypoxia results in lysosomal disruption, leading to cell lysis, propagation of tissue injury, and death (Christy, 1971).

- ***Disseminated intravascular coagulation (DIC):***

The coagulation system comprises circulating proenzymes and factors that, when activated, result in a coordinated cascade of proteolytic events which culminate in the conversion of prothrombin to thrombin. A major action of thrombin is the cleavage of circulating fibrinogen. This leads to the consumption of fibrinogen and to the formation of insoluble fibrin clots. Clot formation is normally regulated by endogenous anticoagulant and fibrinolytic systems which limit the actions to sites of damaged blood vessels. However, under certain pathophysiological conditions such as sepsis and severe

endotoxemia, the coagulation system can become activated systemically, resulting in disseminated intravascular coagulation and consequent tissue injury (Hewett and Roth, 1995).

The coagulation system plays an important role in the pathogenesis of LPS on organ tissues, such as lung, liver, and kidney. Supporting evidence for this was provided by studies with the anticoagulant, heparin, which attenuated the decrease in plasma fibrinogen concentration and afforded protection against liver injury (Hewett and Roth, 1995).

- ***Tissue injury:***

Kidneys: A number of lipid mediators have been implicated in the effects of LPS on kidney function. Among these are certain metabolites of arachidonic acid, including leukotrienes (potent vasoconstrictors), tromboxane, and platelet activating factor (PAF). The latter causes contraction of smooth muscle and glomerular mesangial cells in vitro (Schlondorff *et al.*, 1984). In addition, endothelin may also contribute to decrease in RBF and GFR during LPS exposure. This potent vasoconstrictor is released from vascular endothelial cells in vitro, and its circulating concentration is elevated in vivo after exposure to LPS (Morel *et al.*, 1991; Pernow *et al.*, 1989; Sugiura *et al.*, 1989). Endothelin induces contraction of renal blood vessels and glomerular mesangial cells in vitro (Edwards *et al.*, 1990; Badr *et al.,* 1989). In contrast, IL-1 has been associated with increased Na excretion (Beasley *et al.*, 1988), which was induced by prostaglandins, and the production of these prostaglandins were temporally related to alterations in sodium excretion after administration of IL-1 to rats (Hewett and Roth, 1993).

Gastrointestinal Tract: The mechanism involved in the pathogenesis of gastrointestinal injury has not been clearly elucidated. It is suggested (Hewett and Roth, 1993) that, under severe endotoxemia, the effects of LPS are mediated by leukotrienes and PAF. Leukotrienes induce vasoconstriction and promote increased vascular permeability promoting further leakage of endotoxin and hypotension. Platelet Activating Factor contributes to LPS-induced GIT injury because a PAF concentration in the GIT is increased during LPS exposure (Whittle *et al.*, 1987). Platelet Activating Factor may mediate injury to the GIT in part by stimulating production of thromboxane A_2 (Boughton-Smith *et al.*, 1989). In addition to leukotrienes and PAF, cytokines, most notably, TNF-α is also involved in the pathogenesis of gut injury during LPS exposure (Hewett and Roth, 1993). In contrast, at low doses, LPS exposure causes perturbations in intestinal myoeletric activity. These effects were attenuated by either cyclooxygenase inhibition or oxygen radical scavengers (Pons *et al.*, 1991a, 1991b).

Lungs: Alterations in the lungs have been well characterized (Brigham and Meyrick, 1986). It involves two phases. Phase I occurs within the first hour after LPS exposure and is characterized by a marked increase in pulmonary arterial pressure and pulmonary vascular resistance. Phase I is mediated by thromboxane A_2 (Olson *et al.* 1985). In addition to thromboxane, clotting factors are thought to be involved in lung injury as inhibitors of the clotting system attenuated the increase in pulmonary vascular resistance (Hoffman *et al.*, 1990). These early alterations are also accompanied by an increase in vascular permeability that is associated with polymorphonuclear neutrophil (PMN) infiltration, increased plasma protein leakage, and increased flow of protein rich lung lymph (Hewett and Roth, 1993). Polymorphonuclear neutrophils (PMN) may cause

tissue injury by a mechanism that is dependent on oxygen radicals since oxygen metabolite scavengers (catalase and superoxide dismutase) provided protection against lung injury (Milligan *et al.*, 1988; Koyama *et al.*, 1992).

In phase II, the pulmonary arterial pressure returns towards to normal, but still remains elevated. Unlike the GIT, leukotrienes do not appear to mediate the LPS-induced increase in vascular permeability in the lungs of rats (Cook *et al*., 1990; Koyama *et al*., 1992). The second phase of lung injury may be mediated by PAF; however, the mechanism remains to be elucidated (Hewett and Roth, 1993). Experiments have shown that PAF has a number of effects on PMN function in vitro, including priming and induction of reactive oxygen metabolite production, and adherence of PMNs to cell surfaces (Gay, 1990; Garcia *et al.*, 1988; Tonnesen *et al.*, 1989; Entman *et al.*, 1990). It has also been shown that PMN damage cells in vitro under certain circumstances (Entman *et al.*, 1992). However, if there is a relationship between PAF, PMN, and lung injury, it remains to be elucidated (Hewett and Roth, 1993).

In addition, endothelin-1 has also been implicated as a potent vasoconstrictor because an increase in the circulation is seen under severe endotoxemia in certain animals, such as dogs and sheep (Morel *et al*., 1991; Nakamura *et al.*, 1991). However, as for PAF, the mechanisms are yet to be determined (Hewett and Roth, 1993).

Liver: LPS acts indirectly on liver injury via the coagulation system and via mediated effects such as when endotoxin reacts with macrophages and releases several effector substances. In addition, there is evidence that LPS may directly injure hepatocytes as well (Nolan, 1981).

The mechanisms by which the coagulation system mediates liver injury after LPS exposure remains unknown (Hewett and Roth, 1995). However, the authors suggest that thrombin may play an important role on the expression of LPS-induced liver injury along with PMNs and TNF-α.

Exposure to LPS is associated with the accumulation of large numbers of PMNs in the liver (Hewett *et al.*, 1992) and PMNs have been implicated in the pathogenesis of liver injury. In addition, Hewett *et al.* (1993) suggest that TNF-α is involved in liver injury by a PMN dependent mechanism. Also, TNF-α is needed for activation of the coagulation system and is released under LPS exposure. Therefore, Hewett and Roth *et al.*, 1995 suggest that LPS-induced TNF-α enables PMNs to participate in the activation of the coagulation pathway, which in turn results in the generation of a factor (s) proximal in the pathway to fibrin formation that causes liver injury. This factor is probably thrombin.

Mediated effects result when endotoxin reacts with macrophages and releases several effector substances. Some of the mediators which are released by endotoxin-activated macrophages include lysosomal enzymes, procoagulants, collagenase, pyrogens, prostaglandins, colony-stimulating factor, and a tumoricidal substance (Nolan, 1981).

Nolan (1981) reported that LPS may directly injure hepatocytes as well. Either because of a failure of LPS detoxification or through effector release of lysosomal enzymes and other products from Kuppfer cells, the damaging substance reaches the parenchymal cell which possesses membrane receptors for both LPS and the toxic lipid A component. Lysosomes are more fragile in hepatocytes from mice treated with

endotoxin, and LPS stimulates the degradation of cytochrome P-450 enzymes within hours (Nolan, 1981). In addition, dye uptake and excretion is impaired after endotoxin exposure, and bile salt-independent flow is markedly reduced (Nolan, 1981).

- Augmentation of hepatotoxicity by LPS: Several inflammatory cells are stimulated during LPS exposure, e.g. Kupffer cells. Considerable evidences suggest that activation of Kupffer cells amplifies the hepatotoxic response to several chemicals (Roth *et al.*, 1997). Activated Kupffer cells release reactive oxygen metabolites, and pre-treatment of rats with scavengers of reactive oxygen species prevents the enhancement of some hepatotoxic responses (Roth *et al.*, 1997). In addition to release of reactive oxygen species, Kupffer cells when stimulated by LPS, releases the cytokine TNF. Tumor Necrosis Factor can cause oxidative stress in hepatic parenchymal cells *in vitro* and render these cells more susceptible to injury (Roth *et al.*, 1997). The cytokine-induced increase in hepatocellular sensitivity may be associated with damage to DNA (Roth *et al.*, 1997).

Lipid mediators may also have important roles. Leukotriene C_4, an inflammatory lipid released during Lipopolysaccharide exposure, can kill hepatocytes that have been stressed *in vitro* by hypoxia. LPS elicits the release of large amounts of prostaglandin D_2 from Kupffer cells, and this lipid metabolite alters hepatocellular homeostasis. As a result, many products of activated Kupffer cells have actions that could render them important players in augmenting hepatotoxic responses (Roth *et al.*, 1997).

Other cell types activated by LPS exposure include platelets and neutrophils, both of which release mediators of inflammation when they are activated. Neutrophils release not only reactive oxygen species but also serine proteases such as cathepsin G and

elastase that can injure hepatocytes. Neutrophil-derived proteases are important mediators of hepatocellular injury in rats co treated with LPS and galactosamine. Evidence in a model of LPS-induced liver injury suggests that TNF may act in part by activating these proteases or enhancing their release from neutrophils. Another serine protease that is involved critically in LPS-induced liver injury is thrombin arising from activation of the coagulation system. By altering cell membranes, changing intracellular signaling, influencing gene expression, etc., these and perhaps other inflammatory mediators produced during LPS exposure may render target cells more sensitive to injury from chemical insult.

Finally, an in vivo LPS effect on xenobiotic metabolism is to decrease xenobiotic metabolizing capacity which can contribute to augmentation of hepatotoxicity (Roth *et al.*, 1997).

CHAPTER III

EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON PERFORMANCE, MORTALITY RATE, AND ORGAN WEIGHTS OF CHICKS AND POULTS FED AFLATOXIN

ABSTRACT

Aflatoxin B1 (AFB1) causes poor performance and is hepatotoxic in animals. Endotoxic lipopolysaccharide (LPS), a constituent of cell walls of gram negative bacteria, initiates an inflammatory response in animals. In rodents, nontoxic doses of LPS augment the effects of AFB1. Two studies (Exp.1 and 2) were conducted to determine the effects of chronic exposure to low doses of *E. coli* LPS in chicks and poults fed diets contaminated with non toxic doses of aflatoxin B1. Chicks were fed 0, 2, or 4 mg AFB1/kg diet, whereas poults were fed 0, 100, or 150 µg AFB1/kg diet for 21 days. Beginning on day 7, chicks were injected intraperitoneally with 0, 200, or 400 µg LPS/bird every other day, whereas poults were injected with 0, 100, or 150 µg LPS/bird on alternate days. Due to high mortality in the chick study, the 4 mg/kg AFB1 treatment was not considered in the statistical analysis. AFB1 alone decreased ($P < 0.05$) feed intake (FI) and body weight gain (BWG) in chicks, whereas feed conversion (FDGN) was improved when chicks were given 400 µg LPS. In contrast, LPS decreased ($P < 0.05$) BWG and 150 µg LPS/bird worsened ($P < 0.05$) FDGN in poults. AFB1 at 100 µg/kg decreased ($P < 0.05$) FI and BWG in poults and a further decrease ($P < 0.05$) was

observed in BWG when poults were fed 150 µg AFB1/kg. In addition, 150 µg AFB1/kg worsened ($P < 0.05$) FDGN in poults. In poults, there was also an LPS x AFB1 interaction ($P < 0.05$) for FDGN. Co-treatment of 150 µg LPS/poult with 150 µg/kg AFB1 worsened FDGN compared with other combinations of LPS + AFB1. With respect to relative organ weights, LPS increased ($P < 0.05$) thymus weight in chicks, whereas AFB1 increased ($P < 0.05$) liver and spleen weights and decreased ($P < 0.05$) thymus weight. In poults, LPS increased ($P < 0.05$) spleen weight, whereas AFB1 decreased ($P < 0.05$) liver and thymus weights and increased ($P < 0.05$) spleen weight. Mortality in chicks fed the control diet, 2 mg AFB1/kg, and 4 mg AFB1/kg, was 0, 0, and 37%, respectively. In chicks injected with 200 and 400 µg LPS, mortality was 0 and 5%, respectively. Mortality was 35 and 75% in chicks fed 2 and 4 mg AFB1/kg and injected with 200 µg LPS, whereas mortality was 13 and 85% in chicks fed 2 and 4 mg AFB1/kg and injected with 400 µg LPS. Mortality in poults fed the control diet, 100 µg AFB1/kg, and 150 µg AFB1/kg, was 0, 0, and 7%, respectively. In poults injected with 100 and 150 µg LPS, mortality was 7 and 10%, respectively. Mortality was 33 and 30% in poults fed 100 and 150 µg AFB1/kg and injected with 100 µg LPS, whereas mortality was 23 and 53% in poults fed 100 and 150 µg AFB1/kg and injected with 200 µg LPS. Based on these results, it can be concluded that at the levels chosen chronic exposure to low doses of LPS did not potentiate the effects of dietary non toxic doses of AFB1 on performance of chicks and poults fed from hatch to day 21. However, for mortality rate, results suggest a toxic synergy between AFB1 and LPS in chicks and poults.

INTRODUCTION

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminants of grain worldwide. It has been estimated that 25% of the world's food crops are affected by mycotoxins (CAST, 1989). Many of these mycotoxins can cause serious health problems in animals and their presence in agricultural commodities may result in serious economic losses (estimated to be 1.4 billion dollars annually, CAST, 2003). Poultry producers have a continuous problem with low levels of mycotoxin-contaminated feedstuffs causing poorly defined syndromes.

In the past, it was speculated that many of these unexplained syndromes were caused by synergism between mycotoxins. However, years of research have shown little or no synergy but only additive effects. Another potential explanation for the unexplained syndromes observed in poultry may be concomitant exposure to mycotoxins and stressors (e.g. ammonia, competition for feed and water, heat, disease, and endotoxin exposure) present in commercial poultry operations.

Recent reports suggest that, one of these stressors, endotoxic lipopolysaccharides (LPS) a constituent of the outer membranes of the cell walls of gram-negative bacteria may augment the toxic effects of some mycotoxins (Barton *et* al., 2000a, 2000b; Zhou *et* al., 1999). In rats given a non-toxic dose of aflatoxin (AF), intravenous injections of a non-toxic dose of LPS potentiated the effects of AF (four fold) causing hepatic lesions that were pronounced by 24 hours (Barton *et* al., 2000a, 2000b). In studies with mice, the trichothecene, deoxynivalenol (vomitoxin), became more toxic in the presence of LPS causing increased tissue injury and mortality (Zhou *et* al., 1999). Results of a recent

study comparing nine different occupational settings for personal exposure to endotoxins indicates that the highest exposure occurred in poultry operations (Simpson *et* al., 1999). These results suggest poultry are exposed to high levels of LPS, and exposure may well potentiate the effects of low levels of mycotoxins that would normally be considered non-toxic. The objective of these studies were to determine if LPS will potentiate the effects of AFB1 in broilers and turkeys fed dietary AFB1 from hatch to 21 days of age.

MATERIAL AND METHODS

For these trials, 180 day-old broilers (Exp.1) and 270 day-old turkeys (Exp.2) were obtained from a commercial hatchery, weighed, wing-banded, and randomly assigned to pens in stainless steel chick batteries. Birds were maintained on a 24-h light schedule and allowed *ad libitum* access to feed and water. Birds were monitored daily for signs of morbidity and mortality. Post-mortem evaluation was performed for all birds that died throughout the experiment. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

A 3 x 3 factorial design (three levels of LPS and three levels of AFB1) design was used with four replicates of five birds (chicks) or five replicates of six birds (poults) assigned to each of the nine treatments from day 1 to 21. Treatments included: (A) 0 µg LPS/bird + 0 mg/kg AFB1; (B) 0 µg LPS/bird + 2 mg/kg AFB1 (chicks) or 100 µg/kg AFB1 (poults); (C) 0 µg LPS/bird + 4 mg/kg AFB1 (chicks) or 150 µg/kg AFB1 (poults); (D) 200 µg LPS/bird + 0 mg/kg AFB1 (chicks) or 100 µg LPS/bird + 0 µg/kg AFB1 (poults); (E) 200 µg LPS/bird + 2 mg/kg AFB1 (chicks) or 100 µg LPS/bird + 100 µg/kg

AFB1 (poults); (F) 200 LPS/bird + 4 mg/kg AFB1 (chicks) or 100 µg LPS/bird + 150 µg/kg AFB1 (poults); (G) 400 µg LPS/bird + 0 mg/kg of AB1 (chicks) or 150 µg LPS/bird + 150 µg/kg AFB1 (poults); (H) 400 µg LPS/bird + 2 mg/kg AFB1 (chicks) or 150 µg LPS/bird + 150 µg/kg AFB1 (poults); and (I) 400 µg LPS/bird + 4 mg/kg of AFB1 (chicks) or 150 µg LPS/bird + 150 µg/kg AFB1 (poults).

Lipopolysaccharide (*E. coli* LPS; serotype 0127:B8) was purchased from Sigma Chemical (St. Louis, MO) and reconstituted in sterile and non-pyrogenic saline for injection. The solution was then heated under low heat for approximately 5 minutes or until complete dissolution. All glassware were made endotoxin-free by dry heating to 250°C for at least 3 hours. Chicks and poults were injected intraperitoneally with the appropriate dose of LPS every other day from day 7 to 21. Chicks that did not receive LPS, were injected with sterile saline. Dietary treatments were fed beginning day 1. Chicks and poults were injected intraperitoneally (IP) with the appropriate dose of LPS every other day from day 7 to 21.

At 3 weeks of age (day 21), birds were euthanized with carbon dioxide and samples of liver, spleen, bursa, and thymus (three birds/pen) were collected for histopathologic evaluation as well as relative organ weight calculation (grams organ/100 g body weight).

Due to high mortality in groups fed 4 mg/kg AFB1, this treatment was not included in the statistical analysis in the chick study. Data were analyzed as a 3 x 2 factorial analysis (chicks) or a 3 x 3 factorial analysis (poults) by analysis of variance (ANOVA) using the General Linear Models procedure of SAS software (SAS Institute Inc., 1996). Means showing significant differences in the ANOVA were compared using

Fisher's protected least significant difference procedure (Snedecor and Cochran, 1989). Mortality data were transformed (Arcsin) prior to statistical analysis. All statements of differences were based on a significance of $P < 0.05$.

RESULTS

Experiment 1: Broiler Study: Due to high mortality in the chick study, the 4 mg/kg AFB1 treatment was not included in the statistical analysis of the performance data. AFB1 alone decreased ($P < 0.05$) feed intake (FI) and body weight gain (BWG) in chicks, whereas feed conversion (FDGN) was improved when chicks were given 400 µg LPS (Table 3.1). Figure 3.3 shows treatment effects on final body weight of surviving broiler chicks fed 4 mg/kg AFB1. With respect to relative organ weights (Tables 3.3, 3.4), LPS increased ($P < 0.05$) thymus weight, whereas AFB1 increased ($P < 0.05$) liver and spleen weights and decreased ($P < 0.05$) thymus weight in chicks (Table 3.3). Mortality in chicks fed the control diet, 2 mg AFB1/kg, or 4 mg AFB1/kg, was 0, 0, and 37%, respectively (Figure 3.1). In chicks injected with 200 and 400 µg LPS, mortality was 0 and 5%, respectively. Mortality was 35 and 75% in chicks fed 2 and 4 mg AFB1/kg and injected with 200 µg LPS, whereas mortality was 13 and 85% in chicks fed 2 and 4 mg AFB1/kg and injected with 400 µg LPS.

Experiment 2: Poult Study: In contrast to chicks, LPS decreased ($P < 0.05$) BWG and 150 µg AFB1/kg worsened ($P < 0.05$) FDGN in poults (Table 3.2). AFB1 at 100 µg/kg decreased ($P < 0.05$) FI and BWG in poults and a further decrease ($P < 0.05$) was observed in BWG when poults were fed 150 µg AFB1/kg. In addition, 150 µg

AFB1/kg worsened ($P < 0.05$) FDGN in poults. In poults, there was also an LPS x AFB1 interaction ($P < 0.05$) on FDGN. Co-treatment of 150 µg LPS/poult with 150 µg/kg AFB1 worsened FDGN compared with other combinations of LPS + AFB1 (Table 3.2). In poults, LPS increased ($P < 0.05$) spleen weight, whereas AFB1 decreased ($P < .05$) liver and increased ($P < .05$) spleen weight (Table 3.4). Mortality in poults fed the control diet, 100 µg AFB1/kg, or 150 µg AFB1/kg, was 0, 0, and 7%, respectively (Figure 3.2). In poults injected with 100 and 150 µg LPS, mortality was 7 and 10%, respectively. Mortality was 33 and 30% in poults fed 100 and 150 µg AFB1/kg and injected with 100 µg LPS, whereas mortality was 23 and 53% in poults fed 100 and 150 µg AFB1/kg and injected with 150 µg LPS.

DISCUSSION

Due to high mortality in the chick study, the 4 mg/kg AFB1 treatment was not included in the statistical analysis of the performance data. Two mg/kg AFB1 alone decreased FI and BWG in chicks. Huff *et al.* (1988) showed that feeding 2.5 mg/kg AFB1 to broiler chickens caused a decrease in body weight and a further decrease was observed when birds were co-treated with AFB1 and 4 mg/kg T-2. Verma *et al.* (2002) reported that AFB1 at 3 mg/kg AFB1 had no effect on body weight, serum protein and serum albumin, but increased the relative weights of liver, kidney, and proventriculus. Pimpukdee *et al.* (2004) reported that 5 mg/kg AFB1 decreased performance of 3-week-old chickens.

Lipopolysaccharide at 400 µg/bird improved FDGN in chicks, whereas in poults LPS decreased BWG and 150 µg AFB1/kg worsened FDGN. In addition, there was an LPS x AFB1 interaction on FDGN in poults. The result of the chick study is not in agreement with the literature. In contrast, the result of the poult study is consistent with the reports found in the literature. Because of the diversion of nutrients away from growth in support of immune-related processes, immune challenge is considered a major obstacle to animals' achieving their genetic potential for growth or efficiency of gain (Spurlock, 1997). In addition, during periods of immune challenge, proinflammatory cytokines orchestrate a homeorhetic response in which nutrients are directed away from tissue growth in support of immune function (Spurlock, 1997). Even when disease is subclinical in nature, the cumulative effect of the serial challenges is sufficient to cause significant alterations in metabolism with concurrent losses in performance (Spurlock, 1997). Koh *et al.* (1996) injected broiler chicks 100 µg/ml of *S. typhymurium* LPS on alternate days for 6 days and reported that activation of the acute phase reponse significantly decreased daily BWG, FI, and FDGN. Shimizu *et al.* (1988) immunized chickens with whole cells of *E.coli* and observed elevated levels of anti-LPS activity, suggesting that LPS is a potent antigen in chickens. In addition, a single injection of *S. typhimurium* in 3 week old male broiler chickens decreased body weight 12, 24, and 48 h after LPS challenge (Xie *et al.*, 2000).

Aflatoxin B1 at 100 µg/kg decreased FI and BWG in poults and a further decrease was observed in BWG when poults were fed 150 µg AFB1/kg. In addition, AFB1 worsened ($P < 0.05$) FDGN in poults. In poults, these results are not in agreement with the literature. Witlock and Wyatt (1981) reported that body weight gains of turkey poults

were only affected when 500 µg/kg AFB1 was fed. However, Witlock *et al.* (1981) reported decreased body weights when turkey poults were fed 250 µg/kg AFB1 and a further decrease was observed in poults fed AFB1 and challenged with *Eimeria adenoeides*. Giambrone *et al.* (1985a) reported no significant adverse effects on weight gain or feed conversion in turkey poults fed either 100, 150, or 200 µg/kg AFB1 for 5 weeks. In addition, Giambrone *et al.* (1985b) fed turkey poults 0, 100, 200, 400, or 800 µg/kg AFB1 for 5 weeks and reported that levels greater than or equal to 400 µg/kg AFB1 were highly toxic to turkey poults. This toxicity was characterized by signs and lesions of aflatoxicosis as well as significant decreases in BWG and FDGN during 5 weeks. In addition, microscopic lesions, indicative of aflatoxicosis, were evident as low as 100 µg/kg AFB1, and significant decreases in cell-mediated immunity were noted in the 200 µg/kg AFB1 (Giambrone *et al.*, 1985b).

In this study, the means chosen to administer LPS may have been too invasive which may explain the increased susceptibility of the birds to levels of toxins otherwise considered non toxic. From all the birds that died throughout the experimental period, approximately 50% of the broilers and 15% of the turkeys, independent of treatment, had signs of septicemia with pericarditis, perihepatitis, and retained yolk sack. Interactions demonstrated between mycotoxins presence in the feed and infections, which manifests itself as increased mortality, has important implications for the poultry industry. Prabaharan and George (1999) fed 0.5 and 1.0 mg/kg AFB1 to *Eimeria tenella* infected birds. The authors reported that mortality rate in the combined groups were higher than in the groups receiving either of the treatments alone. Singh *et al.* (1999) reported mortality rate of approximately 50% in 3 week old chicks fed 800 µg/kg AFB1 and

infected with *Salmonella gallinarum*. The authors reported that this increased mortality could be attributed to congestion and enlargement of liver due to aflatoxins and degeneration of liver due to *Salmonella* infection. Boonchuvit *et al.* (1975) reported chickens fed 16 mg/kg T-2 toxin and infected with *Salmonella* had mortality rate increased by 17.5% when compared to either factor alone.

With respect to relative organ weights, AFB1 given alone increased spleen weights in chicks and poults. In addition, AFB1 decreased relative thymus weight in chicks. Also, LPS increased spleen weight in chicks and poults and increased thymus weight in chicks. However, treatments had no effect on relative bursa weight in both species. Hegazy and Adachi (2000) reported that feeding 2.6 mg/kg AFB1 to chicks decreased relative bursal and thymus weights, but spleen weight was increased. The decrease in bursa and thymic weights represents the immunossupressive effect of the treatments. However, the increase in spleen weight observed may be related to the health status of the birds. Chang and Hamilton (1982) found a significant increase in relative spleen weights in chicks with infectious bursal disease virus, which may be enhanced by synergism with aflatoxicosis. In the present study, birds that died during the experimental period were grossly necropsied. From all the birds that died, approximately 50% of the chicks and 15% of the poults, independent of treatment, had signs of septicemia with pericarditis, perihepatitis, and retained yolk sack.

In poults, AFB1 decreased liver, whereas in chicks, AFB1 increased relative liver weight. In chicks, this result is in agreement with the literature. Aflatoxin B1 causes fatty liver in poultry (Hamilton and Garlich, 1971; Carnaghan *et al.*, 1966; Sawhney *et al.*, 1973) leading to increased liver weight due to fat deposition in the liver. In poults,

AFB1 decreased liver weight. Bradley (1985) proposed that inhibition of protein synthesis impairs the ability of the animal to repair cellular damage leading to decreased relative organ weights.

A prominent feature of septic shock is a severe and intractable hypotension coupled with a primary reduction in circulating blood volume, which leads ultimately to circulatory collapse and death (Andrew *et al.*, 2000). Co-treatment with LPS and AFB1 markedly increased mortality rate in broilers compared with the LPS or AFB1 alone. Taylor *et al*. (1991) observed a similar response in mice exposed to LPS and T-2 toxin, with the combination treatment causing 100% mortality, compared with 20% for T-2 alone, and no mortality for LPS alone. Taylor *et al*. (1991) concluded that increased LPS absorption accounted for the increase in mortality.

AFB1 may well have enhanced the effect of LPS in broilers and turkeys in the current study. However, the mechanism of enhancement was probably different from that observed with T-2 toxin. AFB1 is hepatotoxic and damage to the liver caused by AFB1 may well have compromised the ability of the liver to metabolize LPS, resulting in reduced clearance of LPS from circulation, and increased mortality due to hypotension and circulatory collapse.

CONCLUSION

Based on these results, it can be concluded that at the levels chosen, chronic exposure to low doses of LPS did not enhance the effects of dietary non toxic doses of AFB1 on performance of chicks and poults fed from hatch to day 21. However, for mortality rate, results suggest a toxic synergy between AFB1 and LPS in both chicks and poults.

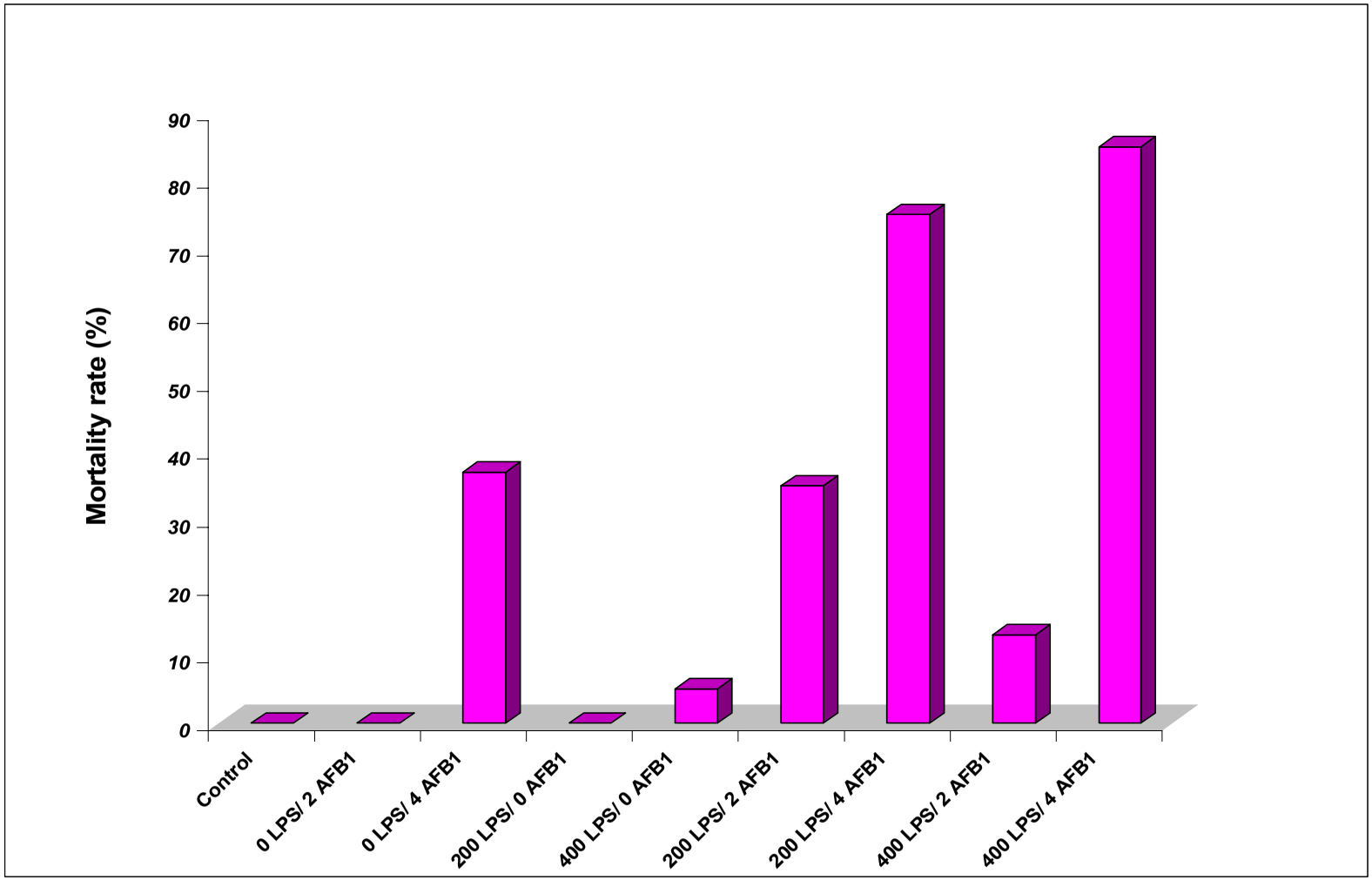


Figure 3.1. Effect of endotoxic lipopolysaccharide on mortality rate of broiler chicks fed Aflatoxin B1

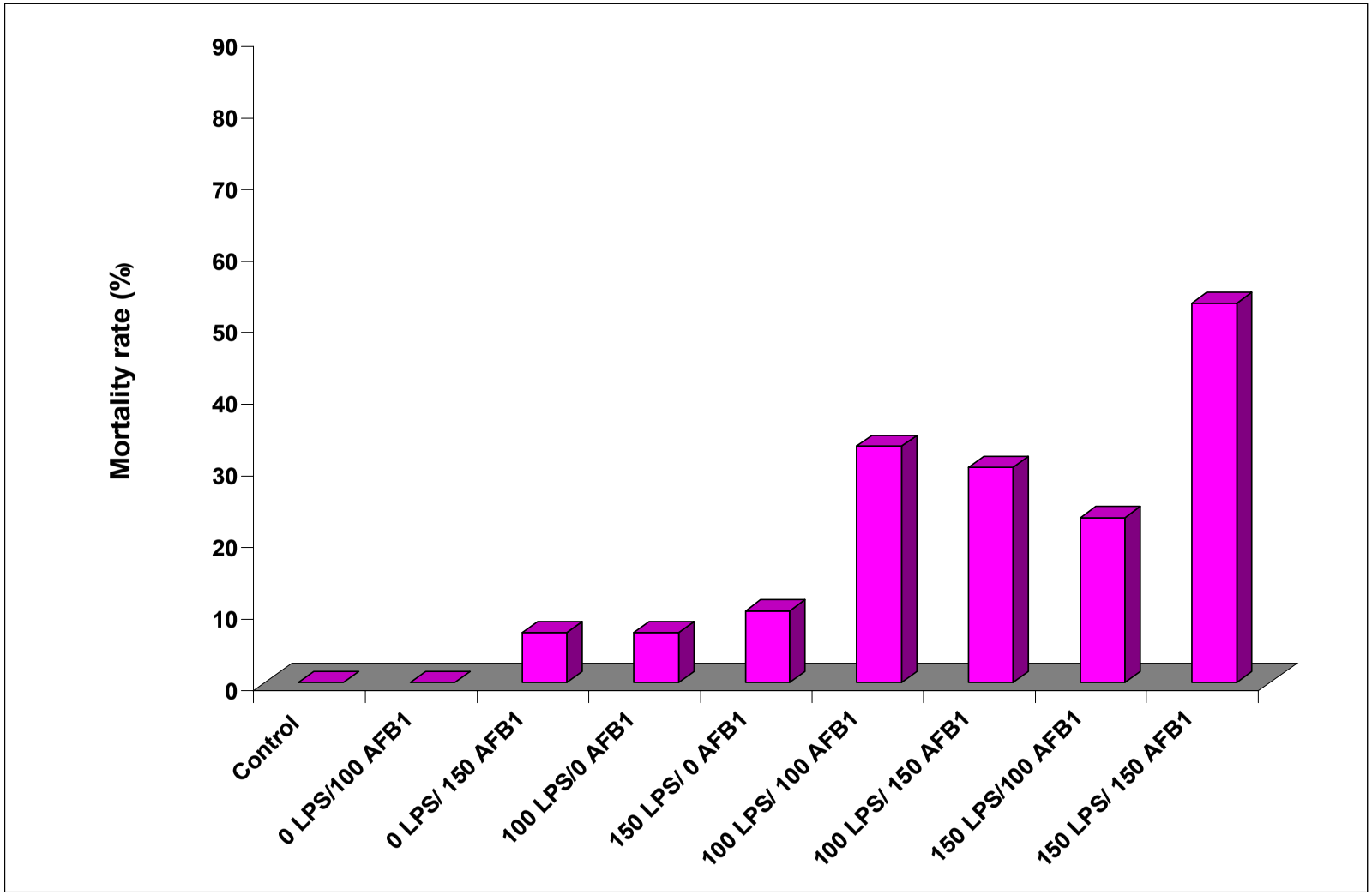


Figure 3.2. Effect of endotoxic lipopolysaccharide on mortality rate of turkey poults fed Aflatoxin B1

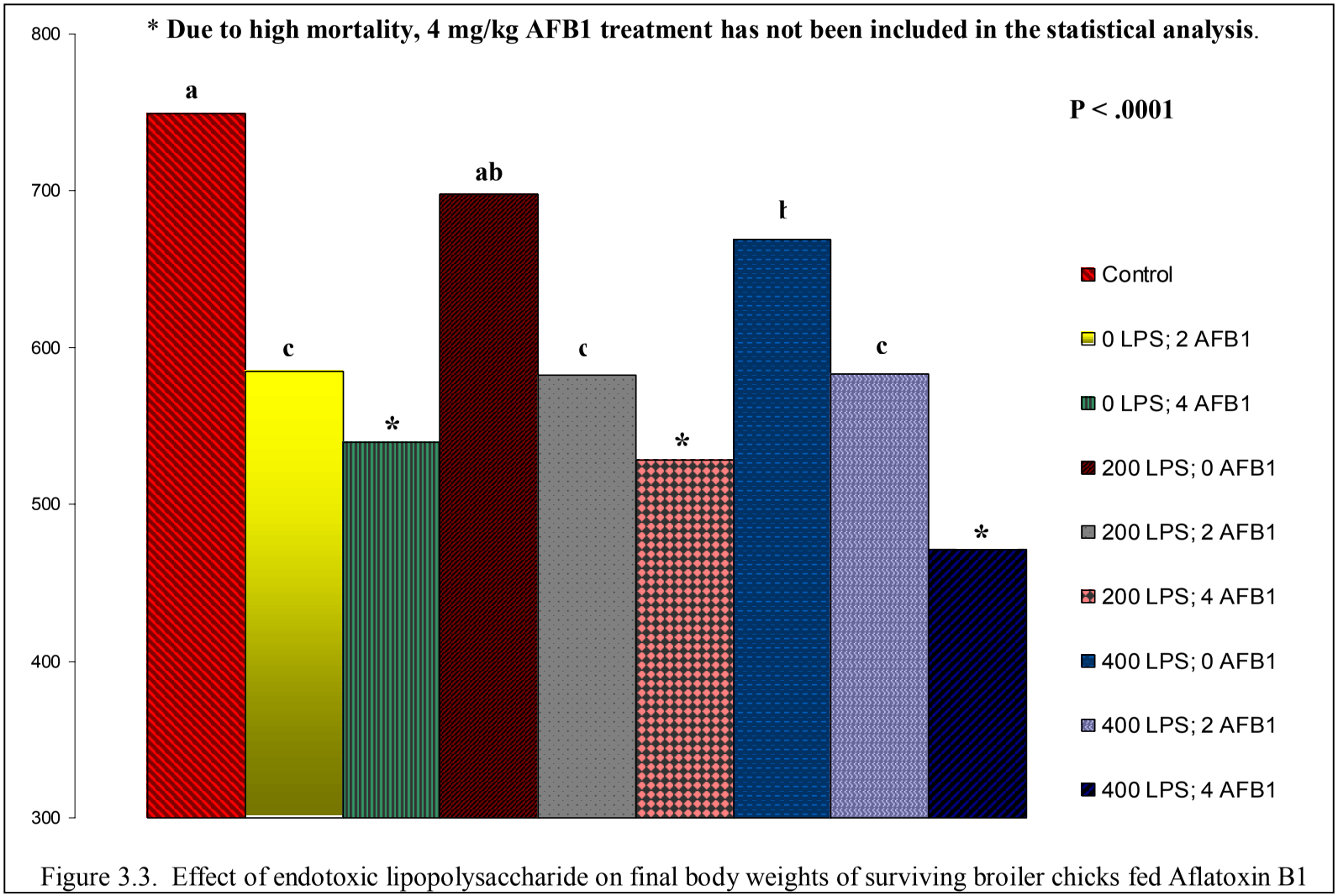


Figure 3.3. Effect of endotoxic lipopolysaccharide on final body weights of surviving broiler chicks fed Aflatoxin B1

TABLE 3.1. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON 3 WEEK PERFORMANCE OF BROILER CHICKS FED AFLATOXIN B1[1,2]

LPS (µg/bird)	AFB1 (ppm)	Feed Intake (g)	Body Weight Gain (g)	Feed Conversion (g:g)
0	0	1047[a]	742[a]	1.414[ab]
0	2	858[ab]	578[b]	1.482[a]
200	0	1013[ab]	688[ab]	1.476[a]
200	2	804[b]	580[b]	1.342[ab]
400	0	878[ab]	667[ab]	1.303[ab]
400	2	722[b]	576[b]	1.252[b]
SEM		70.3	33.3	0.06
		Probability		
LPS		0.1561	0.5358	0.0490
AFB1		0.0111	0.0007	0.4843
LPS X AFB1		0.9430	0.5499	0.3477

[1] Data are means of four replicates of five birds each.

[2] Because of excessive mortality, 4 mg/kg AFB1 treatment has not been included in the statistical analysis.

[a, b] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 3.1: EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON 3 WEEK PERFORMANCE OF BROILER CHICKS FED AFLATOXIN B1[1,2]

Main Effect Mean	Feed Intake (g)	Body Weight Gain (g)	Feed Conversion (g:g)
LPS, µg/bird			
0	952	660	1.448[a]
200	908	634	1.409[a]
400	800	621	1.277[b]
AFB1, mg/kg			
0	980[a]	699[a]	1.398
2	794[b]	578[b]	1.359

[1] Data are main effect means.

[2] Because of excessive mortality, 4 mg/kg AFB1 treatment has not been included in the statistical analysis.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 3.2. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON 3 WEEK PERFORMANCE OF TURKEY POULTS FED AFLATOXIN B1[1]

LPS (µg/bird)	AFB1 (ppb)	Feed Intake (g)	Body Weight Gain (g)	Feed Conversion (g:g)
0	0	792[ab]	435[a]	1.825[b]
0	100	733[ab]	406[ab]	1.806[b]
0	150	721[ab]	385[ab]	1.869[b]
100	0	736[ab]	387[ab]	1.909[b]
100	100	693[b]	346[b]	2.028[b]
100	150	647[b]	300[bc]	2.054[b]
150	0	812[a]	398[ab]	2.068[b]
150	100	725[ab]	311[bc]	1.875[b]
150	150	692[b]	252[c]	2.768[a]
SEM		36.58	24.94	0.102
		Probability		
LPS		0.1262	0.0004	0.0002
AFB1		0.0112	0.0002	0.0008
LPS X AFB1		0.9479	0.4278	0.0011

[1] Data are means of five replicates of six birds each.

[a, b,c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 3.2 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON 3 WEEK PERFORMANCE OF TURKEY POULTS FED AFLATOXIN B1[1]

Main Effect Mean	Feed Intake	Body Weight Gain	Feed Conversion
	----------(g)----------		-----(g:g)----
LPS, µg/bird			
0	749	409[a]	1.833[b]
100	692	344[b]	1.997[b]
150	743	322[b]	2.237[a]
AFB1, µg/kg			
0	780[a]	407[a]	1.934[b]
100	717[b]	354[b]	1.903[b]
150	686[b]	312[c]	2.230[a]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 3.3. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF BROILER CHICKS FED AFLATOXIN B1[1,2]

LPS (µg/bird)	**AFB1 (ppm)**	**LIVER**	**SPLEEN**	**BURSA**	**THYMUS**
		----------(%)----------			
0	0	2.814[b]	0.829[b]	0.233[a]	0.311[ab]
0	2	4.757[a]	1.324[ab]	0.225[a]	0.238[c]
200	0	2.913[b]	0.948[b]	0.246[a]	0.369[a]
200	2	4.116[ab]	1.187[ab]	0.240[a]	0.303[b]
400	0	3.532[b]	0.945[b]	0.234[a]	0.301[b]
400	2	4.468[a]	1.489[a]	0.226[a]	0.303[b]
SEM		0.28	0.142	0.016	0.021
		----------Probability----------			
LPS		0.2533	0.5129	0.6161	0.0492
AFB1		<.0001	0.0018	0.5806	0.0134
LPS X AFB1		0.2097	0.5277	0.9967	0.1728

[1] Data are means of 4 replicates of 3 birds each.

[2] Because of excessive mortality, 4 mg/kg AFB1 treatment has not been included.

[a, b, c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 3.3 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF BROILER CHICKS FED AFLATOXIN B1[1,2]

Main Effect Mean	LIVER	SPLEEN	BURSA	THYMUS
	----------(%)----------			
LPS, µg/bird				
0	3.785	1.076	0.229	0.275[b]
200	3.515	1.068	0.243	0.336[a]
400	4.000	1.217	0.230	0.302[a]
AFB1, mg/kg				
0	3.086[b]	0.907[b]	0.238[a]	0.327[a]
2	4.447[a]	1.333[a]	0.231[a]	0.281[b]

[1] Data are main effect means.

[2] Because of excessive mortality, 4 mg/kg AFB1 treatment has not been included.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 3.4. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF TURKEY POULTS FED AFLATOXIN B1[1]

LPS (µg/bird)	AFB1 (ppb)	LIVER	SPLEEN	BURSA	THYMUS
		(%)			
0	0	3.048[a]	0.100[c]	0.153	0.128[ab]
0	100	2.586[b]	0.116[b]	0.156	0.099[bc]
0	150	2.340[c]	0.117[b]	0.170	0.051[c]
100	0	3.230[a]	0.121[ab]	0.155	0.143[ab]
100	100	2.567[bc]	0.135[a]	0.166	0.101[bc]
100	150	2.472[bc]	0.123[ab]	0.148	0.075[c]
150	0	3.127[a]	0.118[b]	0.150	0.157[a]
150	100	2.590[b]	0.118[b]	0.149	0.120[b]
150	150	2.490[bc]	0.135[a]	0.160	0.058[c]
SEM		0.086	0.005	0.008	0.014
		Probability			
LPS		0.3450	0.0024	0.5999	0.2610
AFB1		<.0001	0.0191	0.6136	<.0001
LPS X AFB1		0.7456	0.0896	0.3040	0.7707

[1] Data are means of 4 replicates of 3 birds each.

[a, b, c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 3.4 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF TURKEY POULTS FED AFLATOXIN B1[1]

Main Effect Mean	LIVER	SPLEEN	BURSA	THYMUS
	----------(%)----------			
LPS, µg/bird				
0	2.658	0.111[b]	0.160	0.093
100	2.756	0.126[a]	0.156	0.107
150	2.735	0.123[a]	0.153	0.112
AFB1, µg/kg				
0	3.135[a]	0.113[b]	0.153	0.143[a]
100	2.581[b]	0.123[a]	0.157	0.107[b]
150	2.434[b]	0.125[a]	0.159	0.062[c]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

CHAPTER IV

EFFECTS OF CONTINUOUS ADMINISTRATION OF LOW-DOSE OF ENDOTOXIC LIPOPOLYSACCHARIDE IN CHICKS AND POULTS FED NON TOXIC DOSES OF T-2 TOXIN

ABSTRACT

Two studies were conducted to determine the effects of chronic exposure to low doses of *E. coli* LPS in combination with feeding diets contaminated with non toxic doses of T-2 toxin in chicks and poults fed from hatch to day 21. Chicks and poults were fed 0, 1, or 2 mg T-2/kg diet. Beginning on day 7, chicks and poults were injected intraperitoneally with 0, 100, or 150 µg LPS/bird every other day. At week two, 150 µg LPS/bird decreased ($P < 0.05$) feed intake (FI) and body weight gain (BWG) in chicks, but no T-2 effect or LPS x T-2 interaction was observed . The mean values were 343, 337, and 318g for FI; 277, 275, and 256 g for BWG for chicks given 0, 100, and 150 µg LPS/bird, respectively. In poults, at week two, birds fed 2 mg T-2 toxin/kg diet showed a decrease ($P < 0.05$) in FI and BWG, but no LPS effect or LPS x T-2 interaction was observed. The mean values were 244, 233, and 209 g for FI; 158, 145, and 126 g for BWG for poults fed 0, 1, and 2 mg/kg T-2, respectively. In chicks, at week three, there was a significant ($P < 0.05$) LPS x T-2 interaction observed for BWG. In poults, at week three, 2 mg/kg T-2 dramatically decreased ($P < 0.05$) FI, BWG, and increased FDGN. The mean values were 394, 383, and 324 g for FI; 198, 194, and 136 g for BWG; 2.05,

1.92, and 2.30 for FDGN for poults fed 0, 1, and 2 mg/kg of T-2, respectively. No LPS effect and no LPS x T-2 interactions were observed. With respect to oral lesions, chicks fed 2 mg/kg T-2 had a higher incidence of oral lesions when compared to chicks fed 1 mg/kg T-2 (2 mg/kg T-2, 18%; 1 mg/kg T-2, 10%), but there was no difference in terms of severity of lesions. At both levels, T-2 caused only mild lesions in chicks. Poults fed 2 mg/kg T-2 had a higher incidence (2 mg/kg T-2, 48%; 1 mg/kg T-2, 30%) and more severe oral lesions (2 mg/kg T-2, scored 1: 40%, scored between 2 and 3; 60%; 1 mg/kg T-2: scored 1, 56%; scored 2, 44%) than birds fed 1 mg/kg T-2. Treatments had no effect on mortality rate of either species. LPS increased relative spleen weight ($P < .05$) in chicks and poults. There was no T-2 effect or LPS x T-2 interaction on relative organ weight in chicks or poults. Based on these results, it can be concluded that, at the levels chosen, chronic exposure to LPS did not potentiate the effects of T-2 toxin in chicks and poults. In poults, however, the decrease in performance observed in birds fed T-2 alone was atypical of a number of studies conducted, in which decrease in performance was only observed when poults were fed at least 5 mg/kg T-2.

INTRODUCTION

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminants of grain worldwide. It has been estimated that 25% of the world's food crops are affected by mycotoxins (CAST, 1989). Many of these mycotoxins can cause serious health problems in animals and their presence in agricultural commodities may result in serious economic losses (estimated to be 1.4 billion dollars annually,

CAST, 2003). Poultry producers have a continuous problem with low levels of mycotoxin-contaminated feedstuffs causing poorly defined syndromes.

In the past, it was speculated that many of these unexplained syndromes were caused by synergism between mycotoxins. However, years of research have shown little or no synergy but only additive effects. Another potential explanation for the unexplained syndromes observed in poultry may be concomitant exposure to mycotoxins and stressors (e.g. ammonia, competition for feed and water, heat, disease, and endotoxin exposure) present in commercial poultry operations.

Recent reports suggest that, one of these stressors, endotoxic lipopolysaccharides (LPS) may augment the toxic effects of some mycotoxins (Barton *et* al., 2000a, 2000b; Zhou *et* al., 1999, 2000; Tai and Pestka, 1988). Lipopolysaccharide is a component of the outer membrane of the cell walls of gram-negative bacteria. Examples of gram-negative bacteria include *Escherichia coli* and *Salmonella typhymurium*. Lipopolysaccharide has been extensively studied as an inflammagen and a major contributing factor to the pathogenesis of bacterial infections. Exposure to LPS causes fever, circulatory shock, disseminated intravascular coagulation, and damage to numerous organs, including the liver (Barton *et al.*, 2001).

With respect to performance, exposure to LPS causes decrease in feed intake, which, in heifers, lasted for 4 hours (Steiger *et al.*, 1999) and in pigs, 48 hours (Frank, 2003) after LPS exposure before returning to control levels. During an immunological challenge, animals undergo a series of physiological events known as the acute-phase response which is designed to help the animal to overcome the challenge (Baumann and Gauldie, 1994). One resulting component of the acute-phase response is suppression in

feed intake (Kelley *et al.*, 1994; Johnson, 1998). Frank (2003) suggested that elevation in proinflammatory cytokines, e.g. TNF-α and IL-2, lead to an acute suppression in feed intake. Warren *et al.* (1997) infused porcine TNF-α i.c.v. and reported dose-dependent reductions in feed intake over an 8-h period.

Exposure to smaller doses of LPS initiates a more modest and noninjurious inflammatory response (Barton *et al.*, 2001). However, exposure to such doses can render the liver more sensitive to injury from xenobiotic agents, such as AFB_1 and T-2 toxin (Roth *et al.*, 1997). In addition to modifying the hepatotoxic responses of xenobiotic agents, it is possible that hepatotoxicity from some agents arises directly from primary damage to the GI tract which increases the translocation of LPS into the portal venous circulation (Roth *et al.*, 1997). The T-2 toxin damages the GI tract (Bratich *et al.*, 1990) increasing LPS translocation from the GI tract and causing liver lesion which resemble that produced by large, injurious doses of LPS (Roth *et al.*, 1997; Taylor *et al.*, 1991).

Animal exposure to bacterial endotoxin occurs through several mechanisms (Roth *et al.*, 1997). However, the two most important types of exposure are exposure to high concentrations of LPS via the respiratory tract from contaminated air, and gram-negative bacterial infections that lead to systemic LPS exposure. Moreover, results of a recent study comparing nine different occupational settings for personal exposure to endotoxins indicates that the highest exposure occurred in poultry operations (Simpson *et* al., 1999). These results suggest poultry are exposed to high levels of LPS via contaminated air, and exposure may well potentiate the effects of low levels of mycotoxins that would normally be considered non-toxic.

The T-2 toxin (T-2) is a mycotoxin produced by the genera *Fusarium* and is a common contaminant of poultry feeds and feedstuffs and its adverse effects have been extensively studied (Leeson *et al.*, 1995). In poultry, exposure to T-2 induces gizzard erosions and oral lesions (Kubena *et al.*, 1997, 1995; Neiger *et al.*, 1994), and causes reduced body weight gain and feed intake. The T-2 toxin is also a potent immunosuppressant (Moss, 2002). Necrosis and depletion of the lymphoid organs has been reported in T-2 treated chickens and turkey poults (Wyatt *et al.*, 1973; Boonchuvit *et al.*, 1975; Richard *et al.*, 1978a, 1978b; Hoerr *et al.*, 1981).

The immunosuppressive effects of T-2 on both cellular and antibody-mediated immunity decreases host resistance to infectious agents. Mortality caused by paratyphoid infections was reported to be increased in chickens exposed to T-2 toxin (Boonchuvit *et al.*, 1975; Ziprin and Elissalde, 1990). In 1988, Tai and Pestka suggested a synergistic interaction between T-2 and *S. typhimurium* LPS in rats. The authors found that a single sublethal oral dose of T-2 increased the acute toxicity of LPS and the LD_{50} values of LPS decreased upon co-administration of the toxin (Tai and Pestka, 1988). In addition, combined treatment with subthreshold doses of *S. typhimurium* LPS and the tricothecene deoxynivalenol (vomitoxin) caused apoptosis in thymus, spleen, and Peyer's patches after a 12-h exposure (Zhou *et al.*, 2000). Furthermore, in mice, acute, simultaneous exposure to T-2 and endotoxin resulted in increased mortality, hypothermia, TNF-α production, and thymic atrophy compared to treatment with either T-2 or endotoxin alone. However, when animals were pretreated with endotoxin, a regime that renders the animals resistant to the effects of endotoxin, there was a reduction in the response to coadmnistration of T-2 and endotoxin (Taylor *et al.*, 1991).

Limited information is available on the chronic effects of a sublethal dose of LPS in combination with feeding subthreshold doses of T-2 toxin in chicks and poults. The objectives of these studies were to determine if administration of low doses of *E. coli* LPS would potentiate the effects of non toxic doses of T-2 in chicks and poults fed dietary T-2 from hatch to 21 days of age.

MATERIAL AND METHODS

For these trials, 270 day-old broilers (Exp.1) and turkeys (Exp.2) were obtained from a commercial hatchery, weighed, wing-banded, and randomly assigned to pens in stainless steel chick batteries. Birds were maintained on a 24-h light schedule and allowed *ad libitum* access to feed and water. Birds were monitored daily for signs of morbidity and mortality. Post-mortem evaluation was performed for all birds that died throughout the experiment. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

For both experiments, a 3 x 3 factorial (three levels of T-2 and three levels of LPS) design was used with five replicates of six birds assigned randomly to each of the nine treatments from day 1 to day 21. Treatments included: (A) 0 µg LPS/bird + 0 mg/kg T-2; (B) 0 µg LPS/bird + 1 mg/kg T-2; (C) 0 µg LPS/bird + 2 mg/kg T-2; (D) 100 µg LPS/bird + 0 mg/kg T-2; (E) 100 µg LPS/bird + 1 mg/kg T-2; (F) 100 µg LPS/bird + 2 mg/kg T-2; (G) 150 µg LPS/bird + 0 mg/kg T-2; (H) 150 µg LPS/bird + 1 mg/kg T-2; and (I) 150 µg LPS/bird + 2 mg/kg T-2.

Lipopolyssacharide (*E. coli* LPS; serotype 0127:B8) was purchased from Sigma Chemical (St. Louis, MO) and reconstituted in sterile and non-pyrogenic saline for injection. The solution was then heated under low heat for approximately 5 minutes or until complete dissolution. All glassware was made endotoxin-free by dry heating to 250°C for at least 3 hours. Chicks and poults were injected intraperitoneally (IP) with 0.5 ml of sterile saline containing the appropriate quantity of LPS. Birds were injected intraperitoneally (IP) with the appropriate dose of LPS every other day from day 7 to 21. Chicks and poults that did not receive LPS, were injected with sterile saline alone. Dietary treatments were fed beginning day 1.

Birds were weighed weekly. At 3 weeks of age (day 21), birds were then euthanized with carbon dioxide and samples of spleen and bursa (three birds/pen) (chicks) or splee, bursa, and thymus (three birds/pen) (poults) were collected for histopathologic evaluation as well as relative organ weight calculation (grams organ/100 g body weight).

Oral lesions were scored in a blind fashion using a 4-point scoring system, ranging from 0 to 4, with 0 = no lesion; 1 = one or two mouth lesions clearly visible on either the lower or upper mandible; 2 = lesions intermediate in appearance to lesions scored as 1 or 3; 3 = large lesions occurring at several sites within the mouth; 4 = large lesions occurring at several sites within the mouth, principally on the upper and lower mandible, the corners of the mouth, and the back of the tongue.

Data were analyzed as a 3 x 3 factorial analysis by analysis of variance (ANOVA) using the General Linear Models procedure of SAS software (SAS Institute Inc., 1996). Means showing significant differences in ANOVA were compared using Fisher's

protected least significant difference procedure (Snedecor and Cochran, 1989). Mortality data were transformed (Arcsin) prior to statistical analysis. All statements of differences were based on a significance of $P < 0.05$.

RESULTS

Experiment 1: Broiler Study: At week two, 150 μg LPS/bird decreased ($P < 0.05$) feed intake (FI) and body weight gain (BWG) in chicks, but no T-2 effect or LPS x T-2 interactions were observed (Table 4.1). The mean values were 343, 337, and 318 g for FI; 277, 275, and 256 g for BWG for chicks given 0, 100, and 150 μg LPS/bird, respectively. In chicks, at week three, there was a significant ($P < 0.05$) LPS x T-2 interaction observed for BWG (Table 4.1). With respect to oral lesions, chicks fed 2 mg/kg T-2 had a higher incidence of oral lesions when compared to chicks fed 1 mg/kg T-2 (2 mg/kg T-2, 18%; 1 mg/kg T-2, 10%), but there was no difference in terms of severity of lesions (Table 4.3). At both levels, T-2 caused only mild lesions. Treatments had no effect on mortality rate of broilers. LPS increased spleen weight in chicks (Table 4.4). There was no T-2 effect or LPS x T-2 interaction on relative organ.

Experiment 2: Poult Study: In poults, at week two, birds fed 2 mg T-2 toxin/kg diet showed a decrease ($P < 0.05$) in FI and BWG, but no LPS effect or LPS x T-2 interaction was observed (Table 4.2). The mean values were 244, 233, and 209 g for FI; 158, 145, and 126 g for BWG for poults fed 0, 1, and 2 mg/kg T-2, respectively. In poults, at week three, 2 mg/kg T-2 dramatically decreased FI, BWG, and increased FDGN (Table 4.2). The mean values were 394, 383, and 324 g for FI; 198, 194, and 136

g for BWG; 2.05, 1.92, and 2.30 for FDGN for poults fed 0, 1, and 2 mg/kg of T-2, respectively. No LPS effect or LPS x T-2 interaction was observed in turkey poults at week 3. With respect to oral lesions, poults fed 2 mg/kg T-2 had a higher incidence (2 mg/kg T-2, 48%; 1 mg/kg T-2, 30%) and more severe oral lesions (2 mg/kg T-2, scored 1: 40%, scored between 2 and 3; 60%; 1 mg/kg T-2: scored 1, 56%; scored 2, 44%) than birds fed 1 mg/kg T-2 (Table 4.3 and Figure 4.1). Treatments had no effect on mortality rate of either species. LPS increased spleen weight in poults (Table 4.4). There was no T-2 effect or LPS x T-2 interaction on relative organ weight in poults.

DISCUSSION

At week two, 150 µg LPS/bird decreased FI and BWG in chicks; however, there was no LPS effect in week three. Acute exposure to LPS decreases FI for a short period of time (Steiger *et al.*, 1999; Frank, 2003). Primary mediators of acute phase response are the cytokines TNF-α, IL-1, IL-2, and Il-6 (Klasing, 1998). Frank (2003) suggested that elevation in proinflammatory cytokines, e.g. TNF-α and IL-2, lead to an acute suppression in feed intake. Warren *et al.* (1997) infused porcine TNF-α i.c.v. and reported dose-dependent reductions in feed intake over an 8-h period. Xie *et al.* (2000) reported that LPS given to broiler chicks produced symptoms of drowsiness, lethargy, and withdrawal from feed and water within 1 hour of injection and that the symptoms persisted for up to 4 hours. In addition, in 1994, Roth *et al.* evaluated the effects of repeated LPS challenges on the febrile and cytokine response of guinea-pigs; however, feed intake was not measured. The febrile and cytokine responses were attenuated after

the third injection of LPS. This phenomenon, known as endotoxin tolerance, was documented over 50 years ago by Beeson (1947).

The state of endotoxin tolerance should not be confused with endotoxin resistance or resilience. Tolerance is a state of a progressively diminishing response to an immunogen. Resistance refers to the capability of physiological system to exclude a pathogen and resilience refers to the ability to maintain productivity during a challenge (Klasing, 1998). Endotoxin tolerance was attributed to reduced TNF-α and IL-6-like activity (Roth *et al.*, 1994); however, other mediators of food intake regulation cannot be eliminated (Frank, 2003).

At week two, LPS caused decrease in performance in chicks. The first LPS injection was administered on day 7 while the third injection was given on day 11 and throughout this period a reduction in FI and BWG was observed. Therefore, the fact that LPS affected performance at 2 weeks of age and no or little effect was observed at week three is consistent with the endotoxin tolerance hypothesis described by Beeson (1947). The injections started at week two which suggest that the birds were adapting to the repeated LPS challenge and the responses to this exposure became less intense at week three. At week two, lipopolysaccharide decreased performance in chicks when LPS-treated group was compared to the birds receiving saline. At week three, there was an LPS x T-2 effect on BWG in chicks; but no other significant difference between the LPS- and saline-treated groups was detected at week three.

In chicks, T-2 had no effect on performance. This result is in agreement with the literature. For broiler chicks, decrease in performance was observed in birds fed 6 mg/kg of T-2 in combination with administration with *Cryptosporidium bailey* at 4 weeks of

age; furthermore, when chickens were fed 2 mg/kg of T-2 alone or combined with *C. bailey* challenge, there was no significant reduction in body weight gain (Békési *et al.,* 1997). In addition, Kubena *et al.* (1997) reported an additive effect on body weight gain when broiler chicks were fed 5 mg/kg of T-2 combined with 300 mg/kg of Fumonisin B1 (FB1) for 19 days. However, no significant difference was observed for intake and feed conversion when 5 mg/kg T-2 was given alone Kubena *et al.* (1997). In addition, Kubena *et al.* (2001) challenged broiler chicks with *Salmonella typhimurium* (ST) in combination with either 0, 7.5, or 15.0 mg T-2/kg of diet from day 3 to 11. The authors determined an increase in susceptibility to ST colonization when chicks were fed the highest level of T-2 (15.0 mg/kg). Moreover, a decrease in body weight gain was only observed in birds fed 15.0 mg/kg of T-2 alone or in combination with ST.

In poults, at week two, birds fed 2 mg T-2 toxin/kg diet showed a decrease in FI and BWG, but no LPS effect or LPS x T-2 interactions were observed. At week three, 2 mg/kg T-2 given alone dramatically decreased FI, BWG, and increased FDGN in poults. The decrease in performance observed in poults fed T-2 alone is atypical of a number of studies conducted in which decrease in performance was observed only when poults were fed at least 5 mg/kg T-2. Kubena *et al.* (1995) reported that female turkey poults fed 5 mg T-2/kg of feed for 21 days had body weight gain 26% lower than the control group and when T-2 treatment was combined with 300 mg FB1/kg of feed, a 47% reduction was observed. In the present study, when 2 mg T-2/kg was given alone, there was a 21% reduction in body weight gain, an 11% reduction in feed intake, and 0.21 increase in feed efficiency. In addition, Sklan *et al.* (2003) found oral lesions in poults fed 200 µg T-2/kg feed after 7 days of feeding which did not influence body weight or feed efficiency.

With respect to oral lesions, chicks fed 2 mg/kg T-2 had a higher incidence of oral lesion when compared to chicks fed 1 mg/kg T-2, but no difference in terms of severity of lesions. At both levels, T-2 caused only mild lesions in chicks. Unlike chicks, poults fed 2 mg/T-2 had a higher incidence and more severe lesions than birds fed 1 mg/kg T-2. Treatments had no effect on mortality rate of either species.

Lipopolysaccharide increased relative spleen weight in chicks and poults. B lymphocytes attain full maturation in the bone marrow, and T lymphocytes mature in the thymus. After the cells have matured, they leave the bone marrow or thymus and populate the peripheral lymphoid organs – e.g. spleen, where the immune responses to foreign antigens occur (Abbas and Lichtman, 2000). In response to antigen and growth factors made by the antigen-stimulated lymphocytes and by other cells, the antigen-specific lymphocytes undergo mitotic division. This results in proliferation and increased size of antigen-specific cells (Abbas and Lichtman, 2000) resulting in enlargement of the spleen. There was no T-2 effect or LPS x T-2 interaction on relative organ weight on chicks and poults. Richard *et al.* (1978) fed broiler chicks diets containing 2 or 10 mg/kg T-2 and found no significant effect of T-2 on relative organ weight of those birds. Ogunbo (1994) fed chicks and poults 4 mg/kg T-2 and found a decrease in absolute spleen weight in chicks, but not in poults. In addition, there was no effect on relative bursa weight for both species.

The T-2 toxin damages the GI tract (Bratich *et al.*, 1990) increasing LPS translocation from the GI tract (Roth *et al.*, 1997; Taylor *et al.,* 1991), suggesting that the natural barriers to systemic LPS exposure via GI tract is more easily broken during T-2 toxicosis (Perry *et al.*, 1972). Therefore, the lack of combined effects between T-2 and

LPS may be due to the fact that the T-2 levels chosen were too low for broiler chicks and turkey poults to cause significant GI tract damage to cause increase in translocation of LPS into the portal blood. In turkey poults, however, the decrease in performance observed in birds fed T-2 alone is atypical of a number of studies conducted in which decrease in performance was only observed when poults were fed at least 5 mg/kg T-2.

CONCLUSION

Based on these results, it can be concluded that LPS did not enhance the effects of T-2 toxin in poults and chicks. In poults, however, a decrease in performance observed in birds T-2 alone was atypical of a number of studies conducted in which decrease in performance was only observed when poults were fed at least 5 mg/kg T-2.

TABLE 4.1. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE IN BROILER CHICKS FED T-2 TOXIN[1]

		2ND WEEK			3RD WEEK		
LPS (µg/bird)	**T-2 (ppm)**	**FI**	**BWG**	**FDGN (g:g)**	**FI**	**BWG**	**FDGN (g:g)**
		--------(g)--------			-------(g)--------		
0	0	346[a]	281[a]	1.2362	477	363[a]	1.3152
0	1	346[a]	275[a]	1.2579	456	330[b]	1.3848
0	2	336[a]	276[a]	1.2198	472	354[ab]	1.3348
100	0	331[a]	272[a]	1.2171	443	327[b]	1.3517
100	1	336[a]	280[a]	1.2008	470	351[ab]	1.3392
100	2	343[a]	272[a]	1.2629	469	326[b]	1.4391
150	0	325[ab]	267[a]	1.2163	449	336[b]	1.3377
150	1	298[b]	234[b]	1.2472	447	343[ab]	1.3026
150	2	331[a]	267[a]	1.2405	483	331[b]	1.3807
SEM		10.52	8.83	0.02	10.03	8.42	0.03
		----------Probability----------					
LPS		0.0322	0.0217	0.7479	0.5766	0.1308	0.2595
T-2		0.5450	0.3935	0.4765	0.0847	0.7750	0.0948
LPS X T-2		0.3618	0.2017	0.0970	0.1374	0.0377	0.0922

[1] Data are means of five replicates of six birds each.
[a, b] Values within column with different superscript differ significantly ($P < 0.05$).

TABLE 4.1 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE IN BROILER CHICKS FED T-2 TOXIN[1]

	2ND WEEK			3RD WEEK		
Main Effect Mean	**FI** --------(g)--------	**BWG**	**FDGN (g:g)**	**FI** -------(g)--------	**BWG**	**FDGN (g:g)**
LPS, µg/bird						
0	343[a]	277[a]	1.2380	468	349	1.3449
100	337[a]	275[a]	1.2269	460	335	1.3767
150	318[b]	256[b]	1.2347	460	337	1.3403
T-2 toxin, ppm						
0	334	273	1.2232	456	342	1.3349
1	327	263	1.2353	457	341	1.3422
2	337	271	1.2411	474	337	1.3849

[1] Data are means of 5 replicates of 6 birds each.
[a, b] Values within column with different superscript differ significantly ($P < 0.05$).

TABLE 4.2. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE IN TURKEY POULTS FED T-2 TOXIN[1]

		2ND WEEK			3RD WEEK		
LPS (µg/kg)	**T-2 (ppm)**	**FI**	**BWG**	**FDGN (g:g)**	**FI**	**BWG**	**FDGN (g:g)**
		------- (g) -------			------- (g) ---------		
0	0	242[ab]	150[ab]	1.6268	395[a]	208[a]	1.9210[b]
0	1	244[ab]	147[ab]	1.6660	391[a]	182[a]	1.9510[ab]
0	2	217[b]	126[b]	1.7378	350[ab]	164[ab]	2.1320[ab]
100	0	254[a]	167[a]	1.5222	390[a]	205[a]	1.9152[b]
100	1	212[b]	131[b]	1.6731	370[a]	194[a]	1.9206[b]
100	2	211[b]	131[b]	1.6366	295[b]	110[b]	2.2300[ab]
150	0	237[ab]	156[ab]	1.5163	397[a]	181[a]	2.3291[ab]
150	1	243[ab]	157[ab]	1.5552	388[a]	205[a]	1.8911[b]
150	2	199[b]	120[b]	1.6631	327[b]	134[b]	2.4778[a]
SEM		9.55	8.35	0.06	16.68	14.03	0.16
		----------Probability----------					
LPS		0.5272	0.9048	0.2096	0.2009	0.4688	0.2573
T-2		0.0007	0.0005	0.0940	<.0001	<.0001	0.0470
LPS X T-2		0.1170	0.2055	0.8312	0.7394	0.1229	0.6365

[1] Data are means of five replicates of six birds each.
[a, b] Values within column with different superscript differ significantly ($P < 0.05$).

TABLE 4.2 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE IN TURKEY POULTS FED T-2 TOXIN[1]

	2ND WEEK			3RD WEEK		
Main Effect Mean	**FI** --------(g)--------	**BWG**	**FDGN (g:g)**	**FI** -------(g)--------	**BWG**	**FDGN (g:g)**
LPS, µg/bird						
0	235	141	1.6769	379	185	2.0013
100	226	143	1.6106	352	170	2.0452
150	226	144	1.5782	370	174	2.2327
T-2 toxin, ppm						
0	244[a]	158[a]	1.5551	394[a]	198[a]	2.0551[b]
1	233[a]	145[a]	1.6314	383[a]	194[a]	1.9209[a]
2	209[b]	126[b]	1.6792	324[b]	136[b]	2.3031[a]

[1] Data are means of 5 replicates of 6 birds each.
[a, b] Values within column with different superscript differ significantly ($P < 0.05$).

TABLE 4.3. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON MOUTH LESION[1] OF BROILER CHICKS AND TURKEY POULTS FED T-2 TOXIN

LPS (µg/bird)	T-2 (ppm)	ORAL LESION SCORE BROILERS	TURKEYS
0	0	0.00[b]	0.00[c]
0	1	0.27[ab]	0.67[b]
0	2	0.40[ab]	2.07[a]
100	0	0.13[b]	0.07[c]
100	1	0.13[b]	0.73[b]
100	2	0.73[a]	1.67[a]
150	0	0.07[b]	0.13[bc]
150	1	0.27[ab]	1.20[ab]
150	2	0.27[ab]	1.43[a]
SEM		0.16	0.23
		----------Probability----------	
LPS		0.5683	0.8405
T-2		0.0170	<.0001
LPS X T-2		0.4250	0.1567

[1] Data are means of five replicates of three birds each.

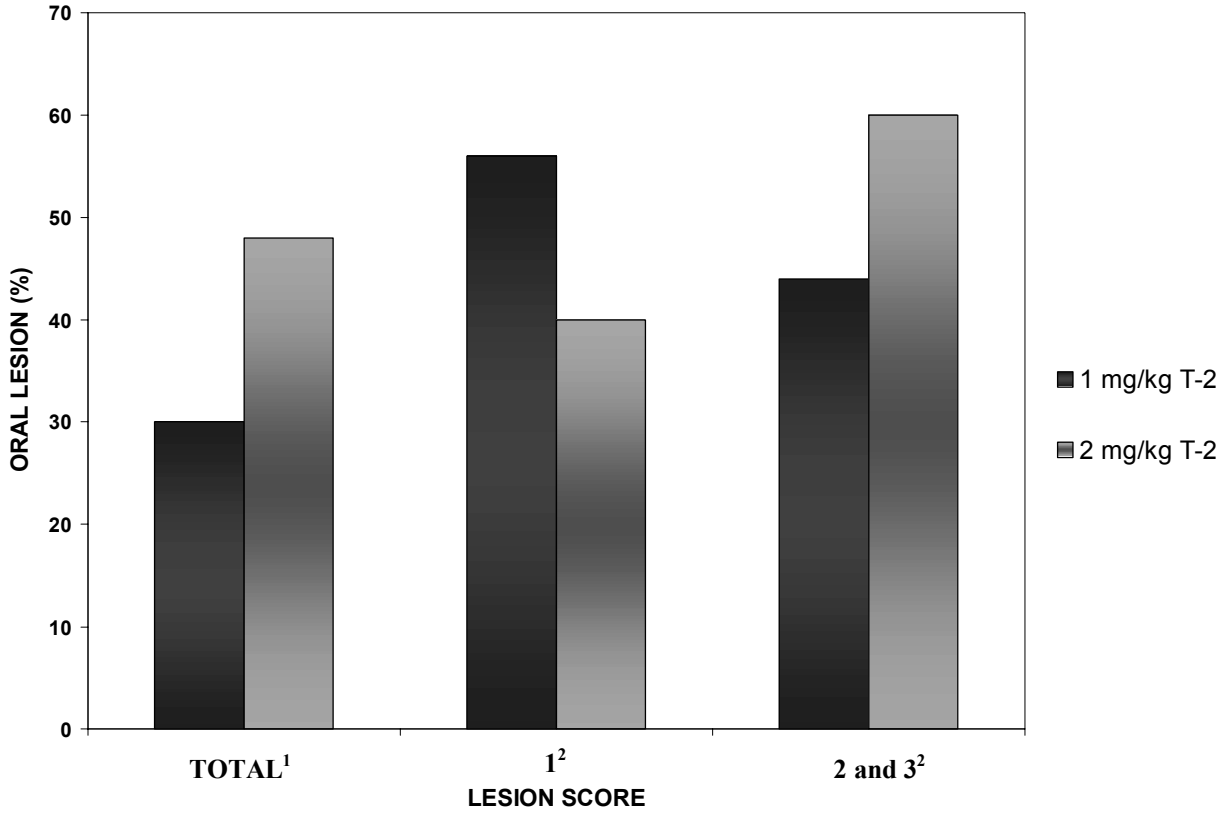
[a, b,c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 4.3 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON HEMATOCRIT[1], MORTALITY RATE[2], AND MOUTH LESION[1] OF POULTS FED T-2 TOXIN AND AFLATOXIN B1

Main effect mean	ORAL LESION SCORE BROILERS	TURKEYS
LPS, µg/bird		
0	0.22	0.91
100	0.33	0.82
150	0.20	0.92
T-2, mg/kg		
0	0.07[b]	0.07[c]
1	0.22[ab]	0.87[b]
2	0.47[a]	1.72[a]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).



[1] Total numbers of chicks observed with oral lesions.
[2] Out of the birds observed with oral lesions, percentage of birds scored between 1 and 3.

Figure 4.1. Incidence and severity of oral lesions in turkey poults fed T-2 toxin

TABLE 4.4. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF BROILER CHICKS FED T-2 TOXIN[1]

		BROILERS		TURKEYS		
LPS (µg/bird)	**T-2 (ppm)**	**SPLEEN**	**BURSA**	**SPLEEN**	**BURSA**	**THYMUS**
		----------	----------	----------(%)----------	----------	----------
0	0	0.104	0.287	0.099	0.176	0.125
0	1	0.092	0.273	0.092	0.160	0.120
0	2	0.108	0.294	0.094	0.182	0.110
100	0	0.167	0.289	0.111	0.165	0.124
100	1	0.165	0.304	0.120	0.172	0.132
100	2	0.154	0.289	0.115	0.191	0.108
150	0	0.166	0.263	0.110	0.167	0.123
150	1	0.145	0.272	0.115	0.184	0.120
150	2	0.143	0.300	0.109	0.175	0.116
SEM		0.01	0.02	0.008	0.005	0.009
		----------Probability----------				
LPS		<.0001	0.6042	<.0001	0.8748	0.9190
T-2		0.3177	0.6147	0.6726	0.1277	0.1290
LPS X T-2		0.5724	0.7293	0.5692	0.1979	0.7844

[1] Data are means of five replicates of three birds each.

[a, b, c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 4.4 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF BROILER CHICKS AND TURKEY POULTS FED AFLATOXIN B1[1]

		BROILERS		TURKEYS		
LPS (µg/bird)	**T-2 (ppm)**	**SPLEEN**	**BURSA**	**SPLEEN**	**BURSA**	**THYMUS**
		----------	----------	----------(%)----------	----------	----------
LPS, µg/bird						
0		0.101[b]	0.284	0.229[b]	0.173	0.119
100		0.162[a]	0.294	0.243[a]	0.176	0.121
150		0.151[a]	0.278	0.240[a]	0.176	0.120
T-2, mg/kg						
0		0.145	0.279	0.107	0.169	0.124
1		0.134	0.282	0.109	0.172	0.124
2		0.135	0.294	0.106	0.183	0.111

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

CHAPTER V

EFFECTS OF CONTINOUS ADMINISTRATION OF LOW-DOSE ENDOTOXIC LIPOPOLYSACCHARIDE IN CHICKS AND POULTS FED NON TOXIC DOSES OF AFLATOXIN B1 AND T-2 TOXIN

ABSTRACT

Two studies were conducted to determine the effects of chronic exposure to low doses of *E. coli* lipopolysaccharide (LPS) in chicks and poults fed diets contaminated with non toxic doses of aflatoxin B1 (AFB1) and T-2 toxin (T-2) from hatch to day 21. Birds were fed diets containing 0 or 2 mg T-2/kg diet singly or in combination with 0 or 2 mg AFB1/kg (chicks) or 0 or 0.15 mg/kg AFB1 (poults). Beginning on day 7, chicks and poults were injected intraperitoneally with 0 or 150 μg LPS/bird every other day. At week 2, LPS, T-2 and AFB1 decreased ($P < 0.05$) feed intake (FI) and body weight gain (BWG) in both chicks and poults. Feed efficiency (FDGN) was increased ($P < 0.05$) by T-2 in both species, by AFB1 in chicks, and by LPS in poults. In chicks, when T-2 was combined with AFB1, there was a further decrease ($P < 0.05$) in FI and BWG whereas in poults, there was a further increase ($P < 0.05$) in FDGN when T-2 was combined with LPS. In chicks, at week three, FI was affected ($P < 0.05$) by AFB1 and T-2, BWG by AFB1, T-2, and LPS, and FDGN by AFB1. Similar to week two, when T-2 was combined with AFB1, there was a further decrease ($P < 0.05$) in FI and BWG of those chicks compared to chicks fed AFB1. In poults, FI was decreased ($P < 0.05$) by T-2,

BWG by T-2 and AFB1, and FDGN was increased by T-2. In chicks, mortality rate at the end of the 3-week period was higher ($P < 0.05$) for the AFB1-fed groups. In poults, LPS increased ($P < 0.05$) mortality rate and poults given the combination of LPS and T-2 had a higher mortality rate compared to those fed T-2 alone or only injected with LPS (LPS, 0; T-2, 0; LPS x T-2, 12%). In chicks, there was an additive interaction ($P < 0.05$) among LPS, AFB1, and T-2 on mortality rate after the first LPS injection (LPS, 6; AFB1, 0; T-2, 0; LPS x T-2, 0; LPS x AFB1, 8; LPS x T-2 x AFB1, 16%); however, in poults, there was no LPS effect ($P > 0.05$) on mortality rate after the first LPS injection. In chicks and poults, LPS decreased FI which lasted for 6 hours after the first LPS injection. Based on these results, it can be concluded that at the levels chosen chronic exposure to low doses of LPS did not potentiate the effects of dietary non toxic doses of T-2 and AFB1 on performance of chicks and poults fed from hatch to day 21. However, LPS did potentiate the effects of T-2 on mortality rate and oral lesions in poults and, after the first LPS injection, decreased feed intake for 6 hours. LPS also enhanced mortality rate in broiler chicks after the first LPS injection and decreased feed intake for 6 hours after the first injection containing LPS.

INTRODUCTION

Mycotoxins are a group of structurally diverse secondary fungal metabolites that occur as contaminants of grain worldwide. It has been estimated that 25% of the world's food crops are affected by mycotoxins (CAST, 1989). Many of these mycotoxins can cause serious health problems in animals and their presence in agricultural commodities

may result in serious economic losses (estimated to be 1.4 billion dollars annually; CAST, 2003). Poultry producers have a continuous problem with low levels of mycotoxin-contaminated feedstuffs causing poorly defined syndromes.

In the past, it was speculated that many of these unexplained syndromes were caused by synergism between mycotoxins. However, years of research have shown little or no synergy but only additive effects. Another potential explanation for the unexplained syndromes observed in poultry may be concomitant exposure to mycotoxins and stressors (e.g. ammonia, competition for feed and water, heat, disease, and endotoxin exposure) present in commercial poultry operations.

Recent reports suggest that, one of these stressors, endotoxic lipopolysaccharides (LPS) may augment the toxic effects of some mycotoxins (Barton *et* al., 2000b; Zhou *et* al., 1999, 2000; Tai and Pestka, 1988). Bacterial lipopolysaccharide is a component of the outer membrane of the cell walls of gram-negative bacteria. Exposure to high doses of LPS causes fever, circulatory shock, disseminated intravascular coagulation, and damage to numerous organs, including the liver (Barton *et al.*, 2001). Acute exposure to LPS causes decrease in feed intake, which, in heifers, lasted for 4 hours (Steiger *et al.*, 1999) and in pigs for 48 hours (Frank, 2003) after LPS exposure before returning to control levels. During an immunological challenge, an animal undergoes a series of physiological events known as the acute-phase response which is designed to help the animal to overcome the challenge (Baumann and Gauldie, 1994). One resulting component of the acute-phase response is suppression in feed intake (Kelley *et al.*, 1994; Johnson, 1998). Frank (2003) suggested that elevation in proinflammatory cytokines, e.g. TNF-α and IL-2, lead to an acute suppression in feed intake. Warren *et al.* (1997)

infused porcine TNF-α i.c.v. and reported dose-dependent reductions in feed intake over an 8-h period.

Exposure to smaller doses of LPS initiates a more modest and noninjurious inflammatory response (Barton *et al.*, 2001). However, exposure to such doses can render the liver more sensitive to injury from xenobiotic agents, such as AFB_1 and T-2 toxin (Roth *et al.*, 1997). In addition, T-2 damages the GI tract (Bratich *et al.*, 1990) increasing the LPS translocation from the GI tract and causing liver lesions which resemble that produced by large, injurious doses of LPS (Roth *et al.*, 1997; Taylor *et al.*, 1991).

Moreover, results of a recent study comparing nine different occupational settings for personal exposure to endotoxins indicates that the highest exposure occurred in poultry operations (Simpson *et* al., 1999). These results suggest poultry are exposed to high levels of LPS via contaminated air, and exposure may well potentiate the effects of low levels of mycotoxins that would normally be considered non-toxic.

Aflatoxin B1 (AFB_1), the most toxic of the aflatoxins, causes a variety of adverse effects in different animal species, especially in poultry which includes retarded growth rate, impaired feed conversion, increased liver weights, liver damage, immunosuppression, negative effects on serum chemistry and hematological parameters, and histopathological lesions. Huff *et al.* (1986) showed that the most sensitive indicators of aflatoxicosis in young broiler chickens are the reduction in serum albumin and total protein levels. Aflatoxin B1 also inhibits protein synthesis and lipid synthesis, depresses glucose metabolism and blood clotting-factor synthesis (Busby and Wogan, 1981). Rastogi *et al.* (2001) reported a decrease in the activities of succinate

dehydrogenase and glucose-6-phosphatase, increased levels of lipid peroxides, and decline in the activities of enzymatic antioxidants levels, such as glutathione-S-transferase, glutathione reductase, and glutathione peroxidase.

The T-2 toxin (T-2) is a mycotoxin produced by the genera *Fusarium* and is a common contaminant of poultry feeds and feedstuffs (Leeson *et al.*, 1995). In poultry, exposure to T-2 induces gizzard erosions and oral lesions (Kubena *et al.*, 1997, 1995; Neiger *et al.*, 1994), reduced body weight gain and feed intake, necrosis and depletion of the lymphoid organs (Wyatt *et al.*, 1973; Boonchuvit *et al.*, 1975; Richard *et al.*, 1978; Hoerr *et al.*, 1981).

The T-2 is also a potent immunosuppressant (Moss, 2002) decreasing host resistance to infectious agents. Mortality caused by paratyphoid infections was reported to be increased in chickens exposed to T-2 (Boonchuvit *et al.*, 1975; Ziprin and Elissalde, 1990). Tai and Pestka 1988's results suggest a synergistic interaction between T-2 and *S. typhimurium* LPS in rats. In addition, T-2 inhibits protein synthesis (Sudakin, 2003) and may stimulate lipid peroxidation (Karppanen *et al.*, 1989).

In the literature, only the acute effects of LPS alone or in combination with high doses of xenobiotics are described. Therefore, there is limited information available on the chronic effects of a sublethal dose of LPS in combination with feeding subtoxic doses of T-2 toxin in chicks and poults. The objective of these studies was to determine the chronic effects of continuous administration of low doses of *E. coli* LPS every other day in chicks and poults fed non toxic doses of AFB1 and T-2 from hatch to 21 days of age.

MATERIAL AND METHODS

For these trials, 200 day-old broilers (Exp.1) and turkeys (Exp.2) were obtained from a commercial hatchery, weighed, wing-banded, and randomly assigned to pens in stainless steel chick batteries. Birds were maintained on a 24-h light schedule and allowed *ad libitum* access to feed and water. Birds were monitored daily for signs of morbidity and mortality. Post-mortem evaluation was performed on all birds that died during the experiment. The animal care and use protocol was reviewed and approved by the University of Missouri-Columbia Animal Care and Use Committee.

For both experiments, a 2 x 2 x 2 factorial (two levels of LPS, two levels of T-2 and two levels of AFB1) design was used with five replicates of five birds assigned randomly to each of the eight treatments from day 1 to 21. Treatments included: (A) 0 µg LPS/bird + 0 mg/kg T-2 + 0 mg/kg AFB1; (B) 0 µg LPS/bird + 2 mg/kg T-2 + 0 mg/kg of AFB1; (C) 0 µg LPS/bird + 0 mg/kg of T-2 + 2 mg/kg AFB1 (chicks) or 0.15 mg/kg AFB1 (poults); (D) 0 µg LPS/bird + 2 mg/kg T-2 + 2 mg/kg AFB1 (chicks) or 0.15 mg/kg AFB1 (poults); (E) 150 µg LPS/bird + 0 mg/kg T-2 + 0 mg/kg AFB1; (F) 150 µg LPS/bird + 2 mg/kg T-2 + 0 mg/kg AFB1; (G) 150 µg LPS/bird + 0 mg/kg T-2 + 2 mg/kg AFB1 (chicks) or 0.15 mg/kg AFB1 (poults); and (H) 150 µg LPS/bird + 2 mg/kg of T-2 + 2 mg/kg AFB1 (chicks) or 0.15 mg/kg AFB1 (poults).

Lipopolysaccharide (*E. coli* LPS; serotype 0127:B8) was purchased from Sigma Chemical (St. Louis, MO) and reconstituted in sterile and non-pyrogenic saline for injection. The solution was then heated under low heat for approximately 5 minutes or until complete dissolution. All glassware were made endotoxin-free by dry heating to

250°C for at least 3 hours. Chicks were injected intraperitoneally with 0.5 ml of sterile saline containing the appropriate quantity of LPS. Chicks that did not receive LPS, were injected with sterile saline. Dietary treatments were fed beginning day 1. Chicks and poults were injected intraperitoneally (IP) with the appropriate dose of LPS every other day from day 7 to 21.

Feed intake (FI) was measured at 1, 2, 4, 6, 12, 18, and 24 hours after the first three injections for Experiments 1 and 2 to determine LPS effect on FI. Birds and feed consumption were weighed weekly. At 3 weeks of age (day 21), birds were then euthanized with carbon dioxide, bled via cardiac puncture for whole blood and serum collection. Whole blood was collected for hematocrit determination and serum for determination of glucose, calcium, uric acid, aspartate aminotransferase, creatine kinase, total protein, albumin, and globulin concentrations. Liver, spleen, bursa, and thymus (three birds/pen) were sampled for histopathologic evaluation as well as relative organ weight calculation. Organ weights are expressed as relative weights (grams organ/100 g body weight).

Liver samples (two birds/rep; eight/trt) were also collected for measurement of hepatic glutathione (GSH). Briefly, 1 g of liver tissue was immediately homogenized in PBS solution (pH 7.0). Homogenates were then centrifuged at 2,800 g for 10 min and stored at - 80ºC until analysis. Hepatic glutathione level in the supernatant was determined using a spectrophotometric assay kit (Calbiochem, Cat. No. 354102) according to the manufacturer's instructions.

Oral lesions were scored in a blind fashion using a 4-point scoring system, ranging from 0 to 4, with 0 = no lesion; 1 = one or two mouth lesions clearly visible on

either the lower or upper mandible; 2 = lesions intermediate in appearance to lesions scored as 1 or 3; 3 = large lesions occurring at several sites within the mouth; 4 = large lesions occurring at several sites within the mouth, principally on the upper and lower mandible, the corners of the mouth, and the back of the tongue.

Data were analyzed as a 2 x 2 x 2 factorial by analysis of variance (ANOVA) using the General Linear Models procedure of SAS software (SAS Institute Inc., 1996). Means showing significant differences in ANOVA were compared using Fisher's protected least significant difference procedure (Snedecor and Cochran, 1989). Mortality data were transformed (Arcsin) prior to statistical analysis. All statements of differences were based on a significance of $P < 0.05$.

RESULTS

Experiment 1: Broiler Study: At week one, there was an AFB1 effect ($P < 0.05$) on feed intake (FI) (Table 5.1). At week two, LPS, T-2 and AFB1 decreased ($P < 0.05$) FI and BWG, whereas feed efficiency (FDGN) was increased ($P < 0.05$) by T-2 and AFB1. In chicks, when T-2 was combined with AFB1, there was a further decrease ($P < 0.05$) in FI (T-2, 291; AFB1, 286; T-2 x AFB1, 228 g) and BWG (T-2, 242; AFB1, 229; T-2 x AFB1, 170 g). Lipopolysaccharide injections affected ($P < 0.05$) feed intake (FI). Independent of the dietary treatments, chicks given LPS did not returne to eating until 6 hours after the first injection (Figure 5.3). In birds given saline alone, these signs were absent. At week three, in chicks, BWG was affected ($P < 0.05$) by AFB1, T-2, and LPS, FI by T-2 and AFB1, and FDGN by AFB1 only. As in week two, when T-2 was

combined with AFB1, there was a further decrease ($P < 0.05$) in FI (T-2, 583; AFB1, 542; T-2 x AFB1, 453 g) and BWG (T-2, 432; AFB1, 385; T-2 x AFB1, 293 g) in chicks (Table 5.1). In chicks, mortality rate at the end of the 3-week period was higher ($P < 0.05$) in the AFB1-fed groups (Table 5.3). In addition, exposure to LPS increased mortality rate measured within 24 hours after the first LPS injection. In chicks, T-2 caused ($P < 0.05$) mild oral lesions in birds fed T-2 singly or in combination with LPS or AFB1 or both (Table 5.3). With respect to relative organ weights of chicks (Tables 5.5), AFB1 increased ($P < 0.05$) liver and spleen weights and decreased thymus weight. Lipopolysaccharide increased spleen weight and decreased bursa weight ($P < 0.05$). T-2 had no effects on relative organ weights of chicks; however, when T-2 was combined with AFB1, there was a further decrease ($P < 0.05$) in thymus weight (T-2, 0.337; AFB1, 0.300; T-2 x AFB1, 0.231 g:100 g BW). In chicks, LPS and AFB1 decreased ($P < 0.05$) serum total protein (TP) and albumin (ALB) concentrations, whereas T-2 decreased only ALB concentrations ($P < 0.05$). There was an LPS x T-2 interaction on TP (LPS, 2.17; T-2, 2.21; LPS x T-2, 2.25 g/100 ml) and ALB (LPS, 1.18; T-2, 1.13; LPS x T-2, 2.25 g/100 ml) (Table 5.7). For serum chemistry, in chicks, AFB1 caused a decrease ($P < 0.05$) in serum concentrations of glucose (GLU) and calcium (Ca) (Table 5.9). In addition, co-treatment with AFB1 and T-2 or AFB1 and LPS decreased ($P < .05$) Ca when compared to LPS and T-2 given alone (LPS, 9.7; T-2, 9.9; AFB1, 8.6; LPS x AFB1, 8.6; T-2 x AFB1, 8.7 mg/dL). There was also an LPS x T-2 interaction ($P < 0.05$) observed in Ca in chicks (LPS, 9.7; T-2, 9.9; LPS x T-2, 9.9). LPS decreased aspartate aminotransferase (AST) in chicks (Table 5.9). In chicks, there were no treatment effects ($P > 0.05$) on hematocrit (HCT) (Table 5.3). In chicks, AFB1 increased ($P < 0.05$) GLUT

(Table 5.11). In addition, a three-way interaction in GLUT was found ($P > 0.05$) in chicks (LPS, 6.24; T-2, 6.70; AFB1, 8.41; LPS x T-2 x AFB1, 6.92 mmol/100 μL).

Experiment 2: Poult Study: At week one, there was a T-2 effect ($P < 0.05$) on FI, body weight gain (BWG), and feed conversion (FDGN) (Table 5.2). At week two, LPS, T-2 and AFB1 decreased ($P < 0.05$) FI and BWG (Tables 5.1 and 5.2). Feed efficiency (FDGN) was increased ($P < 0.05$) by T-2 and by LPS in poults (Tables 5.2). In poults, there was a further increase ($P < 0.05$) on FDGN when T-2 was combined with LPS (LPS, 1.23; T-2, 1.32; LPS x T-2, 1.42 g:g). Lipopolysaccharide injections affected ($P < 0.05$) feed intake (FI). Independent of the dietary treatments, birds given LPS returned to eating 6 hours after the first injection (Figure 5.3). In poults, at week three, FI was increased and FDGN decreased ($P < 0.05$) by T-2, whereas BWG was decreased ($P < 0.05$) by T-2 and AFB1 (Tables 5.2). In poults, LPS increased ($P < 0.05$) mortality rate during the 3 week long study (0 LPS, 2; 150 LPS, 9%). In addition, an LPS x T-2 interaction was also found in poults ($P < 0.05$) (LPS, 0; T-2, 0; LPS x T-2, 12%) (Table 5.4 and Figure 5.2). In poults, T-2 had an effect ($P < 0.05$) on incidence of oral lesions. The incidence of oral lesion in poults fed T-2 was 93%, whereas in poults fed no T-2, no oral lesions were detected (Table 5.4). Lipopolysaccharide increased ($P < 0.05$) the severity of the oral lesions of birds fed T-2 when compared to birds fed T-2 alone. Birds fed T-2 and given LPS had 50% of the oral lesions scored either 3 or 4, whereas birds fed T-2 singly had 93% of the birds scored between 0 and 2 and only 7% scored 3.

With respect to organ weight, in contrast to chicks, T-2 increased liver weight and spleen weight ($P < 0.05$) in poults (Table 5.6), whereas AFB1 increased ($P < 0.05$) spleen weight and decreased liver and thymus weights in poults (Tables 5.6). In poults, LPS

increased ($P < 0.05$) TP (Table 5.8). LPS x T-2 interactions were observed for TP and globulin (GLOB) concentrations (TP: LPS, 3.06; T-2, 2.83; LPS x T-2, 3.36 g/100 ml; GLOB: LPS, 1.65; T-2, 1.47; LPS X T-2, 1.96 g/100 ml). Aflatoxin B1 caused ($P < 0.05$) almost a 2-fold decrease in TP, ALB, and GLOB when compared to the control group. There was also a less than additive AFB1 x T-2 interaction ($P < 0.05$) on ALB (T-2, 1.36; AFB1, 0.78; T-2 x AFB1, 0.81 g/100 ml) (Table 5.8). In poults, AFB1 decreased GLU and Ca and decreased AST ($P < 0.05$) (Table 5.10). In addition, T-2 decreased Ca and LPS decreased creatine kinase (CK) ($P < 0.05$). In poults, no interactions among the factors were observed. In either species, no affects ($P > 0.05$) on uric acid (UA) were observed (Tables 5.9 and 5.10). In poults, T-2 x AFB1 and LPS X T-2 X AFB1 interactions were found (LPS, 35.13; T-2, 34.13; AFB1, 35.33; T-2 X AFB1, 34.23; LPS X T-2 X AFB1, 38.67%) (Table 5.4). Finally, treatments had no effect ($P > 0.05$) on hepatic glutathione (GLUT) concentration (Table 5.12).

DISCUSSION

A prominent feature of septic shock is a severe and intractable hypotension coupled with a primary reduction in circulating blood volume, which leads ultimately to circulatory collapse and death (Andrew *et al.*, 2000). In the present study, at the end of 3 weeks, LPS caused increased mortality in poults, but had no effect in mortality on chicks. In addition, there was a synergistic interaction between T-2 and LPS in poults increasing mortality rate. Furthermore, in poults, T-2 had an effect on incidence of oral lesions. The incidence of oral lesion in poults fed T-2 was 93% whereas in poults fed no T-2, no

oral lesion was detected. Lipopolysaccharide increased the severity of the oral lesions compared to the birds fed T-2 alone. Birds fed T-2 and given LPS had 50% of oral lesions with a score between 3 and 4, whereas birds fed T-2 alone had 93% of the birds with lesion score between 0 and 2 and only 7% with lesion score of 3. These results are in agreement with the literature. The T-2 toxin damages the GI tract (Bratich *et al.*, 1990) increasing LPS translocation from the GI tract (Roth *et al.*, 1997; Taylor *et al.,* 1991), suggesting that the natural barriers to LPS systemic exposure via the GI tract is more easily broken during T-2 toxicosis (Perry *et al.*, 1972). In addition, tricothecenes become more toxic in the presence of LPS, thereby causing markedly elevated tissue injury and mortality (Tai and Pestka, 1988; Taylor *et al.*, 1991; Zhou *et al.*, 1999).

By contrast, in chicks, 2 mg T-2/kg diet, with or without LPS, caused only mild oral lesions suggesting that 2 mg T-2/kg diet may have been too low a concentration to cause significant GI tract damage to allow translocation of LPS into the portal blood and therefore increase mortality when chicks were co-treated with LPS and T-2. However, in chicks, LPS had more of an acute effect. Chicks given LPS had an increase of 12% in mortality after the first injection containing LPS which was not seen in poults. This result suggests that chicks may be more susceptible to an initial dose of LPS when compared to poults.

Acute exposure to LPS decreases FI for a short period of time (Steiger *et al.*, 1999; Frank, 2003). Primary mediators of acute phase response are the cytokines TNF-α, IL-1, IL-2, and IL-6 (Klasing, 1998). Frank (2003) suggested that elevation in proinflammatory cytokines, e.g. TNF-α and IL-2, lead to an acute suppression in feed intake. Warren *et al.* (1997) infused porcine TNF-α i.c.v. and reported dose-dependent

reductions in feed intake over an 8-h period. In the present study, LPS injections affected feed intake immediately. Exposure to LPS caused lethargy and withdrawal from feed and water for at least 4 hours. Birds also presented signs of ruffled feathers and moderate diarrhea. Independent of the dietary treatments, birds given LPS resumed eating 6 hours after the first injection containing LPS. In birds given saline alone, these signs were absent. After the second and third injections, however, LPS had no effect on feed intake. These results are in agreement with the results of Xie *et al.* (2000) who reported that LPS given to broiler chicks produced symptoms of drowsiness, lethargy, and withdrawal from feed and water within 1 hour of injection and that the symptoms persisted for up to 4 hours. In addition, in 1994, Roth *et al.* evaluated the effects of repeated LPS challenges on the febrile and cytokine response of guinea-pigs; however, feed intake was not measured. The febrile and cytokine responses were attenuated after the third injection of LPS. This phenomenon, known as endotoxin tolerance, was documented over 50 years ago by Beeson (1947).

The state of endotoxin tolerance should not be confused with endotoxin resistance or resilience. Tolerance is a state of a progressively diminishing response to an immunogen. Resistance refers to the capability of physiological system to exclude a pathogen and resilience refers to the ability to maintain productivity during a challenge (Klasing, 1998). Endotoxin tolerance was attributed to reduced TNF-α and IL-6-like activity (Roth *et al.*, 1994); however, other mediators of food intake regulation cannot be eliminated (Frank, 2003).

At week two, LPS caused decrease in performance in both species. The first LPS injection was administered on day 7 while the third injection was given on day 11 and

throughout this period a reduction in FI was observed. Therefore, the fact that LPS affected performance at 2 weeks of age and no or little effect was observed at week three is consistent with the endotoxin tolerance hypothesis described by Beeson (1947). The injections started at week 2 which suggest that the birds were adapting to the repeated LPS challenge and the responses to this exposure became less intense at week three. At week two, lipopolysaccharide decreased performance in both species when LPS-treated group was compared to the birds receiving saline. At week three, there was a LPS effect on BWG in chicks; but no other significant difference between the LPS- and saline-treated groups was detected at week three.

The T-2 toxin at 2 mg T-2/kg diet caused a decrease in performance in both species. In chicks, this result is consistent with the literature as the reports are rather controversial. The T-2 toxin reduced growth in chicks fed 4 mg T-2/ kg of diet (Wyatt *et al.*, 1973; Ademoyero and Hamilton, 1991; Leeson *et al.*, 1995). In broiler chicks, decrease in performance at four weeks of age was observed in birds fed 6 mg T-2/kg of diet. In chicks fed 2 mg T-2/kg, there was no significant reduction in body weight gain (Békési *et al.,* 1997). However, Richard *et al.* (1978) reported depression in feed intake and feed efficiency when chickens were fed 2 and 10 mg/kg T-2. Kubena *et al.* (1997) reported an additive effect on body weight gain when broiler chicks were fed 5 mg/kg of T-2 combined with 300 mg/kg of Fumonisin B1 (FB1) for 19 days, but no significant difference was observed for intake and feed conversion when T-2 was given alone. In addition, when Hoehler and Marquardt (1996) fed 4 ppm T-2/kg of diet, only a slight decrease in performance was observed. In contrast, T-2 concentrations as low as 0.9 mg T-2/kg of diet have been reported to cause decreased FI and BWG (Garcia *et al.,* 2003).

In poults, Sklan *et al.* (2003) reported that addition of up to 1 mg/kg T-2 caused no effect on poults performance; however, the authors reported an increase in the severity of oral lesions as the levels of T-2 in the diet increased. Kubena *et al.* (1995) reported that female turkey poults fed 5 mg T-2/kg of feed for 21 days had body weight gain 26% lower than the control group and when T-2 treatment was combined with 300 mg FB1/kg of feed, a 47% reduction was observed. Richard *et al.* (1978) fed turkey poults either 2 or 10 mg/kg T-2 for 4 weeks. Poults fed 10 mg/kg T-2 were a third lighter than the control group, whereas poults fed 2 mg/kg T-2 were slightly lighter the control only at week four (Richard *et al.*, 1978).

In chicks, 2 mg/kg AFB1 caused depressed performance at both two and three weeks of age. In addition, there was a synergistic interaction between T-2 and AFB1 resulting in further decrease in FI and BWG. Verma *et al.* (2002) reported that AFB1 (~3 mg/kg AFB1) co-treatment with OA significantly decreased body weight, serum protein and serum albumin, and increased the relative weights of liver, kidney, and proventriculus. Pimpukdee *et al.* (2004) reported that 5 mg/kg AFB1 had decreased performance of 3-week-old chickens. In addition, Huff *et al.* (1988) showed that feeding 2.5 mg/kg AFB1 to broiler chickens caused a decrease in body weight and a further decrease was observed when birds were co-treated with AFB1 and 4 mg/kg T-2.

In this study, the means chosen to administer LPS may have been too invasive which may explain the increased susceptibility of the birds to levels of toxins otherwise considered non toxic.

Independent of the dietary treatments, LPS significantly increased relative spleen weight and decreased relative bursa weight when compared to chicks receiving saline,

whereas in poults, LPS increased spleen and liver weights compared to the saline-treated groups. Stress-induced bursal atrophy has been suggested to be caused by an increased corticosteroid production (Riddell, 1987). Lipopolysaccharide is an immunological stressor that increases the production of corticosteroid, which may account for the decrease in bursa weight observed in the present study. Xie *et al.* (2000) reported increase in liver weights of chicken exposed to *Salmonella typhimurium* LPS. The increase in the relative weights of liver in LPS-treated birds illustrates the importance of the liver in the acute phase response (Xie *et al.*, 2000). The liver is the site for the synthesis of acute phase proteins and detoxification of xenobiotics. It is likely that the apparent changes in liver weight may be due to an increase in its metabolic function coupled with loss of body weight.

In poults, T-2 increased liver and spleen weights. Friend *et al.* (1983) reported that liver was affected by feeding T-2 in rats. Richard *et al.* (1978) reported decreased relative thymus weight when poults were fed 10 mg/kg T-2, but no effect was observed when poults were fed 2 mg/kg T-2. In addition, the authors reported no effect on spleen and bursa weights when poults were fed either 2 or 10 mg/kg T-2 (Richard *et al.*, 1978). The T-2 toxin induced inhibition of cell proliferation in the murine spleen is a transient effect (Cooray and Lindahl-Kiessling, 1987). During chronic exposure of Swiss mice to T-2 toxin, the spleen decreased in size initially, but subsequently increased in size until it became much larger than that of the control mice (Hayes *et al.*, 1980). Cooray and Lindahl-Kiessling (1987) reported that mice given T-2 toxin for 3 weeks had enlarged spleens and that the increase was dose dependent. In addition, the authors also reported that T-2 treatment increased the metabolic capacity of mouse peritoneal cells and the

number of mature macrophages in the murine spleen which in turn may have been capable of stimulating antibody synthesis in the mouse spleen resulting in increases of its size. In addition, T-2 toxin selectively destroys T suppressor cells (Otakawa *et al.*, 1979; Rosenstein *et al.*, 1981) which may have contributed to enhancement of spontaneous antibody synthesis in the murine spleen as well. In chicks, T-2 had no effect on relative organ weight. Richard *et al.* (1978) fed broiler chicks diets containing 2 or 10 mg/kg T-2 and found no significant effect of T-2 on relative organ weight of those birds. In contrast, Ogunbo (1994) found decreased absolute spleen weights when broilers were fed diets containing 4 mg/kg T-2.

In chicks and poults, AFB1 given alone increased spleen and decreased thymus weight. In addition, a synergistic interaction between T-2 and AFB1 on thymus relative weight was observed in chicks. Hegazy and Adachi (2000) reported that feeding 2.6 mg/kg AFB1 to chicks decreased relative bursal and thymus weights, but spleen weight was increased. The decrease in bursa and thymic weights represents the immunossupressive effect of the treatments. However, the increase in spleen weight observed may be related to the health status of the birds. Chang and Hamilton (1982) found a significant increase in relative spleen weights in chicks with infectious bursal disease virus, which may be enhanced by synergism with aflatoxicosis. In the present study, at the end of the third week, birds were euthanized and grossly necropsied. Some birds (approximately 15%) presented signs of septicemia with perihepatitis and pericarditis. In poults, AFB1 decreased liver and thymus weights and increased spleen weights. AFB1 increased relative liver weight in chicks. In chicks, this result is in agreement with the literature. AFB1 causes fatty liver in poultry (Hamilton and Garlich,

1971; Carnaghan *et al.*, 1966; Sawhney *et al.*, 1973) causing increased liver weight due to fat deposition in the liver. In poults, AFB1 decreased liver weight. Bradley (1985) proposed that inhibition of protein synthesis impairs the ability of the animal to repair cellular damage leading to decreased relative organ weights. In contrast, Ogunbo (1994) fed turkey poults 4 mg/kg T-2 and found no significant difference in liver weight.

In chicks, AFB1 decreased serum Ca. In addition, LPS x T-2, LPS x AFB1, and T-2 x AFB1 interactions were found in serum Ca. In poults, AFB1 and T-2 decreased serum Ca. Sergeev *et al.* (1988) reported that rats exposed to T-2 were hypocalcemic and this condition was accompanied with decreases in the activities of alkaline phosphatase on the intestinal mucosa, the hepatic concentration of 25-OH-D_3, and renal 24,25-OH. In laying hens, the presence of the toxin in the diet resulted in decreased egg production and quality of the egg shell (Chi *et al.*, 1977, 1978). Chan and Tarnawski (2002) suggested that tissue injury may stimulate serum response factor (SRF) which activate genes to promote healing and that Ca^{2+}/calmodulin-dependent kinase (Cam) may be involved in the process of SRF activation by cytoplasmatic Ca^{2+} elevation.

Shen *et al.* (1994) have shown that AFB1 causes lipid peroxidation in rat liver and that pretreatment with vitamin E, selenium, or deferoxamine, an iron chelator, significantly inhibited lipid peroxidation as well as liver cell damage. The tricothecenes deoxynivalenol (DON) and T-2 have also been shown to stimulate lipid peroxidation in rat liver along with depletion of hepatic glutathione (Rizzo *et al.*, 1994). In the present experiment, treatments did not affect GLUT in poults, whereas in chicks there was an increase in GLUT. Hoehler and Marquardt (1996) showed that feeding either 4 mg/kg or

5 mg/kg T-2 did not affect malondialdehyde (MDA), a product from lipid peroxidation, in liver homogenates of chickens.

The results obtained regarding T-2 as a promoter of lipid peroxidation in this study reflect the findings by other authors, as they are rather contradictory. Schuster *et al.* (1987) suggested that lipid peroxidation is not involved in the toxicity of T-2. Segal *et al.* (1983), in contrast, reported that T-2 and another tricothecene, DON, stimulated lipid peroxidation in the livers of rats. Hoehler and Marquardt (1996) suggested that the assays utilized (TBARS) are not always accurate because of their lack of specificity. In addition, the vitamin E status of the animal may also affect the degree of lipid peroxidation.

In chicks fed AFB1, there was an increase in GLUT. Rastogi *et al.* (2001) reported a decrease in the activities of succinate dehydrogenase and glucose-6-phosphatase, increased levels of lipid peroxides, and decline in the activities of enzymatic antioxidants levels, such as glutathione-S-transferase, glutathione reductase, and glutathione peroxidase. This may explain the increase in the concentration of GLUT in chicks fed AFB1. The low activities of the glutathione enzymes probably resulted in an increase in the concentration of the substrate. In addition, one important route of AFB1 8,9-epoxide detoxification is via conjugation to glutathione (GSH) (Degen and Neumann, 1978; Eaton *et al.*, 1994) which may increase the concentration of this compound. Furthermore, a three-way interaction in glutathione concentration was found in chicks, but not in poults. Glutathione concentration was depressed in the treatments receiving LPS and fed T-2 and AFB1. Luyendyk *et al.* (2002) reported that it is conceivable that LPS might decrease liver GSH concentration, thereby decreasing capacity to detoxify the

AFB1 8,9 epoxide, and resulting in enhanced hepatotoxicity. Unfortunately, it is beyond the scope of this study to suggest that GSH may have been decreased by LPS.

CONCLUSION

Based on these results, it can be concluded that at the levels chosen chronic exposure to low doses of LPS did not potentiate the effects of dietary non toxic doses of T-2 and AFB1 on performance of chicks and poults fed from hatch to day 21. However, LPS did potentiate the effects of T-2 on mortality rate and oral lesion in poults. In addition, acute exposure to LPS also enhanced mortality rate in broiler chicks and cause a decrease in feed intake in chicks and poults, independent of dietary treatments.

TABLE 5.1. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON PERFORMANCE OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

			1ST WEEK[2]			2ND WEEK			3RD WEEK		
LPS (µg/bird)	T-2 (ppm)	AFB1 (ppm)	FI	BWG	FDGN (g:g)	FI	BWG	FDGN (g:g)	FI	BWG	FDGN (g:g)
			----- (g) ------			----- (g) --------			------- (g) ---------		
0	0	0	120	115	1.0489	289[a]	250[a]	1.1550[b]	565[a]	439[a]	1.3078[b]
0	2	0	117	115	1.0174	291[a]	242[ab]	1.2011[ab]	583[a]	432[a]	1.3446[b]
0	0	2	114	115	1.0000	286[a]	229[b]	1.2454[ab]	542[a]	385[b]	1.4041[ab]
0	2	2	109	107	1.0220	228[b]	170[c]	1.3177[ab]	453[b]	293[c]	1.4935[a]
150	0	0	N/A	N/A	N/A	277[a]	251[a]	1.1298[b]	582[a]	432[a]	1.3536[b]
150	2	0	N/A	N/A	N/A	272[a]	229[b]	1.1900[b]	526[ab]	385[b]	1.3659[ab]
150	0	2	N/A	N/A	N/A	256[ab]	212[b]	1.2119[ab]	502[ab]	357[b]	1.4327[ab]
150	2	2	N/A	N/A	N/A	203[b]	150[d]	1.3400[a]	381[b]	264[c]	1.4513[ab]
SEM			4.38	3.17	0.04	13.91	6.57	0.046	28.18	14.81	0.045
			----------Probability----------								
LPS			N/A	N/A	N/A	0.0435	0.0109	0.7268	0.0672	0.0132	0.6890
T-2			0.1769	0.1451	0.9851	0.0085	<.0001	0.0320	0.0038	<.0001	0.2440
AFB1			0.0264	0.1938	0.0977	0.0006	<.0001	0.0031	<.0001	<.0001	0.0043
LPS X T-2			N/A	N/A	N/A	0.9347	0.3757	0.6133	0.1882	0.3276	0.4758
LPS X AFB1			N/A	N/A	N/A	0.5418	0.2041	0.8568	0.3721	0.9452	0.5464
T-2 X AFB1			0.8502	0.1544	0.0548	0.0122	<.0001	0.4958	0.0387	0.0040	0.6594
LPS X T-2 X AFB1			N/A	N/A	N/A	0.7868	0.5656	0.7630	0.5957	0.3777	0.7276

[1] Data are means of five replicates of five birds each.

[a, b, c, d] Values within columns with different superscripts differ significantly ($P < 0.05$).

[2] LPS treatments begun on day 7. Considering only T-2 and AFB1 effects. (N/A = not applicable).

TABLE 5.1 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON PERFORMANCE OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	1ST WEEK[2]			2ND WEEK			3RD WEEK		
	FI	BWG	FDGN	FI	BWG	FDGN	FI	BWG	FDGN
	------- (g) ---------		(g:g)	------- (g) ---------		(g:g)	------- (g) ---------		(g:g)
LPS, µg/bird									
0		N/A		274[a]	223[a]	1.2300[a]	536[a]	387[a]	1.3875[a]
150		N/A		252[b]	210[b]	1.2178[a]	498[b]	359[b]	1.4009[a]
T-2 toxin, ppm									
0	117	115	1.0194	277[a]	235[a]	1.1856[b]	548[a]	403[a]	1.3746[a]
2	113	111	1.0197	249[b]	198[b]	1.2621[a]	486[b]	344[b]	1.4138[a]
AFB1, ppm									
0	118[a]	115	1.0332	282[a]	243[a]	1.1690[b]	564[a]	422[a]	1.3430[b]
2	111[b]	111	1.0060	243[b]	190[b]	1.2786[a]	470[b]	325[b]	1.4454[a]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

[2] LPS treatments begun on day 7. Considering only T-2 and AFB1 effets. (N/A = not applicable).

TABLE 5.2. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON PERFORMANCE OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

			1ST WEEK[2]			2ND WEEK			3RD WEEK		
LPS (µg/bird)	**T-2 (ppm)**	**AFB1 (ppb)**	**FI**	**BWG**	**FDGN (g:g)**	**FI**	**BWG**	**FDGN (g:g)**	**FI**	**BWG**	**FDGN (g:g)**
			----- (g) ------			----- (g) ------			------ (g) --------		
0	0	0	108[a]	99[a]	1.0927[b]	284[a]	229[a]	1.1940[c]	437[a]	264[a]	1.5721[b]
0	2	0	91[b]	73[b]	1.2496[a]	199[d]	146[d]	1.3159[b]	320[b]	184[b]	1.7363[ab]
0	0	150	109[a]	94[a]	1.1491[b]	256[b]	208[b]	1.2272[bc]	357[b]	208[b]	1.6801[ab]
0	2	150	90[b]	71[b]	1.2704[a]	192[d]	147[d]	1.2693[bc]	325[b]	184[b]	1.7645[a]
150	0	0				248[bc]	201[bc]	1.2325[bc]	394[ab]	249[a]	1.5358[b]
150	2	0				194[d]	136[de]	1.4235[a]	286[b]	174[bc]	1.6585[ab]
150	0	150				233[c]	188[c]	1.2360[bc]	349[b]	223[b]	1.5659[b]
150	2	150				178[d]	121[e]	1.4715[a]	286[b]	145[c]	1.7721[a]
SEM			4.38	3.17	0.037	7.72	5.64	0.034	25.77	12.21	0.056
			----------Probability----------								
LPS			N/A	N/A	N/A	0.0012	<.0001	0.0013	0.0974	0.1697	0.1960
T-2			<.0001	< .0001	<.0001	<.0001	<.0001	<.0001	0.0001	<.0001	0.0017
AFB1			0.9847	0.1049	0.1602	0.0048	0.0055	0.7072	0.1035	0.0029	0.1039
LPS X T-2			N/A	N/A	N/A	0.0714	0.4197	0.0139	0.7614	0.1539	0.6331
LPS X AFB1			N/A	N/A	N/A	0.8444	0.5715	0.5227	0.6842	0.9807	0.9642
T-2 X AFB1			0.7104	0.5334	0.5117	0.3479	0.2137	0.7271	0.0824	0.1381	0.9819
LPS X T-2 X AFB1			N/A	N/A	N/A	0.3425	0.1385	0.2247	0.5848	0.0938	0.3355

[1] Data are means of five replicates of five birds each.

[2] LPS treatments begun on day 7. Considering only T-2 and AFB1 effets. (N/A = not applicable).

[a, b, c, d, e] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.2 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON PERFORMANCE OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	1ST WEEK[2]			2ND WEEK			3RD WEEK		
	FI	BWG	FDGN	FI	BWG	FDGN	FI	BWG	FDGN
	------- (g) ---------		(g:g)	------- (g) ---------		(g:g)	------- (g) ---------		(g:g)
LPS, µg/bird									
0		N/A		233[a]	183[a]	1.2516[b]	360[a]	210[a]	1.6882[a]
150		N/A		213[b]	162[b]	1.3409[a]	329[a]	198[a]	1.6331[a]
T-2 toxin, ppm									
0	109[a]	96[a]	1.1209[b]	255[a]	207[a]	1.2224[b]	384[a]	236[a]	1.5885[b]
2	91[b]	72[b]	1.2600[a]	191[b]	138[b]	1.3701[a]	304[b]	172[b]	1.7329[a]
AFB1, ppb									
0	100[a]	86[a]	1.1711[a]	231[a]	178[a]	1.2915[a]	360[a]	218[a]	1.6257[a]
150	100[a]	82[a]	1.2100[a]	215[b]	166[b]	1.3010[a]	329[a]	190[b]	1.6957[a]

[1] Data are main effect means.

[2] LPS treatments begun on day 7. Considering only T-2 and AFB1 effets. (N/A = not applicable).

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.3. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON HEMATOCRIT[1], MORTALITY RATE[2], AND MOUTH LESION[1] OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppm)	HEMATOCRIT	MORTALITY	LESION
			----------(%)----------		
0	0	0	31.47[a]	4[b]	0.00[b]
0	2	0	31.80[a]	0[c]	0.60[a]
0	0	2	33.40[a]	20[ab]	0.00[b]
0	2	2	31.90[a]	12[b]	0.60[a]
150	0	0	31.40[a]	8[b]	0.00[b]
150	2	0	30.63[a]	0[c]	0.73[a]
150	0	2	29.93[a]	12[b]	0.00[b]
150	2	2	31.07[a]	32[a]	0.53[a]
SEM			1.49	---	0.10
			----------Probability----------		
LPS			0.1975	0.3052	0.8223
T-2			0.8503	0.6993	<.0001
AFB1			0.8135	<.0001	0.5018
LPS X T-2			0.7177	0.1762	0.8223
LPS X AFB1			0.4711	0.7465	0.5018
T-2 X AFB1			0.9874	0.0883	0.5018
LPS X T-2 X AFB1			0.3812	0.0438	0.5018

[1] Data are means of five replicates of three birds each.

[2] Mortality data were transformed (Arcsin) prior to statistical analysis.

[a, b] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.3 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON HEMATOCRIT[1], MORTALITY RATE[2], AND MOUTH LESION[1] OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1

Main Effect Mean	HEMATOCRIT	MORTALITY	LESION
	----------(%)----------		
LPS, µg/bird			
0	32.14[a]	9[a]	0.30[a]
150	30.76[a]	13[a]	0.32[a]
T-2 toxin, ppm			
0	31.55[a]	11[a]	0.00[b]
2	31.35[a]	11[a]	0.62[a]
AFB1, ppm			
0	31.32[a]	3[b]	0.33[a]
2	31.57[a]	19[a]	0.28[a]

[1] Data are main effect means.

[2] Mortality data were transformed (Arcsin) prior to statistical analysis.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.4. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON HEMATOCRIT[1], MORTALITY RATE[2], AND MOUTH LESION[1] OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppb)	HEMATOCRIT (%)	MORTALITY (%)	LESION
0	0	0	35.40[b]	0[b]	0.00[c]
0	2	0	34.13[b]	0[b]	1.60[b]
0	0	150	35.33[b]	8[a]	0.20[c]
0	2	150	34.23[b]	0[b]	2.00[ab]
150	0	0	35.13[b]	0[b]	0.20[c]
150	2	0	33.43[b]	12[a]	2.40[a]
150	0	150	34.47[b]	8[a]	0.20[c]
150	2	150	38.67[a]	16[a]	2.33[a]
SEM			0.96	---[2]	0.50
			Probability		
LPS			0.3436	0.0177	0.0446
T-2			0.9610	0.3460	<.0001
AFB1			0.0987	0.0934	0.4092
LPS X T-2			0.0814	0.0177	0.1531
LPS X AFB1			0.1034	0.8551	0.3037
T-2 X AFB1			0.0319	0.1831	0.8357
LPS X T-2 X AFB1			0.0419	0.8551	0.6786

[1] Data are means of five replicates of three birds each.

[2] Mortality data were transformed (Arcsin) prior to statistical analysis.

[a, b,c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.4 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON HEMATOCRIT[1], MORTALITY RATE[2], AND MOUTH LESION[1] OF POULTS FED T-2 TOXIN AND AFLATOXIN B1

Main Effect Mean	HEMATOCRIT (%)	MORTALITY (%)	LESION
LPS, µg/bird			
0	34.77[a]	2[b]	0.95a
150	35.42[a]	9[a]	1.28[a]
T-2 toxin, ppm			
0	35.08[a]	4[a]	2.08[b]
2	35.12[a]	7[a]	0.15[a]
AFB1, ppb			
0	34.52[a]	3[a]	1.05[a]
150	35.67[a]	8[a]	1.18[a]

[1] Data are main effect means.

[2] Mortality data were transformed (Arcsin) prior to statistical analysis.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.5. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppm)	LIVER	SPLEEN	BURSA	THYMUS
			----------(%)----------			
0	0	0	3.279^{c}	0.086^{d}	0.250^{ab}	0.300^{ab}
0	2	0	2.850^{c}	0.089^{c}	0.278^{a}	0.337^{a}
0	0	2	4.600^{b}	0.153^{bc}	0.259^{ab}	0.300^{ab}
0	2	2	5.243^{a}	0.162^{b}	0.282^{a}	0.231^{b}
150	0	0	3.158^{c}	0.119^{c}	0.237^{ab}	0.298^{ab}
150	2	0	3.360^{c}	0.125^{c}	0.252^{ab}	0.312^{ab}
150	0	2	4.878^{ab}	0.179^{b}	0.200^{b}	0.268^{b}
150	2	2	5.266^{a}	0.227^{a}	0.209^{b}	0.232^{b}
SEM			0.182	0.013	0.019	0.019
			----------Probability----------			
LPS			0.1889	<.0001	0.0040	0.2904
T-2			0.1268	0.0719	0.1818	0.3282
AFB1			<.0001	<.0001	0.2284	0.0003
LPS X T-2			0.4737	0.2556	0.6493	0.8810
LPS X AFB1			0.8674	0.5245	0.0973	0.9432
T-2 X AFB1			0.0200	0.1803	0.8641	0.0068
LPS X T-2 X AFB1			0.0940	0.3175	0.9904	0.2960

[1] Data are means of five replicates of three birds each.

[a, b, c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.5 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	LIVER	SPLEEN	BURSA	THYMUS
	(%)			
LPS, µg/bird				
0	3.993[a]	0.123[b]	0.267[a]	0.292[a]
150	4.165[a]	0.162[a]	0.225[b]	0.277[a]
T-2 toxin, ppm				
0	3.978[a]	0.134[a]	0.237[a]	0.291[a]
2	4.180[a]	0.151[a]	0.255[a]	0.278[a]
AFB1, ppm				
0	3.162[b]	0.105[b]	0.255[a]	0.312[a]
2	4.996[a]	0.180[a]	0.238[a]	0.257[b]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.6. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppb)	LIVER	SPLEEN	BURSA	THYMUS
			(%)			
0	0	0	2.407^{bc}	0.117^{bc}	0.188^{a}	0.135^{a}
0	2	0	2.443^{b}	0.114^{c}	0.200^{a}	0.134^{a}
0	0	150	2.257^{c}	0.157^{ab}	0.181^{a}	0.115^{a}
0	2	150	1.933^{d}	0.125^{bc}	0.196^{a}	0.117^{a}
150	0	0	2.739^{a}	0.139^{ab}	0.188^{a}	0.128^{a}
150	2	0	2.495^{b}	0.138^{b}	0.202^{a}	0.130^{a}
150	0	150	2.366^{bc}	0.160^{a}	0.187^{a}	0.118^{a}
150	2	150	2.222^{c}	0.146^{ab}	0.200^{a}	0.118^{a}
SEM			0.057	0.007	0.014	0.008
			Probability			
LPS			<.0001	0.0025	0.7642	0.6793
T-2			0.0002	0.0231	0.1758	0.9310
AFB1			<.0001	0.0006	0.7329	0.0114
LPS X T-2			0.5382	0.3354	0.9991	0.9184
LPS X AFB1			0.9297	0.3006	0.8247	0.5031
T-2 X AFB1			0.1199	0.0623	0.9638	0.9893
LPS X T-2 X AFB1			0.0075	0.4732	0.9115	0.8156

[1] Data are means of five replicates of three birds each.

[a, b, c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.6 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON RELATIVE ORGAN WEIGHTS OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	LIVER	SPLEEN	BURSA	THYMUS
	(%)			
LPS, µg/bird				
0	3.993[b]	0.123[b]	0.267[a]	0.292[a]
150	4.165[a]	0.162[a]	0.225[a]	0.277[a]
T-2 toxin, ppm				
0	3.978[b]	0.134[b]	0.237[a]	0.291[a]
2	4.180[a]	0.151[a]	0.255[a]	0.280[a]
AFB1, ppb				
0	2.521[a]	0.127[b]	0.195[a]	0.132[a]
150	2.195[b]	0.147[a]	0.191[a]	0.117[b]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.7. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CONCENTRATIONS OF TOTAL PROTEIN, ALBUMIN, AND GLOBULIN OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppm)	TOTAL PROTEIN	ALBUMIN	GLOBULIN
			(g/100 ml)		
0	0	0	2.51[a]	1.40[a]	0.92[a]
0	2	0	2.21[ab]	1.13[b]	1.09[a]
0	0	2	1.92[b]	0.82[c]	1.10[a]
0	2	2	1.59[c]	0.61[d]	0.91[a]
150	0	0	2.17[b]	1.18[b]	0.99[a]
150	2	0	2.25[ab]	1.14[b]	1.11[a]
150	0	2	1.57[c]	0.73[cd]	0.82[a]
150	2	2	1.52[c]	0.60[d]	0.91[a]
SEM			0.11	0.05	0.12
			Probability		
LPS			0.0232	0.0287	0.5688
T-2			0.0530	<.0001	0.5759
AFB1			<.0001	<.0001	0.2820
LPS X T-2			0.0417	0.0243	0.5025
LPS X AFB1			0.6830	0.4714	0.2849
T-2 X AFB1			0.5812	0.7632	0.2494
LPS X T-2 X AFB1			0.7665	0.2807	0.3153

[1] Data are means of four replicates of three birds each.

[a, b,c, d] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.7 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CONCENTRATIONS OF TOTAL PROTEIN, ALBUMIN, AND GLOBULIN OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	TOTAL PROTEIN	ALBUMIN	GLOBULIN
	----------(g/100 ml)----------		
LPS, µg/bird			
0	2.06[a]	0.99[a]	1.00[a]
150	1.88[b]	0.91[b]	0.96[a]
T-2 toxin, ppm			
0	2.05[a]	1.03[a]	0.96[a]
2	1.89[a]	0.87[b]	1.00[a]
AFB1, ppm			
0	2.29[a]	1.21[a]	1.03[a]
2	1.65[b]	0.69[b]	0.93[a]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.8. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CONCENTRATIONS OF TOTAL PROTEIN, ALBUMIN, AND GLOBULIN OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppb)	TOTAL PROTEIN	ALBUMIN	GLOBULIN
			(g/100 ml)		
0	0	0	3.16[ab]	1.43[a]	1.80[ab]
0	2	0	2.83[b]	1.36[a]	1.47[b]
0	0	150	1.75[c]	0.78[c]	0.92[c]
0	2	150	1.71[c]	0.81[c]	0.92[c]
150	0	0	3.06[ab]	1.41[a]	1.65[ab]
150	2	0	3.36[a]	1.40[a]	1.96[a]
150	0	150	1.73[c]	0.81[c]	0.95[c]
150	2	150	2.00[c]	0.97[b]	1.12[c]
SEM			0.12	0.04	0.12
			Probability		
LPS			0.0433	0.0687	0.0891
T-2			0.5451	0.3647	0.6669
AFB1			<.0001	<.0001	<.0001
LPS X T-2			0.0099	0.0728	0.0211
LPS X AFB1			0.6405	0.1258	0.7400
T-2 X AFB1			0.4279	0.0131	0.5740
LPS X T-2 X AFB1			0.3359	0.4655	0.1496

[1] Data are means of four replicates of three birds each.

[a, b,c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.8 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CONCENTRATIONS OF TOTAL PROTEIN, ALBUMIN, AND GLOBULIN OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	TOTAL PROTEIN	ALBUMIN	GLOBULIN
	----------(g/100 ml)----------		
LPS, µg/bird			
0	2.37[b]	1.10[a]	1.28[a]
150	2.54[a]	1.14[a]	1.42[a]
T-2 toxin, ppm			
0	2.43[a]	1.11[a]	1.33[a]
2	2.48[a]	1.13[a]	1.37[a]
AFB1, ppb			
0	3.10[a]	1.40[a]	1.72[a]
150	1.80[b]	0.84[b]	0.98[b]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.9. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CHEMISTRY PARAMETERS OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppm)	Glucose	Calcium	Uric Acid	AST[2] (U/L)
			----------(mg/dL)----------			
0	0	0	297[a]	10.6[a]	6.1[a]	178[ab]
0	2	0	291[a]	9.9[ab]	4.9[a]	163[a]
0	0	2	228[b]	8.6[c]	6.7[a]	182[ab]
0	2	2	223[b]	8.7[c]	4.8[a]	195[a]
150	0	0	312[a]	9.7[b]	5.6[a]	170[ab]
150	2	0	281[a]	9.9[ab]	5.3[a]	143[b]
150	0	2	223[b]	8.6[c]	5.9[a]	153[b]
150	2	2	243[b]	9.5[b]	6.2[a]	133[b]
SEM			12.7	0.25	0.67	11.3
			----------Probability----------			
LPS			0.5869	0.8092	0.7799	0.0010
T-2			0.5606	0.5588	0.1233	0.1318
AFB1			<.0001	<.0001	0.3500	0.7908
LPS X T-2			0.9698	0.0268	0.1334	0.1686
LPS X AFB1			0.7561	0.0281	0.7404	0.0596
T-2 X AFB1			0.1638	0.0460	0.9879	0.2859
LPS X T-2 X AFB1			0.1731	0.8630	0.4940	0.5261

[1] Data are means of four replicates of three birds each.

[2] AST = aspartate aminotransferase.

[a, b, c] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.9 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CHEMISTRY PARAMETERS OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	Glucose	Calcium	Uric Acid	AST[2] (U/L)
	----------(mg/dL)----------			
LPS, µg/bird				
0	260[a]	9.4[a]	5.6[a]	180[a]
150	265[a]	9.4[a]	5.8[a]	150[b]
T-2 toxin, ppm				
0	265[a]	9.4[a]	6.1[a]	171[a]
2	260[a]	9.5[a]	5.3[a]	159[a]
AFB1, ppb				
0	295[a]	10.0[a]	5.5[a]	164[a]
2	230[b]	8.9[b]	5.9[a]	166[a]

[1] Data are main effect means.

[2] AST = Aspartate aminotransferase

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.10. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CHEMISTRY PARAMETERS OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	T-2 (ppm)	AFB1 (ppb)	Glucose	Calcium	Uric Acid	AST[2]	Creatine Kinase
			----------(mg/dL)----------			----------(U/L)---------	
0	0	0	332[a]	10.9[a]	5.5[a]	264[b]	1600[b]
0	2	0	318[ab]	10.4[a]	5.2[a]	255[b]	1571[b]
0	0	150	273[b]	9.2[b]	6.2[a]	277[ab]	1800[ab]
0	2	150	293[ab]	8.7[b]	5.6[a]	296[a]	2115[a]
150	0	0	289[ab]	10.7[a]	7.1[a]	245[b]	1379[b]
150	2	0	298[ab]	10.2[a]	5.7[a]	252[b]	1340[b]
150	0	150	268[b]	8.8[b]	5.7[a]	274[ab]	1333[b]
150	2	150	267[b]	8.6[b]	6.0[a]	283[ab]	1442[b]
SEM			18.5	0.26	0.88	8.16	148
			----------Probability----------				
LPS			0.0822	0.2542	0.4152	0.1181	0.0009
T-2			0.8043	0.0228	0.4190	0.2551	0.4057
AFB1			0.0153	<.0001	0.9828	<.0001	0.0683
LPS X T-2			0.9610	0.7931	0.9274	0.8331	0.6105
LPS X AFB1			0.5532	0.8890	0.3722	0.7616	0.1139
T-2 X AFB1			0.6421	0.6597	0.6118	0.2098	0.2532
LPS X T-2 X AFB1			0.4018	0.7505	0.4456	0.2727	0.6446

[1] Data are means of four replicates of three birds each.

[2] AST = aspartate aminotransferase.

[a, b] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.10 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON SERUM CHEMISTRY PARAMETERS OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	Glucose	Calcium	Uric Acid	AST[2]	Creatine Kinase
	----------(mg/dL)----------			----------(U/L)---------	
LPS, µg/bird					
0	304[a]	9.8[a]	5.6[a]	273[a]	1772[a]
150	280[a]	9.6[a]	6.1[a]	264[a]	1374[b]
T-2 toxin, ppm					
0	291[a]	9.9[a]	6.1[a]	265[a]	1528[a]
2	294[a]	9.5[b]	5.6[a]	272[a]	1617[a]
AFB1, ppb					
0	309[a]	10.6[a]	5.9[a]	254[a]	1472[a]
150	275[b]	8.8[b]	5.9[a]	282[b]	1673[a]

[1] Data are main effect means.

[2] AST = Aspartate aminotransferase

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$).

TABLE 5.11: EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON GLUTATHIONE (GSH) CONCENTRATION OF LIVER HOMOGENATES OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	**T-2 (ppm)**	**AFB1 (ppm)**	**GLUTATHIONE (mmol/100 uL)**
0	0	0	7.89[ab]
0	2	0	6.70[b]
0	0	2	8.41[a]
0	2	2	8.29[a]
150	0	0	6.24[b]
150	2	0	7.55[ab]
150	0	2	8.10[ab]
150	2	2	6.92[ab]
SEM			0.49
			Probability
LPS			0.0950
T-2			0.4201
AFB1			0.0280
LPS X T-2			0.3220
LPS X AFB1			0.5435
T-2 X AFB1			0.3288
LPS X T-2 X AFB1			0.0200

[1] Data are means of four replicates of two birds each.

[a, b] Values within columns with different superscripts differ significantly ($P < 0.05$).

TABLE 5.11 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON GLUTATHIONE (GSH) CONCENTRATION OF LIVER HOMOGENATES OF BROILER CHICKS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	GLUTATHIONE (mmol/100 uL)
LPS, µg/bird	
0	7.82[a]
150	7.20[a]
T-2, ppm	
0	7.66[a]
2	7.37[a]
AFB1, ppm	
0	7.10[b]
2	7.93[a]

[1] Data are main effect means.

[a, b] Values within columns, in main effect, with different superscripts differ significantly ($P < 0.05$)

TABLE 5.12. EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON GLUTATHIONE (GSH) CONCENTRATION OF LIVER HOMOGENATES OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

LPS (µg/bird)	**T-2 (ppm)**	**AFB1 (ppb)**	**GLUTATHIONE (mmol/100 uL)**
0	0	0	2.58
0	2	0	2.40
0	0	150	2.43
0	2	150	2.51
150	0	0	2.48
150	2	0	2.56
150	0	150	2.25
150	2	150	2.38
SEM			0.12
			Probability
LPS			0.4271
T-2			0.7367
AFB1			0.1893
LPS X T-2			0.3537
LPS X AFB1			0.2767
T-2 X AFB1			0.3511
LPS X T-2 X AFB1			0.5477

[1] Data are means of four replicates of two birds each.

TABLE 5.12 (CONT). EFFECTS OF ENDOTOXIC LIPOPOLYSACCHARIDE ON GLUTATHIONE (GSH) CONCENTRATION OF LIVER HOMOGENATES OF TURKEY POULTS FED T-2 TOXIN AND AFLATOXIN B1[1]

Main Effect Mean	GLUTATHIONE (mmol/100 uL)
LPS, µg/bird	
0	2.48
150	2.42
T-2, ppm	
0	2.44
2	2.46
AFB1, ppb	
0	2.51
150	2.39

[1] Data are main effect means.

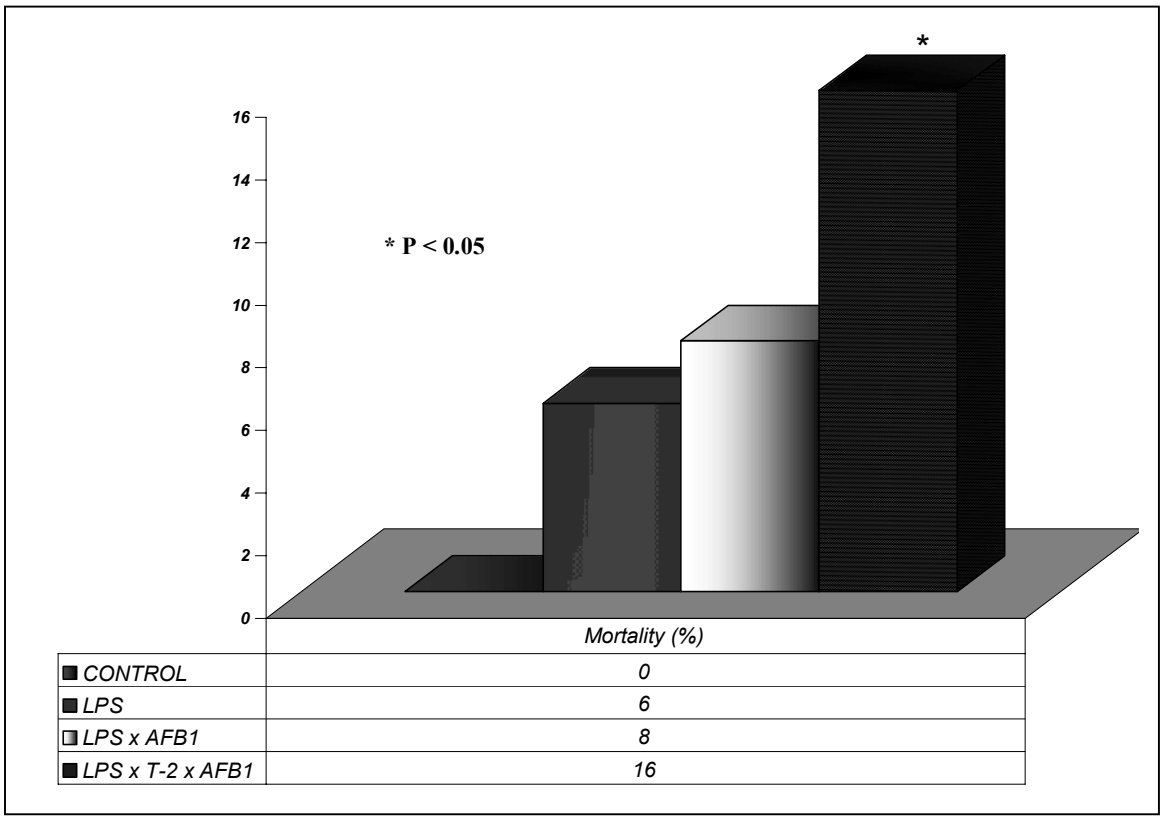


Figure 5.1. Mortality of broiler chicks after the first injection containing endotoxic LPS

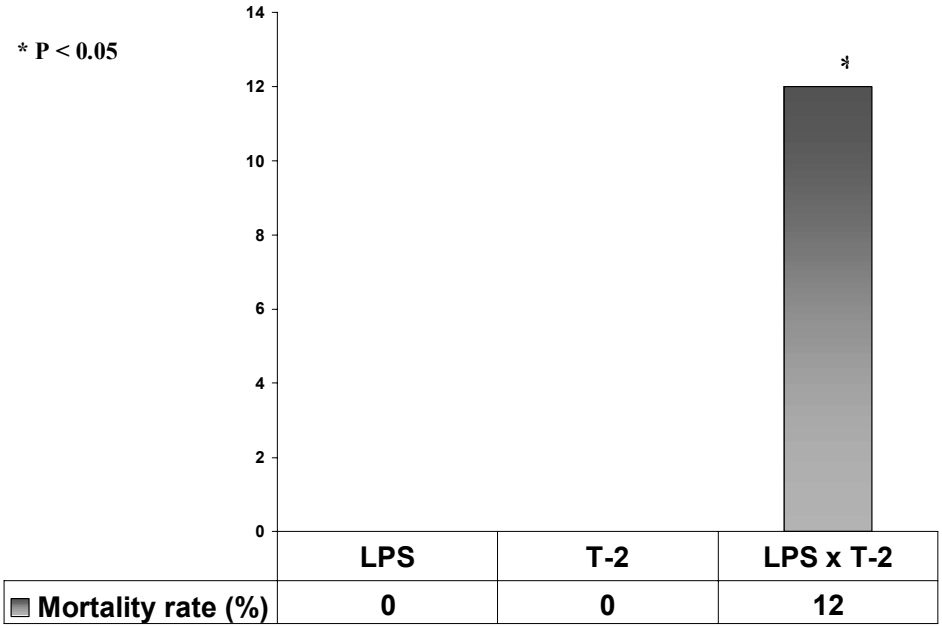
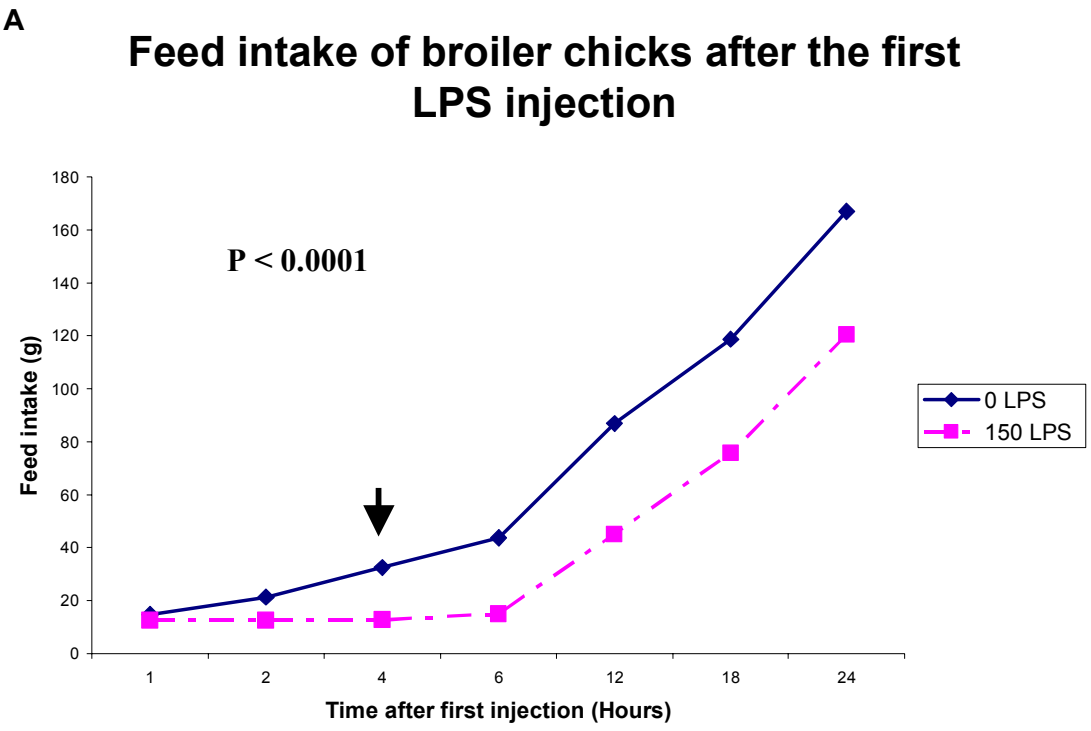
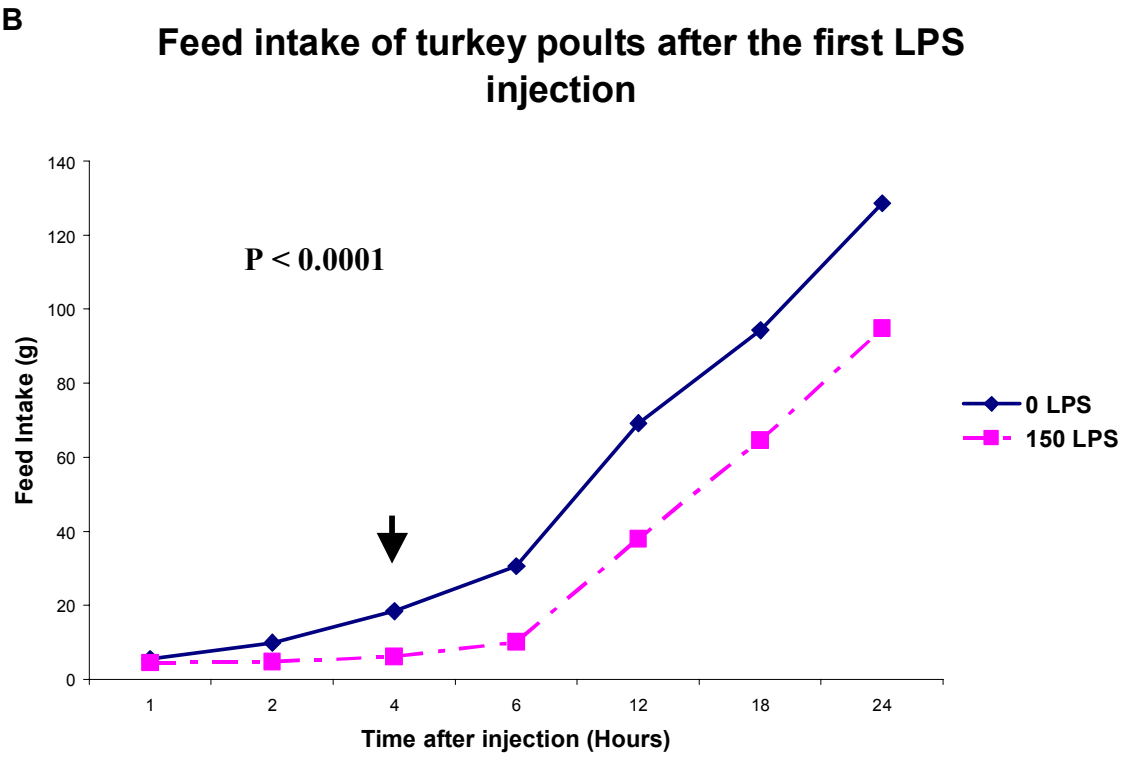


Figure 5.2. Interaction of endotoxic lipopolysaccharide and T-2 on mortality rate of turkey poults

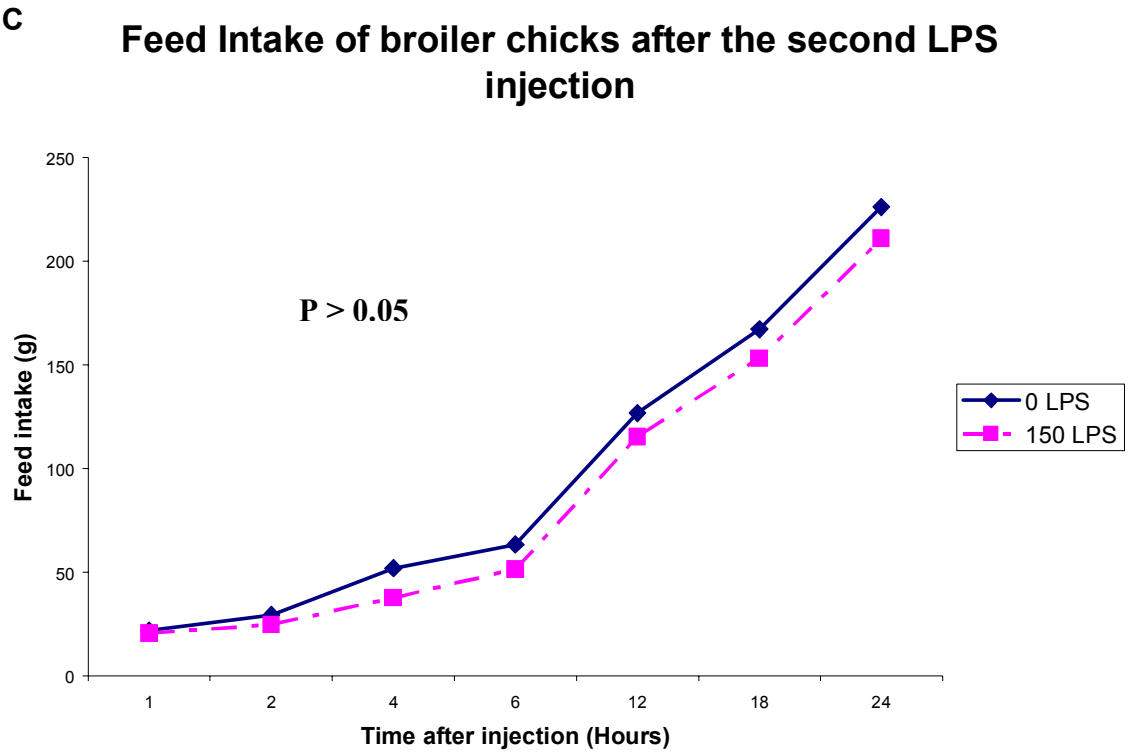
A


Feed intake of broiler chicks after the first LPS injection
P < 0.0001
Feed intake (g)
180
160
140
120
100
80
60
40
20
0
1
2
4
6
12
18
24
Time after first injection (Hours)
0 LPS
150 LPS

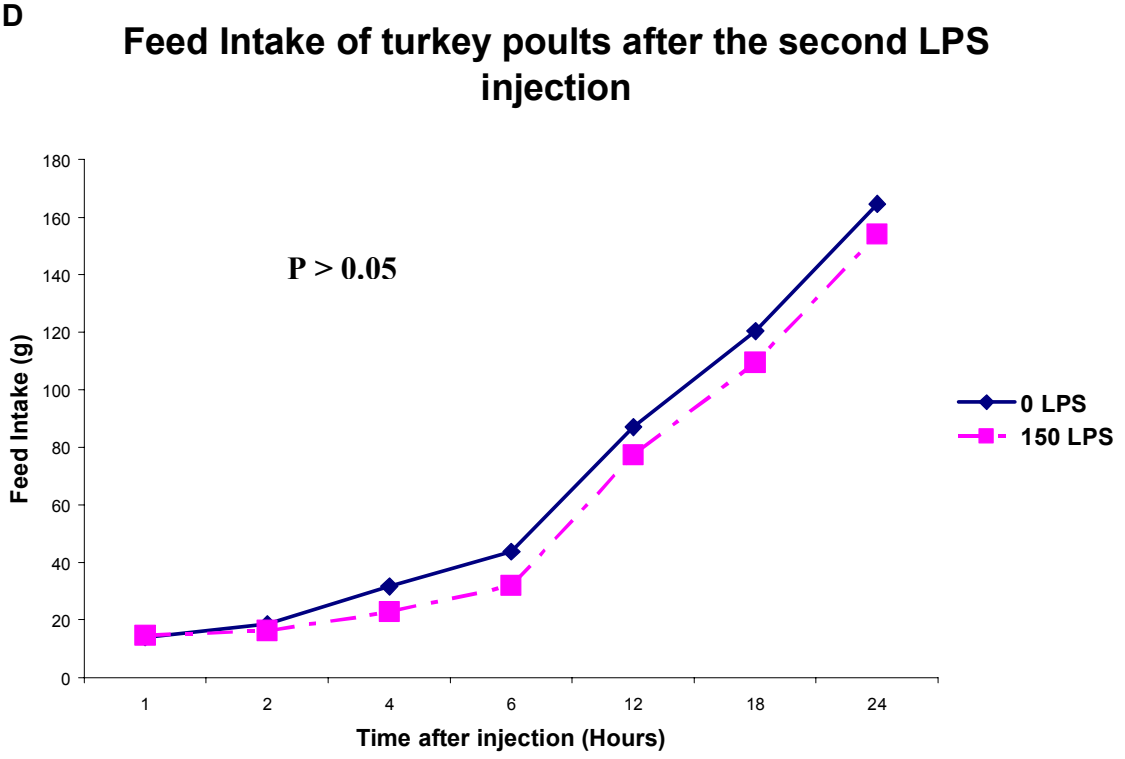
B


Feed intake of turkey poults after the first LPS injection
P < 0.0001
Feed Intake (g)
140
120
100
80
60
40
20
0
1
2
4
6
12
18
24
Time after injection (Hours)
0 LPS
150 LPS

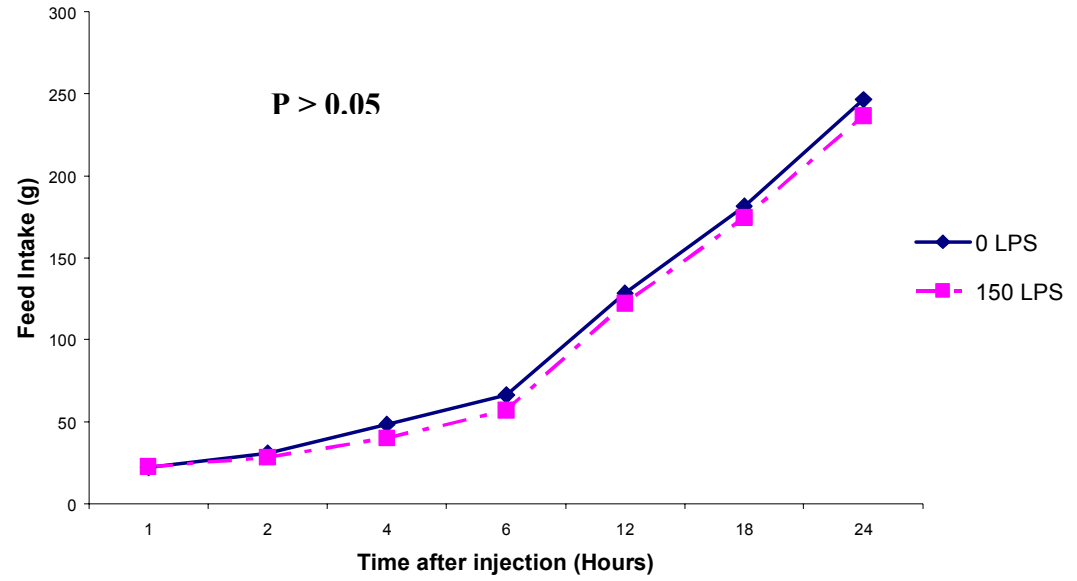
C


Feed Intake of broiler chicks after the second LPS injection
P > 0.05
Feed intake (g)
250
200
150
100
50
0
1
2
4
6
12
18
24
Time after injection (Hours)
0 LPS
150 LPS

D


Feed Intake of turkey poults after the second LPS injection
P > 0.05
Feed Intake (g)
180
160
140
120
100
80
60
40
20
0
1
2
4
6
12
18
24
Time after injection (Hours)
0 LPS
150 LPS

E



F

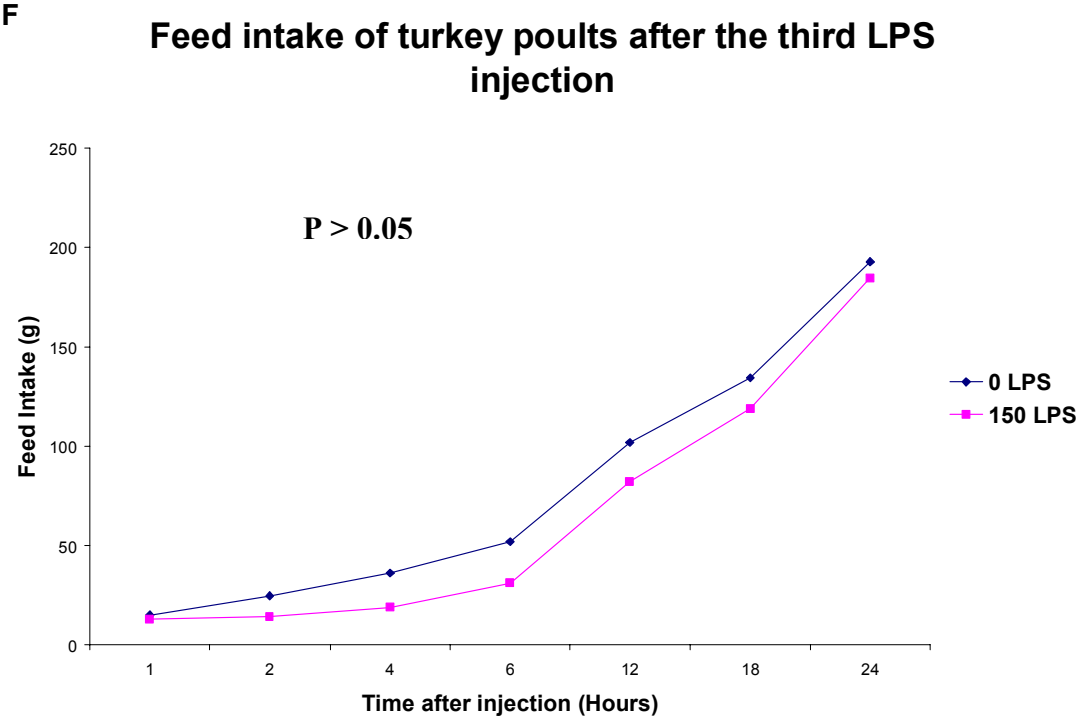


Figure 5.3. Effect of three consecutive LPS injections on feed intake of chicks and poults. Orthogonal contrasts were designed to compare LPS- to saline-treated groups.

CHAPTER VI

CONCLUSION

Aflatoxin B1 (AFB1) causes poor performance and is hepatotoxic in animals. Endotoxic lipopolysaccharide (LPS), a constituent of cell walls of gram negative bacteria, initiates an inflammatory response in animals. In rodents, nontoxic doses of LPS augment the effects of AFB1. Two studies (Exp.1 and 2) were conducted to determine the effects of chronic exposure to low doses of *E. coli* LPS in chicks and poults fed diets contaminated with non toxic doses of aflatoxin B1. Chicks were fed 0, 2, or 4 mg AFB1/kg diet, whereas poults were fed 0, 100, or 150 µg AFB1/kg diet for 21 days. Beginning on day 7, chicks were injected intraperitoneally with 0, 200, or 400 µg LPS/bird every other day, whereas poults were injected with 0, 100, or 150 µg LPS/bird on alternate days. Based on the results obtained, it can be concluded that at the levels chosen chronic exposure to low doses of LPS did not potentiate the effects of dietary non toxic doses of AFB1 on performance of chicks and poults fed from hatch to day 21. However, for mortality rate, results suggest a toxic synergy between AFB1 and LPS in chicks and poults.

In Exp. 3 and 4, the effects of chronic exposure to low doses of *E. coli* LPS in combination with feeding diets contaminated with non toxic doses of T-2 toxin were determined in chicks and poults fed from hatch to day 21. Chicks and poults were fed 0, 1, or 2 mg T-2/kg diet. Beginning on day 7, chicks and poults were injected intraperitoneally with 0, 100, or 150 µg LPS/bird every other day. Based on the results

from these experiments, it can be concluded that, at the levels chosen, chronic exposure to LPS did not potentiate the effects of T-2 toxin in chicks and poults. In poults, however, the decrease in performance observed in birds fed T-2 alone was atypical of a number of studies conducted in which decrease in performance was only observed when poults were fed at least 5 mg/kg T-2.

Finally, Exp. 5 and 6 were conducted to determine the effects of chronic exposure to low doses of *E. coli* lipopolysaccharide (LPS) in chicks and poults fed diets contaminated with non toxic doses of aflatoxin B1 (AFB1) and T-2 toxin (T-2) from hatch to day 21. Birds were fed diets containing 0 or 2 mg T-2/kg diet singly or in combination with 0 or 2 mg AFB1/kg (chicks) or 0 or 0.15 mg/kg AFB1 (poults). Beginning on day 7, chicks and poults were injected intraperitoneally with 0 or 150 µg LPS/bird every other day. In chicks and poults, LPS decreased FI which lasted for 6 hours after the first LPS injection. Based on the results obtained, it can be concluded that at the levels chosen chronic exposure to low doses of LPS did not potentiate the effects of dietary non toxic doses of T-2 and AFB1 on performance of chicks and poults fed from hatch to day 21. However, LPS did potentiate the effects of T-2 on mortality rate and oral lesions in poults and, after the first LPS injection, decreased feed intake for 6 hours. LPS also enhanced mortality rate in broiler chicks after the first LPS injection and decreased feed intake for 6 hours after the first injection containing LPS.

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