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H. V. Shirley, Major Professor

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Accepted for the Council:

Vice Chancellor
Graduate Studies and Research

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A STUDY OF OCHRATOXIN TOXICITY IN LAYING HENS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Saad Hussain Tohala

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ABSTRACT

Ochratoxin at levels of 0, 0.25, 0.5, 1, and 2 ppm (mg per kg feed) was fed to 280 S.C.W.L. hens for twelve weeks. Economic traits such as egg production, egg weight, egg quality, body weight, feed consumption, water consumption, urine and urate excretion, comb color, and mortality were studied. At the end of the test, twelve birds from each treatment were killed to observe macroscopical renal and liver changes. Liver weights were taken and kidney slides were made for histological examination. Analysis of variance was conducted for each trait and comparisons between treatment one, the control, and each of the other treatments were made.

All of the treated groups were lower than the control group in percent hen-day egg production, but differences were not statistically significant. Egg weights were reduced significantly ($p \leq .05$) for the 2 ppm group during weeks 8 through 12 and for the 1 ppm group during weeks 11 and 12. The 0.5, 1, and 2 ppm levels of ochratoxin increased significantly ($p \leq .05$) the percentages of egg blood and meat spots at 8 weeks of the experiment. All levels of ochratoxin reduced significantly the egg specific gravity score at the end of the experiment and the extent of reduction was proportional to the level of ochratoxin. The 0.5, 1, and 2 ppm levels of ochratoxin significantly reduced body weight at 4 weeks, but the highest body weight loss occurred at 12 weeks for the 1 and 2 ppm groups. Water consumption, mortality rate, and average liver weight were significantly increased ($p \leq .05$) only in the hens receiving 2 ppm of ochratoxin.

There was no significant effect of any level of ochratoxin on egg Haugh units, feed consumption, or urine and urate excretion.

The 1 and 2 ppm ochratoxin groups were significantly lower than the control group in the percent of hens with red comb color ($p \leq .05$) and had a lower number of birds with normal livers and kidneys. They were significantly higher than the control group in the percent of hens with pale comb color ($p \leq .05$) and had a higher number of birds with abnormal livers and pale kidneys. Microscopical examination did not show clear histophathologic alterations in the kidneys.

Generally, it can be concluded that each trait studied was affected by some level of ochratoxin. Some traits were affected by 0.25 ppm ochratoxin and above, while other traits were not affected by even 2 ppm ochratoxin. Some symptoms of ochratoxicosis appeared early in the experiment, while other symptoms appeared only towards the end of the experiment. Some of the effects were proportional to the level of ochratoxin or became significant as the duration of exposure increased.

The effects of ochratoxin at levels used in this study on laying hens were generally not severe. These levels can be considered lower than the level required to produce acute ochratoxicosis.

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CHAPTER I

INTRODUCTION

Ochratoxins, mold-produced substances, comprise a small group of structurally related metabolites. Several species of the genera Aspergillus and Penicillium have been reported as ochratoxin producers. The ochratoxins have been designated A, B, C, and D. Ochratoxin A is the major metabolite both in respect to occurrence and toxicity. Thus, for all practical purposes, only ochratoxin A is a natural contaminant of foods and feedstuffs. The fungi and the ochratoxin are widely distributed in stored feed ingredients that might be used in poultry feeds.

Ochratoxicosis, a disease caused by ochratoxin, has been documented in broilers, layers, turkeys, and several mammalian species. In most species the biological target organ of ochratoxin A appears to be the kidney although the liver is also affected to a lesser degree. Livers from affected birds exhibit a tan colored appearance, and the kidneys are pale and enlarged. The changes of renal structure are characterized by degeneration of the tubular epithelium in the form of dilated tubules. The changes in renal function are characterized by impairment of glomerular and tubular functions resulting in polyuria and polydipsia.

Effects of ochratoxin on laying hens depend on the level of ochratoxin and duration of exposure. Studies of ochratoxicosis in laying hens reveal that ochratoxin A cause a decrease in egg

production, egg weight, feed consumption, and body weight. Other clinical signs are paleness of the comb and wattles, diarrhea, and increased mortality rate.

It was reported that four outbreaks of disease involving six million chickens were associated with consumption of corn or corn-gluten meal containing ochratoxin A. Therefore the potential for serious economic losses resulting from ochratoxin contamination of feed is great.

Ochratoxins continue to receive attention and concern by researchers and poultry producers throughout the world, but they have not been studied thoroughly enough to fully predict their effects on poultry.

An objective of the present study was to associate graded levels of ochratoxin in the feed with the appearance of each of the several symptoms generally associated with ochratoxicosis.

CHAPTER II

REVIEW OF LITERATURE

Chemical Structure

The ochratoxins were the first major group of fungal toxins to be characterized after the discovery of the aflatoxins (van der Merwe et al., 1965). The ochratoxins comprise a group of chemically closely related secondary metabolites produced by species of the genera Aspergillus and Penicillium. Chemically, they are isocoumarin compounds linked to L-B-phenylalanine (Figure 1). The ochratoxins have been designated A, B, C, and D (4-hydroxyochratoxin A). These and the methyl and ethyl esters have been isolated from fungal cultures. Ochratoxin A contains chlorine, ochratoxin B is the dechloro analog and ochratoxin C is the ethylester of ochratoxin A (Steyn, 1977). Ochratoxin A is the major metabolite both in respect to occurrence and toxicity. Under natural, non-laboratory conditions, only ochratoxin A and, very rarely, ochratoxin B have been encountered. Thus, for all practical purposes, only ochratoxin A is a natural contaminant of foods and feedstuffs. Ochratoxin A is the product of an enzymic reaction (ochratoxin A synthetase) that couples L-B-phenylalanine to the dihydroisocoumarin moiety (Steyn, 1977; Chu, 1974).

Stability

Ochratoxin A is a stable compound and can be stored in ethanol at refrigeration temperatures for more than a year without loss (Chu

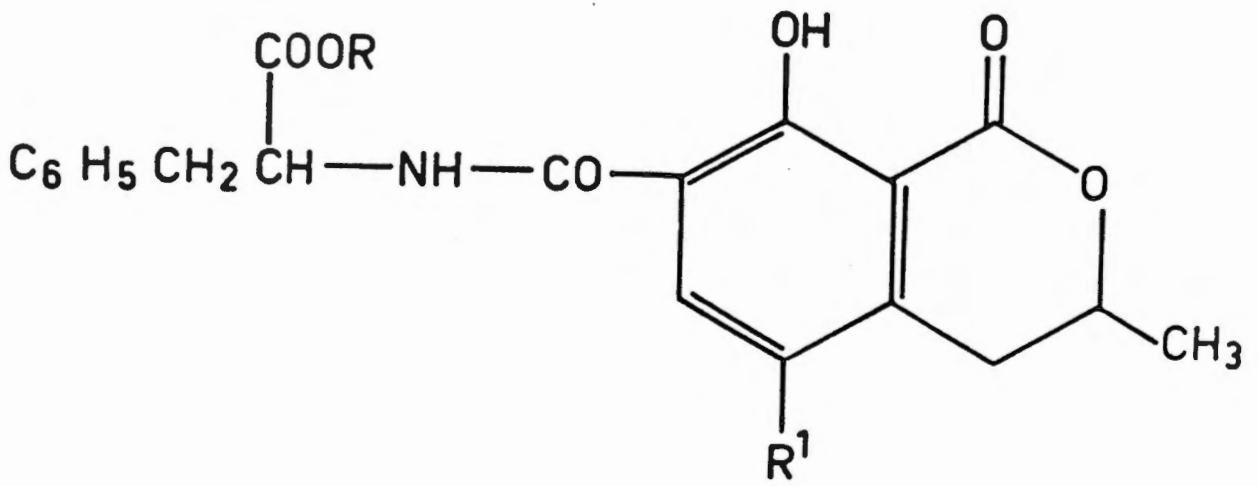


Figure 1. The chemical structure of the ochratoxins.

Ochratoxin A: R=H; R_1=Cl;
Ochratoxin B: R=H; R_1=H;
Ochratoxin C: R=C_2H_5; R_1=Cl;
Ochratoxin D: 4-Hydroxyochratoxin A
Methyl ester of ochratoxin A: R=CH_3;
R_1=Cl; methyl and ethylester of ochratoxin B:
R=CH_3 or C_2H_5; R_1=H.

and Butz, 1970). In cereal products some degradation occurs with time. Thus, Trenk et al. (1971) reovered only 36-47% of ochratoxin A added to oatmeal and rice and stored for 12 weeks at 28°C. During autoclaving of cereals, 12.5-17% of added ochratoxin A remained after 0.5 to 3 hours; when water was added, 26-35% survived the same treatment (Trenk et al., 1971). Harwig et al. (1974) concluded that a significant amount of any ochratoxin A in raw beans would likely survive practical canning procedures.

Considerable degradation of ochratoxin A occurs during the brewing of beer. Krogh et al. (1974) studied the fate of ochratoxin A in malting and brewing experiments using naturally contaminated barley and by the addition of crystalline mycotoxin to the mash. There was considerable loss of ochratoxin A during the brewing processes and the final beer contained 2-7% of the inital amount of ochratoxin A representing concentrations of 11-20 µg/liter.

Fungal Formation

The ochratoxin-producing fungi are included in the genera *Aspergillus* and *Penicillium*. Seven species of *Aspergillus* and seven species of *Penicillium* have been reported as producers (Krogh, 1978; Ciegler, 1972). The ochratoxins were first isolated from *A. ochraceus* (van der Merwe et al., 1965) and members of this group may be important toxin producers in tropical-subtropical areas. The penicillia, in particular *P. viridicatum*, are the major producers under colder climatic conditions (Krogh, 1978). Not all strains of the species mentioned are capable of ochratoxin production. Toxin

formation can occur in most plant products and production is determined by two factors: temperature and water activity (water content) of the substrate. The temperature and water activity conditions required for fungal growth and ochratoxin A production have been determined for several strains of the three species, A. ochraceus, P. cyclopium and P. viridicatum (Bacon et al., 1973; Harwig and Chen, 1974; Northolt et al., 1979). It appears that the minimum temperature for ochratoxin A production is 4°C for the penicillia and 12°C for A. ochraceus. For ochratoxin production by P. viridicatum, 18.5% water is sufficient in wheat, whereas 21.6% water is needed in corn (Northolt et al., 1979). Fungal growth, however, can occur at lower water content (water activity) than toxin production. Water is generated during fungal metabolism and once fungal growth is established the water activity conditions suitable for toxin production may be created. Thus, prevention of fungal growth is the only efficient means of prevention of ochratoxin contamination of grains and other feedstuff.

Yamazaki et al. (1970) reported the production of large amounts of ochratoxin A by A. ochraceus in a nutrient solution containing 1% L-phenylalanine and 2% yeast extract. Sansing et al. (1973) found that peak production of ochratoxin A by an isolate of A. ochraceus occurred at 25°C after 10-12 days of incubation on a nutrient solution of 4% sucrose and 2% yeast extract in stationary cultures. High pH values of 7.6-8.2 coincided with high ochratoxin production.

Lai et al. (1970) investigated culture conditions for ochratoxin A production by species of Aspergillus. Greatest yields of

ochratoxin A (155 mg/liter) by A. sulphureus was obtained at pH 6.0-6.3 within 8-10 days of incubation. Glucose and sucrose were the best carbon sources for toxin production. Some species such as A. ochraceus and A. melleus required the addition of certain trace minerals for toxin production.

Occurrence

Ochratoxin A, as a natural contaminant, was first reported by Shotwell et al. (1969) in a survey of corn. Since that time the mycotoxin has been found in a variety of plant products in countries of North America and Europe (Carlton and Krogh, 1979).

Most of the field contamination has occurred in cereals, especially corn, oats, wheat, and barley. However, the mycotoxin has been found in samples of coffee, peanuts, and beans. Concentrations have ranged from 5 to 27,500 µg/kg of commodity (Krogh et al., 1973; Shotwell et al., 1976).

When farm animals (pigs, poultry) are exposed to ochratoxin A-contaminated feed, a portion of the ingested toxin will be retained as residues in the tissue; these residues are eliminated exponentially, with RL_{50} values in the range of 3.3-4.5 days (Krogh et al., 1976). Thus, carryover of ochratoxin A from contaminated feed to food of animal origin is possible. Surveys at meat plants in Denmark and Sweden have revealed that tissues from 25-35% of pigs suffering from nephropathy contain residues of ochratoxin A (Krogh, 1977). Concentrations of $\geq$2 ppb were found in 21/60 kidneys (35%) from slaughtered pigs; highest concentration was 68 ppb (Krogh, 1976).

A feed-associated chronic renal disease in pigs, porcine nephropathy, has been known in Denmark for more than half a century (Krogh and Elling, 1977). The disease is characterized by tubular atrophy, interstitial cortical fibrosis, and hyalinized glomeruli in advanced cases. At slaughter, the kidneys are enlarged and pale with an uneven cortical surface. The cortical fibrosis is visible on the cut surface in severely affected kidneys. Ochratoxin A is the major causal determinant of this disease, but other nephrotoxic mycotoxins such as citrinin, may be involved (Krogh, 1976b, 1978). Using meat inspection data from slaughter houses, the epidemiology of porcine nephropathy in Denmark was elucidated (Krogh, 1976a). The disease is endemic with a prevalence rate of 10-20 cases per 10,000 pigs. After unfavorable climatic conditions, such as high precipitation during harvest or drought in the growing season of cereal plants, outbreaks have been encountered during the subsequent feeding period of these crops, reaching 80-90 cases per 10,000 pigs. Samples obtained from the epidemic districts in 1971 were analyzed and ochratoxin A was found in 50-65% of the samples and citrinin was present in 0-11% of the feed samples (Krogh et al., 1973). In a survey in Denmark of kidneys of pigs with nephropathy, 21 out of 60 kidneys (=35%) contained residue of ochratoxin A (Krogh, 1977). Cases of porcine nephropathy causually associated with feedborne ochrotoxin A have been reported in Sweden (Rutgvist et al., 1978).

Methods of Analysis of Ochratoxin

The ochratoxin acids and esters exhibit fluorescence when exposed to ultraviolet (uv) light and this phenomenon is the basis for a number of thin-layer chromatographic (TLC) and minicolumn procedures. All methods rely on final estimation of the toxin concentration by visual or fluorodensitometric comparison with standard ochratoxin A (Carlton and Krogh, 1979). On TLC, ochratoxin A is seen as a green fluorescent spot under longwave uv light (Chu, 1970). By exposing the TLC plates to ammonia fumes, the fluorescence is blue and more intense and, thereby, the sensitivity is increased (Trenk and Chu, 1971). This modification has been used in analysis of animal tissue for residues of ochratoxin A and as little as 2-3 μg/kg can be measured in renal tissues (Krogh et al., 1974). The TLC procedure by Nesheim et al. (1973) has been collaboratively studied (Nesheim, 1973), and has been accepted by the Association of Official Analytical Chemists as the official, first action method. The procedure detects both ochratoxin acids and esters; however, because only ochratoxin A, and vey rarely ochratoxin B, are naturally occurring contaminants, the procedure can be simplified by omitting the step for ester direction.

A spectrophotometric procedure has been developed (Hult and Gatenbech, 1976) based upon enzymatic cleavage of ochratoxin A by carboxypeptidase A. The quantitation of ochratoxin A is based on the loss of fluorescence intensity (sensitivity: 4 μg/kg). This procedure has been used in Sweden for analysis of pig kidneys for residues of ochratoxin A.

Ochratoxin Influence on Poultry

Elling et al. (1975) described a spontaneous nephropathy in chickens at a poultry slaughterhouse. Macroscopically, affected kidneys were enlarged and pale. In 5 of the 14 birds with abnormal kidneys, ochratoxin A was detected in muscle in amounts ranging from 4.3 to 29.2 ppb. Four of the birds with ochratoxin A residues had renal disease characterized by atrophy and degeneration of the epithelium of the proximal and distal tubules and by the presence of interstitial fibrosis.

Hamilton et al. (1977) reported that four outbreaks of disease involving 6 million chickens were associated with consumption of corn or corn gluten meal containing ochratoxin A at concentrations up to 2 ppm. Clinical signs included poor growth and feed conversion.

Chu and Chang (1971) administered ochratoxin A, the methylester of ochratoxin A, ochratoxin C, and dihydroisocoumarin to one-day-old New Hampshire Leghorn cross chicks by oral intubation. Ochratoxin A and dihydroisocoumarin were dissolved in sterilized water and ochratoxin C and the methylester of ochratoxin A were dissolved in corn oil. The LD_{50} was 166 µg/chick for ochratoxin A and it was 216 µg/chick for ochratoxin C. Chicks given doses greater than 300 µg/chick died within 24-76 hours post-administration. All chicks given 74 or 100 µg/chick of the methylester of ochratoxin A survived. Weight gains were reduced in all ochratoxin-administered groups. Hemorrhages in the liver and kidney as well as enteritis have been reported. Dihydroisocoumarin given at 500 µg/chick was

not toxic; none of the chicks died and the weight gains were comparable to control chicks.

Peckham et al. (1971) orally administered ochratoxin A and ochratoxin B dissolved in 0.1 M aqueous sodium bicarbonate to one-day-old Babcock B-300 cockerels. The single oral dose 7-day LD_{50} for ochratoxin A was 116-135 µg/chick (3.3-3.9 mg/kg body weight). The estimated 7-day LD_{50} for ochratoxin B was 1890 µg/chick (54 mg/kg body weight). Sublethal doses of both ochratoxin A and ochratoxin B resulted in reduced body weight gains and the reduction was roughly proportional to the dose of the ochratoxin. Some of the chicks had lesions of visceral gout consisting of white, flakelike deposits on the kidney, in the ureter and over the surface of the heart, pericardium, liver, and spleen.

Histopathologic alterations were acute tubular nephrosis characterized by proteinaceous and urate casts and focal necrosis of renal tubular epithelium. Some chicks had mild diffuse cytoplasmic vacuolation and focal necrosis of hepatocytes. Hematopoiesis was suppressed in the bone marrow and the lymphoid cells of the spleen and bursa of Fabricius were depleted.

Tucker and Hamilton (1971) administered ochratoxin A to one-day-old male broiler chicks at dietary concentrations of 0.5, 1, 2, 4, or 8 ppm for 3 weeks. Growth was depressed at all dietary concentrations. Mortality was high at dietary concentrations of 4 and 8 ppm. Relative weights of the crop, pancreas, liver, and gizzard were increased significantly while the weight of the bursa of Fabricius was decreased.

Huff and coworkers (1974) fed ochratoxin A to one-day-old male broiler chicks at dietary concentrations of 0.5, 1, 2, 4, and 8 ppm for 2 weeks. Their studies also included determination of LD_{50}'s in one-day-old and 3-week-old chicks by oral intubation of ochratoxin A dissolved in 0.1 M sodium bicarbonate. Dietary concentrations of 2 ppm and above reduced body weight gains. The reduction was dependent on the dietary concentration of ochratoxin A and on the period of administration. The oral 7-day LD_{50} of ochratoxin A was 2.14 ± 0.37 mg/kg body weight for day-old chicks and for 3-week-old chickens it was 3.60 ± 0.57 mg/kg body weight. The clinical signs of acute ochratoxicosis A in 3-week-old chickens included depression, huddling, diarrhea, and prostration followed by death within 22-25 hours after a single oral dose of 16 mg/kg body weight. Necropsy findings included paleness of the liver, pancreas, and kidneys. The weight of the kidney relative to body weight was increased at dietary concentrations of 1 ppm and above. The results of dry weight ratios indicated that some part of the renal enlargement was due to edema. Plasma uric acid concentrations were increased and serum potassium concentrations were decreased at dietary concentrations of 4 and 8 ppm. Histopathologic alterations in the kidney were swelling of tubular epithelium, dilatation of tubular lumens and presence of proteinaceous casts in tubular lumens (Huff et al., 1975). Huff and Hamilton (1975) reported that the concentration of plasma carotenoids was significantly decreased at dietary concentrations of 4 and 8 ppm and the reduction was about 80% at the higher dietary concentration. Ochratoxin A at dietary concentrations of 4 and 8 ppm increased

recalcification and prothrombin times, but did not alter whole blood clotting time (Doerr et al., 1974). Doerr et al. (1976) reported that ochratoxin A reduced in the blood of broiler chickens the concentrations of clotting factors V, VII, and X with V being reduced at ochratoxin A dietary levels too small to affect body weight gains. Mean survival time of chicks fed 4.0 and 8 ppm ochratoxin was decreased by exposure to 4°C and 90% relative humidity, but survival time was increased when birds were exposed to 43°C and 45% relative humidity. A water source of 2% saline decreased survival time in birds fed 8 ppm ochratoxin A (Huff and Hamilton, 1975). The effects of mycotoxins on certain parameters of digestion in broiler chicks was reported (Osborne et al., 1976). The study included fecal lipid content, bile concentration, activities of pancreatic lipase, amylase, trypsin, and DNase and RNase. Ochratoxin A had no effect on any of the parameters except for a slight increase in volume of bile.

Studies of bone characteristics in broiler chickens was reported (Huff et al., 1977). Ochratoxin A feeding increased tibial diameter relative to body weight. The force required to break the tibia was decreased by ochratoxin A-feeding and the displacement at failure was increased.

Galtier et al. (1976) administered ochratoxin A dissolved in isotonic sodium bicarbonate (dosage volume 5 ml/kg body weight) by oral intubation to Leghorn chicks as a single dose varying from 4 to 16 mg/kg on day 10 post-hatching or as multiple doses varying from 8 to 24 mg/kg on days 5 to 14 post-hatching. The single dose LD_{50} was 10.7 mg/kg body weight (10 day old chicks) and for the multiple

dose regiment, the LD_{50} was about 14 mg/kg body weight. Clinical signs of toxicity were reduced spontaneous activity, huddling, hypothermia, diarrhea, and reduced body weight gains. At necropsy, the liver and kidneys were pale. Histopathologic alterations were focal necrosis and fatty change in the liver, cytoplasmic vacuolation of renal tubular epithelium and the presence of hyaline casts in tubular lumens.

Prior et al. (1976) administered by oral (esophageal) intubation ochratoxin A dissolved in 0.1 M sodium bicarbonate solution to one-day-old H and N White Leghorn pullets. The single dose 7-day LD_{50} was 3.4 $\pm$ 0.14 mg/kg body weight. Body weight gains were decreased at doses of 1.9 mg/kg and above. Signs of toxicity included depression, huddling, diarrhea, prostration, and death. Necropsy findings was urate deposits over the heart. Tubular nephrosis was observed in some chicks.

Chang et al. (1979) described the development of lymphopenia in broiler chicks administered ochratoxin A. The mycotoxin was incorporated into a commercial, non-medicated broiler feed at concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 ppm and fed 1 day to 3 weeks of age to broiler chickens. Total leukocyte counts determined after 3 weeks of feeding were decreased by every dose level of ochratoxin A and this was due to a reduction in the number of lymphocytes. The total number of circulating heterophils was not altered, but total number of circulating monocytes was decreased by dose levels of 2.0 ppm and higher.

Huff and coworkers (1979) reported on the induction of an iron deficiency anemia in chickens by ochratoxin A. The mycotoxin was fed at concentrations of 0.5, 1.0, 2.0, 4.0, and 8.0 ppm to broiler chicks from 1 day to 3 weeks of age. After 3 weeks of feeding, the packed cell volume and hemoglobin were decreased significantly in the birds fed 8.0 ppm ochratoxin A, but the number of circulating erythrocytes was not significantly altered at any dietary concentration of ochratoxin A. The mean corpuscular volume and mean corpuscular hemoglobin concentration were also reduced in the high dose birds. Serum iron was significantly decreased in both the 4.0 and 8.0 ppm ochratoxin A-fed groups. Total iron binding capacity was not significantly different among the experimental groups. Transferrin saturation was reduced in both the 4.0 and 8.0 ppm groups.

Choudhury et al. (1971) administered ochratoxin A at dietary concentrations of 1, 2, and 4 ppm to White Leghorn pullets from 14 weeks to one year of age. The dietary levels of 2 and 4 ppm resulted in 12.5 and 35.4% mortality, respectively, during the growing period, and in 17.9 and 64.5% mortality, respectively, during the laying period. The amount of feed consumed per bird during the growing period was higher but not significant for the group fed 4 ppm. Body weights were reduced at dietary concentration of 4 ppm. The hens on the 4 ppm diet did not attain sexual maturity (no eggs laid) during the 9 month experimental period. Sexual maturity was delayed at the other dietary concentrations. Egg production of hens fed 1 ppm ochratoxin A was moderately reduced whereas egg production of the 2 ppm group was much poorer (control--68.5%; 1 ppm--33.3%; 2 ppm--

8.1%). The group fed 4 ppm produced no eggs. Hatchability was reduced by dietary ochratoxin A concentrations of 1 and 2 ppm. Growth of hatched chicks was reduced during the first 2 weeks post-hatching. Necropsy examination of the ochratoxin treated birds revealed pale tan to yellow colored livers with focal hemorrhages. No other gross lesions were observed in the treated group.

Prior and Sisodia (1978) administered ochratoxin A at dietary concentrations of 0.5, 1, and 4 ppm to H and N White Leghorn hens from week 26 to week 32. The hens were in full production at the beginning of ochratoxin A administration. Egg production was reduced significantly below the contro group after 2, 4, and 6 weeks for the groups fed 4.0, 1.0, and 0.5 ppm, respectively. Egg weights were reduced in the 1 and 4 ppm groups during weeks 2 through 5. Body weight was significantly reduced in the group fed 4 ppm. Feed consumption was decreased during weeks 2-5 for the group fed 4 ppm ochratoxin A. No significant difference in hatchability of fertile eggs was found at any time during the experimental period. Prothrombin times were significantly increased and total serum proteins significantly reduced in the groups fed 1 and 4 ppm ochratoxin A. Detectable concentration of ochratoxin A was not found in eggs, but occurred in red and white muscle and in the liver and kidney. Detectable concentrations disappeared from the red and white muscle within 24 hours post-withdrawal of ochratoxin A, but persisted in the liver and kidney for more than 48 hours.

Page et al. (1980) conducted a trial for 3 weeks to evaluate the effect of ochratoxin A on egg production and egg-shell stains.

One hundred twenty adult Leghorn-type were assigned to cages randomly for this trial. Ochratoxin A was added to a laying ration to obtain levels of 0.0, 0.5, or 1.0 ppm of ochratoxin A. They found that ochratoxin A at the levels used did not have any effect on body weight, ochratoxin A did, however, produce a significant reduction in egg production (control--94.10%, 0.5 ppm--89.06%, 1 ppm--80.73%) and a significant increase in the number of eggs with stained shells. All of the birds receiving ochratoxin A were said to develop diarrhea with a high urate excretion. Post-mortem lesions were confined to the kidneys and consisted of an increase in size and an alteration in color. Histological lesions were confined to the tubular epithelium of the kidney. In describing a case report which was suggested to be a chronic ochratoxicosis, Page et al. (1980) said that the prominent clinical signs were paleness of the comb and wattles, diarrhea, and emaciation.

Svendsen and Shadhauge (1976) found that White Leghorn hens consuming feed containing 1 ppm of ochratoxin A developed renal changes characterized by a decrease in the renal concentrating ability and glomerular filtration rate.

Krough et al. (1976) administered ochratoxin A at dietary concentrations of 0.3 and 1 ppm to White Leghorn chicks from day 1 to day 341 of age. The results indicated that the water consumption, as measured at the end of the experiment, was increased in the experimental groups. Renal function in these hens was impaired. Impairment of glomerular and tubular function was indicated by decreased inulin clearance, decreased Tm_{PAH} (PAH = p-aminohippurate)

and decreased urine concentrating capacity (osmotic urine to plasma ratio after injection of vasopressin). At necropsy, the kidneys appeared normal, but mild microscopic changes were present in all treated birds. Damage was limited to tubules and lesions were found in the proximal and distal tubules of both the reptilian and mammalian nephrons. The alterations included epithelial cell necrosis, ballooning of epithelial cells, the presence of karyomegalic cells, and reduction of brush border. Mitotic figures, some bizarre, were seen in tubules of most test birds. Ochratoxin A was detected in muscle, liver, and kidney with the higher concentrations found in the group exposed to 1 ppm for 2 weeks. Maximum concentration was 49.5 ppb in the kidney and 9.1 ppb in muscle. Ochratoxin A was not detected in eggs.

Hamilton et al. (1977) reported that three outbreaks of disease involving 360,000 turkeys was associated with ochratoxin A contamination of feed and corn at concentrations up to 16 ppm. Clinical observations included high mortality, pale swollen kidneys, and decreased feed intake. Renal lesions consisted of necrosis of proximal tubular epithelium.

Prior et al. (1976) administered by oral intubation ochratoxin A dissolved in 0.1 M sodium bicarbonate to one-day-old Nicholas Broad Breasted white turkey poults. The single dose 7-day LD_{50} was 5.9 ± 0.72 mg/kg body weight. Body weight gains were slightly reduced at doses of less than 4 mg/kg. Reduction in body weight gains was marked at the higher dose levels (6 and 8 ppm). No gross and histopathologic alteration was reported.

Doster et al. (1973) administered ochratoxin A and a crude Aspergillus ochraceus culture extract at dietary concentrations of ochratoxin A of 4, 8, or 16 ppm to one-day-old Japanese quail. Body weight gains were reduced by all ochratoxin A concentrations. Test quail were inactive and had less feather growth than controls. Mortality after 10 days of feeding varied from 60 to 90% and was similar for the pure ochratoxin A groups and the crude extract groups. Necropsy findings were pale tan to orange-colored livers. Histopathologic alterations were swollen hepatic nuclei, biliary duct proliferation, and diffuse cytoplasmic vacuolation of hepatocytes. Apparently the kidneys were not examined histologically.

Prior et al. (1976) administered by oral intubation ochratoxin A dissolved in 0.1 M sodium bicarbonate solution to one-day-old Japanese quail. The single dose LD_{50} was 16.5 ± 0.56 mg/kg body weight. Quail dying of acute ochratoxicosis had clinical signs of listlessness, huddling, diarrhea, prostration, and death. No gross lesions were observed and histopathologic alterations were not described.

Prior et al. (1978) administered ochratoxin A at dietary concentrations of 1, 2, 4, 8, or 16 ppm to female Japanese quail aged 67, 109, or 151 days at the beginning of the feeding period that extended for 3 to 6 weeks. These dietary concentrations had no adverse effect on egg production, egg weight, feed consumption, or body weight. Fertility and hatchability were greatly reduced when the dietary concentration of ochratoxin A was 16 ppm.

Purchase and Nel (1967) revised an earlier reported LD_{50} of 25 µg/duckling (van der Merwe et al., 1965) to 150 µg/duckling. Theron et al. (1966) administered ochratoxin A dissolved in 0.1 M sodium bicarbonate to day-old Pekin ducklings at a dose of 100 µg/duckling. Alterations in the liver were studied by light and electron microscopy and by enzymic histochemistry (apparently, other tissues were not examined). The most prominent pathologic change was an increase in fatty vacuolation of hepatocytes. The lipid was present as small discrete vacuoles of relatively uniform size throughout the cytoplasm. Ultrastructural alterations were present in mitochondria and the endoplasmic reticulum.

Ochratoxin Influence on Other Animals

Szczech and associates (1973) studied acute ochratoxicosis A produced in swine by feeding a diet containing a rice culture of an ochratoxin A-producing strain of Apsergillus ostianus and by daily oral doses of 1.0 or 2.0 (25-50 ppm in feed) mg/kg body weight of ochratoxin A. The mycotoxicosis was characterized by depression, reduction of feed intake, and loss of body weight followed by diarrhea, polyuria, polydipsia, and dehydration. Pigs given ochratoxin A were moribund or dead in 5 to 6 days. Packed cell volume, hemoglobin, and total plasma protein were increased, probably a reflection of dehydration. Blood urea nitrogen was increased at determinations made on days 4 and 6. Necropsy findings included dehydration, enteritis, pale-tan livers, and edema and hyperemia of the mesenteric and other lymph nodes. Histopathologic alterations were frequent and severe in the kidney and gut. Renal lesions were tubular

dilation and necrosis of epithelium of the proximal convoluted tubules. Focal necrosis occurred in several areas of the gut and fatty change was not found in most livers. In lymphoid tissue, edema, hyperemia, and necrosis of lymphocytes were the principal alterations.

Krogh and associates (1974) studied changes in renal structure and function in swine fed an ochratoxin A-contaminated diet containing 0.2, 1, or 4 ppm. Most of the pigs were fed the diet for 4 months, but some were terminated after 9 and 68 days of feeding. Growth rate and feed conversion were reduced in ochratoxin A-fed pigs. Water consumption was increased in the group fed 4 ppm of the mycotoxin. Renal damage was indicated by elevations of serum creatinine and urea, a decrease of Tm_{PAH}/C_{IN}, lowering the inulin and PAH clearances, reduction in ability to concentrate urine, by increased urinary content of glucose and proteins, and by increased urinary activity of leucine aminopeptidase. All of the pigs fed 1 and 4 ppm ochratoxin A and 4/9 fed 0.2 ppm had microscopically detectable renal alterations. The severity of the renal lesions varied with dietary ochratoxin A concentration. Mild lesions were found in affected kidneys of the 0.2 ppm group and consisted of degenerative changes in the proximal tubules and slight increase in the interstitial fibrous tissue in the medullary rays, in the cortex corticis and about glomeruli. In the pigs fed the middle dose, the renal lesions varied from slight degenerative changes of tubular epithelium with tubular dilatation and interstitial fibrosis to tubular atrophy, thickened basement membranes, sclerosis of glomeruli and marked interstitial fibrosis. Nuclear changes included enlargement and necrosis.

Basement membranes were most thickened about dilated and degenerated proximal tubules. Renal lesions in pigs fed 4 ppm ochratoxin were of the same kind but were more severe.

Krogh and coworkers (1976) studied the changes of renal structure and function in pigs fed ochratoxin A in amounts corresponding to a feed concentration of 1 ppm for 3 months. Ochratoxin A-fed pigs had polydipsia, reduced growth rate and feed conversion, elevation of seum creatinine, glucosuria, a reduction of Tm_{PAH} and ratio of Tm_{PAH}/C_{IN}, and reduced ability to concentrate urine after water deprivation. Renal lesions consisted of degeneration of proximal tubules, tubular atrophy, and interstitial fibrosis. Focal areas of marked peritubular and periglomerular fibrosis contained a few hyalinized glomeruli, dilated tubules, and proximal tubules with thickened basement membranes.

Shreene and associates (1977) fed ochratoxin A and B to two pregnant sows (equivalent to 0.38 and 0.13 mg/kg body weight respectively; 9.5-3.3 ppm in feed) from 21 to 28 days of pregnancy. The sows were killed at 30 days. The sows remained clinically normal during the period of feeding. No abnormalities were found in the reproductive tracts or in the fetuses and no histopathologic alterations were found in the organs examined.

Pier et al. (1976) administered ochratoxin A orally by gelatin capsules to four one-month-old Jersey calves. The daily doses were 0.1, 0.5, 1, or 2 mg/kg body weight and the treatment was continued for 30 days (6.5, 32.5, 65, and 130 ppm in feed). At doses of 0.1 and 0.5 mg/kg body weight, detectable abnormalities were limited to

polyuria. Depression, reduced weight gains, low urinary specific gravity, and dehydration were observed at the two higher doses. Necropsy findings were pale kidneys and mild enteritis. Histopathologic alterations were mild tubular degeneration, protein material in tubular lumens, and interstitial fibrosis in calves given the two higher dose levels.

Ribelin et al. (1978) administered ochratoxin A by stomach tube to two 5-week-old Holstein calves and to four pregnant Holstein cows. The single doses in the calves were 11 and 25 mg/kg body weight, respectively. Three cows were given ochratoxin A for 4-5 days at doses of 0.2, 0.75, or 1.66 mg/kg body weight. One cow was given a single dose of 13.3 mg/kg body weight. The two calves died within 24 hours of dosing and each had epicardial hemorrhages as the only gross or microscopic abnormality. Cows treated with the lower doses remained clinically normal and delivered normal calves. The cow given the single dose of 13.3 mg/kg ochratoxin A had clinical signs of toxicity including difficulty in rising, diarrhea, anorexia, and cessation of milk production. Recovery was complete by day 4 post-administration except milk production never returned to pre-treatment level.

Ribelin et al. (1978) administered ochratoxin A to three American La Mancha female goats by stomach tube at daily doses of 1, 2, and 3 mg/kg body weight. The goat given 3 mg/kg body weight died on the sixth day. The other two goats were treated for 14 days. The high-dose goat developed a watery diarrhea, became dehydrated, and died. There were no gross lesions and microscopic changes were

confined to centrilobular cloudy swelling of hepatocytes. The goats given 1 and 2 mg/kg for 14 days remained clinically normal and had no gross lesions.

Munro et al. (1973) administered by jugular vein infusion ochratoxin A dissolved in 0.01 M sodium bicarbonate to two pregnant Horned Dorset ewes that had surgically implanted cannulae in the saphenous vein of the fetus and cannulae in the amniotic cavity. The total dose was equivalent to 1 mg/kg maternal body weight. One ewe had died by 12 hours post-administration of mycotoxin and the other died between 12 and 24 hours post-infusion. The necropsy findings were pale, friable maternal livers and congested edematous lungs. The fetuses were free of lesions. Histopathologic alterations in the ewes were centrilobular necrosis of the liver, perilobular hepatic cell vacuolation, and pulmonary congestion.

No Effect Concentration

Whether a given dietary concentration of ochratoxin A has no toxic effect depends upon the animal species, the duration of administration, and the sensitivity of the methods used to determine if an adverse effect has been produced (Carlton and Krogh, 1979).

For broiler chickens, a no effect concentration in acute (3 week) studies was approximately 0.5-1.0 ppm (Huff et al., 1975). At these doses body weight gain was not significantly reduced but at a dietary concentration of 1.0 ppm ochratoxin A, the average renal weight was significantly increased. For continuous chronic exposure a no effect level based on renal function tests would be less than

0.3 ppm (Krogh et al., 1976). Chickens fed 0.3 ppm ochratoxin A from 1 day to 341 days had detectable alterations of renal function and structure. Other studies in which egg production was evaluated during dietary exposure to ochratoxin A have indicated a no effect concentration of less than 0.5 ppm. Thus, Prior and Sasodia (1978) reported that egg production and feed consumption of White Leghorn hens were reduced by feeding 0.5 ppm ochratoxin A for 6 weeks. Turkeys are less sensitive and Japanese quail much less so to ochratoxin A toxicosis than are Leghorn chickens (Prior et al., 1976). Consequently, the no effect concentration for these species would be higher than one that could be proposed for the chicken.

Metabolism and Biochemical Effects

van Walbeek et al. (1971) studied the toxicity and excretion of ochratoxin A in rats intubated with ochratoxin A and fed cultures of Penicillium viridicatum. Male Wistar rats (83-110 g) were given 500 μg ochratoxin A in 0.1 M sodium bicarbonate for 6 days and excretion in feces and urine was followed. The average total amount of ochratoxin A excreted daily in urine and feces was about 10% of the administered dose. A portion of the ochratoxin A was metabolized to ochratoxin α and excreted mainly in the urine (greatest concentration was 27 μg). Small amount of ochratoxin α (up 10 μg) was present in the feces. The liver and kidney from rats killed on day 5 contained small amounts of ochratoxin A (2.0 μg/g liver and 6.2 μg/g kidney).

Galtier (1974a) studied the transport of ochratoxin A in male Wistar rats (250 g) given a single oral dose of 10 mg/kg body weight

ochratoxin A dissolved in isotonic sodium bicarbonate. The toxin was found in trace amounts in blood cells, but large amounts were found in the plasma and the maximum of 56.7 μg/ml was obtained at 8 hours after dosing. Binding of ochratoxin A to plasma albumin was 95% and saturation of binding sites occurred at a concentration of 70 μg/ml. The tissue distribution and excretion of ochratoxin A and ochratoxin α was studied in male Wistar rats given a single oral dose of 10 mg/kg body weight of ochratoxin A (Galtier, 1974b). The mycotoxin was detected in all organs examined with the highest concentration in the kidney, liver, and skeletal muscle. The data indicated that ochratoxin A was absorbed in the stomach as little was detected in the small intestine. Ochratoxin α was recovered from the colon and cecum indicating hydrolysis of ochratoxin A by microbial flora. Urinary and fecal excretion was mainly in the form of ochratoxin α .

Patterson et al. (1976) studied the metabolism of ochratoxins A and B in the pig. A mixture of the two mycotoxins (0.38 and 0.13 mg/kg body weight, respectively) was fed to pigs daily for 8 days during early pregnancy. Ochratoxin A but not ochratoxin B accumulated in maternal tissues and in the placenta. Neither toxin was detected in the fetus. Ochratoxin B was poorly absorbed and was hydrolyzed in the gut. Ochratoxins A, α and β were excreted in the feces and in the urine.

Pitout (1969) studied the <u>in vitro</u> hydrolysis of ochratoxin A by some proteolytic enzymes and found that the toxin can be hydrolyzed to ochratoxin α by carboxypeptidase A and α-chymotrypsin, but not by trypsin.

Meisner and Selanik (1979) examined the effects of ochratoxin A on gluconeogenesis in kidney cortex slices from Sprague-Dawley rats fed 0.5 to 2.0 mg/kg body weight of ochratoxin A. Glucose formation from pyruvate was inhibited 25-30%. The activity of renal phosphoenolpyruvate carboxykinase of principals was only 37-52% of control values, but activity of this enzyme in the liver was unaffected by the mycotoxin. Toxin administration of 1.43 mg/kg body weight for several days reduced gluconeogenesis to about 50% of control values and phosphoenolpyruvate carboxykinase activity declined to about 40% of controls. In rats fed 1.9 mg/kg ochratoxin for 7 days, gluconeogenesis from malate, glutamine, lactate, and pyruvate decreased by 28%, 32%, 39%, and 61% respectively.

Purchase and Theron (1968) reported that a single dose of 10 mg/kg body weight ochratoxin A produced an accumulation of glycogen in the liver of rats killed 6 and 10 days after mycotoxin administration. Pitout (1968) studied the effects of ochratoxin A on nucleic acids and their constituents and on the hepatic phosphorylase enzyme system. It was concluded that ochratoxin A did not interact with nucleic acids and their derivatives. Ochratoxin A inhibited the phosphorylase enzyme system. The inhibition was about 70% when the concentration was 0.108 mg/ml of reaction mixture. Ochratoxin A had no inhibitory effect on the enzymes glucomutase, glucose-6-phosphate dehydrogenase, phosphorylase A, or hexokinase.

Suzuki and Satoh (1973) studied the effects of ochratoxin A on tissue glycogen concentration in both intact and adrenalectomized rats. Male Winstar rats (230-300 g) were given a single oral dose

of 15 mg/kg body weight of ochratoxin A suspended in 0.1% carboxymethylcellulose. A significant reduction in hepatic glycogen concentration was found in intact ochratoxin A-treated rats 4 hours after toxin administration. Hepatic glycogen concentrations gradually returned to control values within 5 days. Adrenalectomy also resulted in marked reduction in hepatic glycogen concentration after 4 hours. Glycogen concentration in the heart was increased within 4-6 hours after ochratoxin A administration in intact rats. No significant changes in hepatic or cardiac glycogen concentrations were found after the administration of a dose of 9.5 mg/kg ochratoxin.

Huff et al. (1979) studied glycogen mobilization in the liver of male broiler chicks fed graded doses of ochratoxin A (0.5, 1.0, 2.0, 4.0, and 8.0 ppm) for 3 weeks. Hepatic glycogen concentrations were increased in birds fed the two higher concentrations of 4.0 and 8.0 ppm. The mycotoxin inhibited the mobilization of glycogen by glucagon at these same dietary concentrations.

Control

Ochratoxin A is produced in natural substrates such as cereal grains and mixed feedstuffs under conditions of suitable temperature and water activity. Prevention of fungal growth by proper drying appears presently to be the most efficient means of controlling contamination with ochrotoxin A (Carlton and Krogh, 1979).

Chemical treatment can be used to reduce or prevent fungal growth in cereal grains and animal feeds. Various insecticides and fumigants have been used to treat stored grains in an effort to reduce contamination with mycotoxins. None of the insecticides or fumigants reduced

aflatoxin or ochratoxin formation by inoculated fungi to make their use practical (Vandegraft et al., 1973).

Wu and Ayres (1974) studied the effect of the organophosphate insecticide dichlorvos on the production of ochratoxin A by strains of A. ochraceus grown in liquid medium and on corn. The insecticide at the lowest concentration of 0.1 mg/100 ml of medium or 0.1 mg/100 g of corn had no effect on fungal growth or ochratoxin A production. Production of ochratoxin A was reduced by a concentration of 1 mg/100 ml or g of substrate and the reduction varied from 21-29%. When the concentration of dichlorvos was 10 mg/100 ml or g of substrate, ochratoxin A production was reduced 42-72% depending on fungal strain and substrate.

Vandegraft et al. (1975) studied the effects of grain preservatives on production of aflatoxin and ochratoxin. Ammonia (2%) was applied to corn at a moisture level that would be encountered under normal storage conditions and propionic acid (1%) to high-moisture corn and these samples were stored for various periods of time. The treated corn was tested for resistance to mold invasion and subsequent toxin production by inoculating with aflatoxin and ochratoxin-producing fungi. A heavy inoculum was used and corn samples were raised to moisture content of 36%. Propionic acid prevented mold growth and ochratoxin formation. Some mold growth occurred on ammoniated corn after storage, but aflatoxin and ochratoxin production by the strains of fungi used for inoculation was drastically reduced. Ochratoxins A and B were not detected in ammonia-treated corn stored for 5 and 7 weeks. Corn stored for 19 weeks became more vulnerable

to mold invasion and toxin production, but toxin production, when it occurred, was markedly reduced as compared to control values.

Specific studies of the chemical or physical reduction of ochratoxin A content in grains and feedstuffs are not available. Although several solvent systems have been used to extract ochratoxins from grain samples, none of these appear adaptable for practical use (Carlton and Krogh, 1979). Procedures, such as roasting, developed for the reduction of aflatoxin contamination, might successfully be applied to ochratoxin A decontamination. Under simulated coffee roasting conditions, ochratoxins were largely destroyed (Levi et al., 1974).

Detoxification procedures such as those investigated for decontamination of the aflatoxins (Beckwith et al., 1976) have not been studied in relation to reduction of ochratoxin A concentrations in grains and other materials. Vandegraft et al. (1975) suggest that ammonia may be effective as a detoxifying agent for ochratoxins.

CHAPTER III

EXPERIMENTAL PROCEDURE

Experimental Animals

Single-combed White Leghorn chicks were obtained from a local commercial hatchery. They were hatched on February 26, 1980, and were maintained under identical conditions and fed the same diet at the poultry farm of The University of Tennessee until they were about 9 months of age. They were vaccinated for Marek's disease at the hatchery and were debeaked and vaccinated at the farm for infectious bronchitis, Newcastle disease, and fowl pox. They were fed a coccidiostatic drug in the starting and growing diets. At 40 weeks of age, 280 laying hens were selected from these birds on the basis of uniform egg production and body weight. They were moved to laying cages (25 by 45 cm) at the Poultry Service Laboratory on the Agricultural Campus of the University for use in this experiment.

Experimental Design

In two environmentally identical rooms, the birds were assigned randomly to 10 groups of 28 hens each, 5 groups in each room (block). Five treatments were assigned at random to the experimental groups within each block. Each group was divided into two lots of 14 hens each in order to get two replicates from each group. Each replicate consisted of seven cages containing two hens each.

Treatments

The basal diet was a commercial cage layer ration which was fed to all birds during a 4-week equilibration period prior to the start of the experiment. Ochratoxin A was produced by the Food Technology Department in sterilized artificial media incubated with toxin producing isolate of Aspergillus ochraceus NRRL 3174 which was obtained from the U. S. D. A. Solutions of ochratoxin A in chloroform was thoroughly mixed with the basal diet to give final levels of 0, 0.25, 0.5, 1, and 2 ppm ochratoxin corresponding to treatment 1, 2, 3, 4, and 5 respectively. After a 12-week period of toxin feeding, all birds were returned to the basal ration. Artificial lights, controlled by a time clock, provided 14 hours of light per day. Feed and water were available ad libitum.

Methods of Data Collection

Dead birds were collected daily and percentage of birds that died during the 12-week experimental period was calculated. Egg production was recorded daily for each lot and the percent hen day production was calculated from the number of eggs laid per hen housed divided by average number of days of each hen in the laying house. Eggs were gathered by lot for egg weight determination and averaged for each lot. Average weight in grams computed from bulk weighing of all eggs laid in one day each week throughout the study. Measurements of specific gravity, Haugh units, and of blood and meat spots were made on a sample of 10 eggs from each lot one day each for 4 weeks. Eggs were scored according to the specific gravity of

the salt solution in which they would barely float. The higher the specific gravity score, the thicker the shell. Specific gravity of the solutions and their scores are shown in Table 1.

For the purpose of determining albumen quality, Haugh units were computed from egg weights and albumen heights by applying the Card and Nesheim formula (1966):

$$HU = 100 \log [H + 7.57 - 1.7W^{0.37}]$$

where (H) is the albumen height in millimeters measured by micrometer and (W) is the egg weight in grams.

Meat and blood spots were easily seen on the surface of the yolk of the egg. By the use of a clear glass break-out table with mirrors to view all sides of the egg, all spots could be readily detected.

Body weights of the birds were obtained at the beginning and after 4, 8, and 12 weeks of the experiment by weighing seven birds from each lot (one from each cage) individually and averaged for each lot. Therefore, weight gain or loss were calculated for each period.

Food consumption was determined for each lot for each 14-day period and calculated as gram/day/bird.

Water consumption was measured during a 3-day period just before the end of the experiment. Water cans were fixed in two cages of each replicate and the automatic water troughs of those cages were covered. Known amounts of water were added every day. A can was placed in each room to measure the amount of evaporated water. The average amount of the water consumed was measured as ml/day/bird.

TABLE 1

SPECIFIC GRAVITY OF SALT WATER SOLUTIONS USED IN SPECIFIC GRAVITY DETERMINATIONS

Specific Gravity	Score*
1.068	0
1.072	1
1.076	2
1.080	3
1.084	4
1.088	5
1.092	6
1.096	7
1.100	8
sink in 1.100 solution	9

*Scores used in random sample laying test (Shirley, 1976). Eggs floating in various specific gravity solutions are given the corresponding score except eggs which sink in the 1.100 specific gravity solution are scored 9.

Hens with excessive wetness of abdominal feathers and with adhering urates (wet rear end) were counted one time at the end of the experiment for each lot and expressed as a percentage to be used as an indication of the excessive urinary excretion.

Comb color was observed at the end of the test and classified as pale, median, or red. The percentages of birds with pale and red comb color for each lot were calculated.

At the termination of the trial, three birds from each replicate were killed for macroscopical renal and liver changes. Liver weights were taken and pieces of kidney were fixed, embedded, sectioned, and stained for microscopical renal changes.

Analysis of Data

The data were statistically analyzed by the analysis of variance procedure for a randomized block design. The following mathematical model describes the variables studied:

$$Yijk = u + ti + Bj + eijk$$

Yijk = observed value from the Kth lot in the jth block received the ith treatment,

u = overall mean,

ti = effect of the ith treatment (level of ochratoxin), i = 1, 2, . . . 5,

Bj = effect of the jth block, j = 1, 2 blocks,

eijk = effect of the experimental error and sampling error together.

k = 1, 2 lots received the same level of ochratoxin.

Nonorthogonal contrast was used to compare treatment one (control) with each one of the other treatments and tested at 0.05, 0.01, and 0.001% levels of probability.

CHAPTER IV

RESULTS AND DISCUSSION

The tables presented in this chapter will show the treatments, the level of ochratoxin for each treatment, and the treatment mean which represents the average of four readings, one from each lot. They do not show the block mean since the block effect was not significant for any trait studied.

Appendix Table 1 is an example of the analysis of variance tables used to show sources of variance and their degrees of freedom. Treatment one, the control, was compared with each one of the other treatments.

Readings, either as percentages or averages, of all the traits studied for each lot are shown in Appendix Tables 2 through 41.

The Effects of Ochratoxin on Traits

Egg Production

The results of the effects of ochratoxin on egg production are presented in Table 2. At any period, all of the treated groups were lower than the control group in percent hen-day egg production, but differences were not statistically significant. Egg production of the 2 ppm group was lower than the other treated groups during the experiment. The lowest percent was during the second 4 weeks of the experiment (76.28%). The effects of ochratoxin on egg production in this trial were less severe than those described in the literature.

TABLE 2

THE EFFECTS OF OCHRATOXIN ON EGG PRODUCTION

Treatment		% Hen-Day Egg Production			
No.	Ochratoxin (ppm (mg/kg)	1st 4 weeks	2nd 4 weeks	3rd 4 weeks	Entire period
1 (control)	0	81.62	80.48	79.39	80.50
2	0.25	79.52	78.44	78.75	78.90
3	0.5	78.78	79.16	78.76	78.90
4	1	79.39	79.26	79.05	79.24
5	2	77.90	76.28	76.54	76.91

Choudury et al. (1971) reported that egg production of hens fed 1 ppm ochratoxin was moderately reduced whereas egg production of the 2 ppm group was much poorer (control--68.5%, 1 ppm--33.3%, 2 ppm--8.1%) during 9-month experimental period. Prior and Sisodia (1978) found that the 0.5 and 1 ppm ochratoxin reduced significantly the egg production below the control group after 6 and 4 weeks of the experiment respectively. The results obtained by Page et al. (1980) tended to partially agree with the results in the present study. Although they have reported a significant reduction in egg production with ochratoxin feeding for 3 weeks, but the reduction was less severe (control--94.10%, 0.5 ppm--89.06%, 1 ppm--80.73%).

Egg Weight

The effects of ochratoxin on egg weight are presented in Table 3. During the first 7 weeks of the experiment, there was no significant effect of ochratoxin at any level on average egg weight. At 8 and 10 weeks only the 2 ppm ochratoxin group was significantly lower than the control group ($P \leq .05$). While at 11 and 12 weeks, both 1 and 2 ppm ochratoxin groups were significantly lower than the control group ($P \leq .05$). This indicates that as the duration of exposure increased the effects of the lower levels became significant. During weeks 6 to 10, the 2 ppm group was not only lower than the control group in average egg weight, but also lower than the other treated groups. These results agree generally with those of Prior and Sisodia (1978) who reported that egg weights were reduced in the 1 and 4 ppm

TABLE 3

THE EFFECTS OF OCHRATOXIN ON EGG WEIGHT

Treatment		Average Egg Weight (Grams)[a]											
		weeks											
No.	Ochratoxin PPM (mg/kg)	1	2	3	4	5	6	7	8	9	10	11	12
1 (control)	0	62.57	62.36	63.30	63.86	63.04	63.84	63.70	63.72	63.80	63.93	64.13	64.47
2	0.25	61.92	62.87	62.57	63.93	63.67	64.24	64.79	63.23	63.37	64.15	64.65	65.13
3	0.5	62.66	62.15	61.64	62.53	62.18	63.82	63.31	62.96	63.11	63.82	64.31	64.02
4	1	61.86	62.13	62.46	61.87	62.84	63.38	63.28	62.21	62.62	63.27	62.50*	62.85*
5	2	61.97	62.14	62.06	62.55	62.67	62.93	62.54	62.02*	62.31	62.11*	62.56*	62.61*

[a]All eggs laid in one day each week.

*Significantly different from control group ($P \leq .05$).

groups during weeks 2 through 5. It seems that egg weight is affected by 1 ppm ochratoxin and above.

Egg Blood and Meat Spots

The results of the effects of ochratoxin on egg blood and meat spots are summarized in Table 4. Two ppm ochratoxin in the ration increased significantly ($P \leq .05$) the percentages of egg blood and meat spots when measured at 4, 8, and 12 weeks of the experiment. Ochratoxin (0.5 and 1 ppm) in the ration increased the percentages too, but this increase was significant only at 8 weeks of the experiment ($P \leq .05$). At 4, 8, and 12 weeks the percentages of the egg spots of the 2 ppm group were higher than the other treated groups, they were 22.5, 17.5, and 22.5% respectively. The effects of ochratoxin on blood and meat spots of eggs have not been previously reported, thus comparisons with other studies cannot be made. It can be concluded that the ovary is sensitive to as low as 0.5 ppm level of ochratoxin, since the group at this level was significantly higher than the control group in percent egg blood and meat spots ($P \leq .05$) at 8 weeks of the experiment.

Egg Haugh Units

Haugh unit is a measure of albumen quality. The results of the effects of ochratoxin on egg Haugh units are presented in Table 5. There was no significant effect of ochratoxin at any level on egg Haugh units during the entire period of the experiment. Generally, Haugh units shown in Table 5, were relatively low, and this might be due to the fact that egg production was relatively high for

TABLE 4

THE EFFECTS OF OCHRATOXIN ON EGG BLOOD AND MEAT SPOTS

Treatment No.	Ochratoxin PPM (mg/kg)	% Blood and Meat Spots[a] At 4 weeks	At 8 weeks	At 12 weeks
1 (control	0	10.0	5.0	7.5
2	0.25	7.5	10.0	10.0
3	0.5	15.0	15.0*	20.0
4	1	17.5	15.0*	17.5
5	2	22.5*	17.5*	22.5*

[a] n = 40 eggs per treatment level (10 eggs from each lot).

*Significantly different from control group. ($P \leq .05$)

TABLE 5

THE EFFECTS OF OCHRATOXIN ON EGG HAUGH UNITS

Treatment No.	Ochratoxin PPM (mg/kg)	Average Haugh Units[a] At 4 weeks	At 8 weeks	At 12 weeks
1 (control)	0	73.25	72.50	73.00
2	0.25	72.75	70.75	72.25
3	0.5	72.75	74.25	73.25
4	1	73.75	71.75	73.50
5	2	73.00	73.25	72.00

[a] n = 40 eggs per treatment level (10 eggs from each lot).

all groups. Harms and Douglas (1960), Srinivasan (1972), and Al-Mohammadi (1973) found negative correlations between egg production and Haugh units.

Egg Specific Gravity

The results are shown in Table 6. Generally, all levels of ochratoxin reduced egg specific gravity score. The lower the specific gravity score, the thinner the shell. At 4 weeks of the experiment, although there was no significant difference between the control and any one of the treated groups in egg specific gravity score, all of the treated groups had scores lower than the control and the lowest score was (2.42) that of the 2 ppm group. At 8 weeks, the 0.5 and 2 ppm ochratoxin groups were significantly lower than the control group at 5 and 1% level of probability respectively. The 2 ppm group had the lowerst score also (1.95). At the end of the experiment the effects were more prominent, all levels of ochratoxin caused a significant decrease in egg specific gravity score and the degree of significance was proportional to the level of ochratoxin.

The thinner egg shells as a result of ochratoxin feeding might be due to the reduction in the absorption of Ca^{++} from the intestine due to a deficiency of the 1 25-dihydroxycholecaliferol, the most potent biological active form of vitamin D. This theoretical conclusion needs further study.

Body Weight

The results of the effects of ochratoxin on body weight are shown in Table 7. Body weights of hens receiving 0.5, 1, and 2 ppm

TABLE 6

THE EFFECT OF OCHRATOXIN ON EGG SPECIFIC GRAVITY

Treatment No.	Ochratoxin PPM (mg/kg)	Average Specific Gravity[a] At 4 weeks	At 8 weeks	At 12 weeks
1 (control)	0	3.40	3.10	2.97
2	0.25	3.32	2.40	2.32*
3	0.5	2.45	2.25*	2.10**
4	1	3.05	2.35	2.00***
5	2	2.42	1.95**	2.07***

[a]n = 40 eggs per treatment level (10 eggs from each lot).

*Significantly different from control groups. ($P \leq .05$)

**Significantly different from control group. ($P \leq .01$)

***Significantly different from control group. ($P \leq .005$)

TABLE 7

THE EFFECTS OF OCHRATOXIN ON BODY WEIGHT

Treatment No.	Ochratoxin PPM (mg/kg)	Average Weight Gain or Loss (gms)[a] At 4 weeks	At 8 weeks	At 12 weeks
1 (control	0	13.60	14.60	59.46
2	0.25	4.82	9.92	12.25
3	0.5	-28.00*	10.00	20.00
4	1	-24.96*	3.46	-40.35***
5	2	-41.32**	-47.53**	-75.20***

[a]n = 28 hens per treatment level (7 hens from each lot).

*Significantly different from control group. ($P \leq .05$)

**Significantly different from control group. ($P \leq .01$)

***Significantly different from control group. ($P \leq .005$)

ochratoxin were significantly reduced at 4 weeks of the experiment. At 8 weeks, the 0.5 and 1 ppm groups compensated the loss and the 2 ppm group lost more and the loss was significant at 1% level of probability. At 12 weeks, only the higher levels of ochratoxin (1 and 2 ppm) significantly reduced body weight ($P \leq .005$). During the entire experimental period, the 2 ppm level caused the highest body weight loss compared with the other levels of ochratoxin. These results agree generally with most of the results obtained by many investigators such as Peckham et al. (1971), Chu and Chang (1971), Huff et al. (1974), Choudhury et al. (1971), and Prior and Sisodia (1978) who reported that body weights were reduced with ochratoxin administration and the reduction was dependent on the dietary concentration of the ochratoxin and on the period of administration.

Feed Consumption

The results are shown in Table 8. There was no significant difference between the control group and any of the treated groups in the amount of feed consumed at any period of the experiment, however, feed consumption did tend to increase as ochratoxin levels increased. Since feed consumption is generally correlated with rate of egg production, egg size, and body size, the ochratoxin treated hens may have actually been less efficient users of feed. Choudhury et al. (1971) found no effect of ochratoxin on feed consumption during the growing period of the laying hens when 1, 2, and 4 ppm ochratoxin were used. While Prior and Sisodia (1978) reported a decrease in the amount of feed consumed only for the group fed 4 ppm ochratoxin.

TABLE 8

THE EFFECTS OF OCHRATOXIN ON FEED CONSUMPTION

Treatment		Average Amount of Feed Consumed (gm/day/bird)					
No.	Ochratoxin PPM (mg/kg)	1st 2 weeks	2nd 2 weeks	3rd 2 weeks	4th 2 weeks	5th 2 weeks	6th 2 weeks
1 (control)	0	108.25	106.75	110.25	108.50	103.50	106.50
2	0.25	108.25	110.75	108.50	108.50	104.75	104.50
3	0.5	112.00	107.25	112.75	111.00	106.00	105.25
4	1	108.75	108.25	113.50	108.50	103.50	108.75
5	2	110.75	111.00	112.00	113.50	109.25	110.50

[a]n = 56 hens per treatment level.

Water Consumption and Urine and Urate Excretion

The results of the effects of ochratoxin on water consumption are shown in Table 9. Water consumption, as measured at the end of the experiment, was significantly increased ($P \leq .05$) only in the hens received 2 ppm ochratoxin. The 0.25, 0.5, and 1 ppm levels were not significantly different from control group in the amount of water consumed. Krogh et al. (1976) found that 0.3 and 1 ppm ochratoxin given for 341 days to White Leghorn chicks increased water consumption as measured at the end of the experiment. Sevendsen and Shadhauge (1976) found that White Leghorn hens consuming feed containing 1 ppm of ochratoxin developed renal changes characterized by a decrease in the renal concentrating ability and glomerular filtration rate. In the present experiment, measurements of renal function were not made, but urine and urate excretion as judged by degree of wetness and amounts of urates adhering to abdominal feathers was observed and the results are shown in Table 9. There was no significant difference between the control group and any of the treated groups in the percentage of hens with wet rear end. The 2 ppm ochratoxin group had higher percentage than the control and other treated groups. Diarrhea with a high urate content was one of the prominent clinical signs of ochratoxin toxicity in the findings of Huff et al. (1974), Galtier et al. (1976), and Page et al. (1980). In this study the effects were less severe than those described in the literature.

TABLE 9

THE EFFECTS OF OCHRATOXIN ON WATER CONSUMPTION AND URINE AND URATE EXCRETION[a]

Treatment No.	Ochratoxin PPM (mg/kg)	Water Consumed[b] ml/day/bird	% of Hens Wet Rear End
1 (control)	0	181.72	19.63
2	0.25	185.91	17.85
3	0.5	182.22	19.91
4	1	182.39	21.69
5	2	202.18*	30.83

[a]As judged by degree of wetness and amounts of urates adhering to abdominal feathers.

[b]n = 16 hens per treatment level (4 hens from each lot).

*Significantly different from control group. ($P \leq .05$)

Comb Color

Comb color was observed at the end of the test and classified as pale, median, or red. Figure 2 shows the three classifications. The results of the effects of ochratoxin on comb color are shown in Table 10. The 1 and 2 ppm ochratoxin groups were significantly lower than the control group in the percent of hens with red comb color ($P \leq .05$). At the same time, they were significantly higher than the control group in the percent of hens with pale comb color ($P \leq .05$). In describing a case report which was suggested to be a chronic ochratoxicosis, Page et al. (1980) reported that the paleness of the comb and wattles was one of the prominent clinical signs. The pale comb color is an indication of anemia. Peckham et al. (1971) found that the hematopoiesis was suppressed in the bone marrow and the lymphoid cells of the spleen as a result of ochratoxin administration to one-day-old cockerels. Huff et al. (1979) found that PCV and hemoglobin were decreased significantly in broiler chicks fed 8.0 ppm ochratoxin for 3 weeks.

A possible explanation may be that ochratoxin interferes with the production in the kidney of erythropoietin which is important in hematopoiesis.

Mortality

Table 10 also shows the results of the effects of ochratoxin on mortality. Only the 2 ppm group was significantly higher than the control group ($P \leq .05$). The mortality rates in this trial were less severe than those described by Choudhury et al. (1971) who reported



2A. Pale Comb Color



2B. Median Comb Color



2C. Red Comb Color

Figure 2. The three classifications of the comb color.

TABLE 10

THE EFFECTS OF OCHRATOXIN ON COMB COLOR AND MORTALITY

Treatment		% of Hens		
No.	Ochratoxin PPM (mg/kg)	Red Comb Color	Pale Comb Color	% Mortality
1 (control)	0	24.99	7.14	0
2	0.25	30.35	12.49	0
3	0.5	16.61	12.63	1.78
4	1	10.98*	16.47*	1.78
5	2	11.71*	15.28*	7.14*

*Significantly different from control group ($P \leq .05$)

that the dietary levels of 2 and 4 ppm ochratoxin resulted in 17.9 and 64.5% mortality respectively, during the laying period. The levels of ochratoxin used in this study were much lower than the levels reported by many investigators to be the LD_{50} for young chickens.

Liver Weight and Color

The effects of ochratoxin on liver weight are shown in Table 11. The average liver weight of the 2 ppm group was significantly higher than the control group. There was no significant difference between the control group and any of the other treated groups in liver weight. Tucker and Hamilton (1971) found that the liver weight was increased significantly after administration of ochratoxin for 3 weeks to one-day-old broiler chicks.

At necropsy, the livers were classified as normal, slightly abnormal, or abnormal according to the paleness of the liver and the amount of hemorrhages. Figure 3 shows the three classifications. The number of birds of each class for each level of ochratoxin are shown in Table 11. The 1 and 2 ppm ochratoxin groups had a higher number of birds with abnormal livers than the control group. These results agree with most of the results obtained by many investigators such as Chu and Change (1971), Huff et al. (1974), Galtier et al. (1976), and Choudhury et al. (1971) who studied the effects of different levels of ochratoxin on young chickens and laying hens and reported pale tan to yellow colored livers with focal hemorrhages.

TABLE 11

THE EFFECTS OF OCHRATOXIN ON LIVER WEIGHT AND COLOR[a]

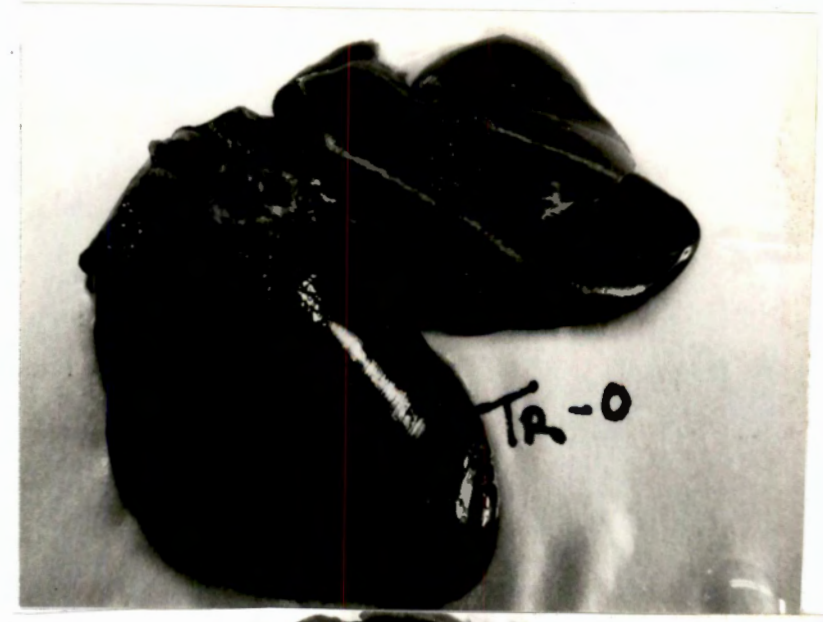
Treatment			Liver Color		
No.	Ochratoxin PPM (mg/kg)	Average Liver Weight (gm)	Normal No.	Slightly Abnormal[b] No.	Abnormal[c] No.
1 (control	0	41.16	8	1	3
2	0.25	39.41	5	1	6
3	0.5	38.66	4	3	5
4	1	42.41	3	2	7
5	2	45.16*	3	1	8

[a]n = 12 hens per treatment level (3 hens from each lot).

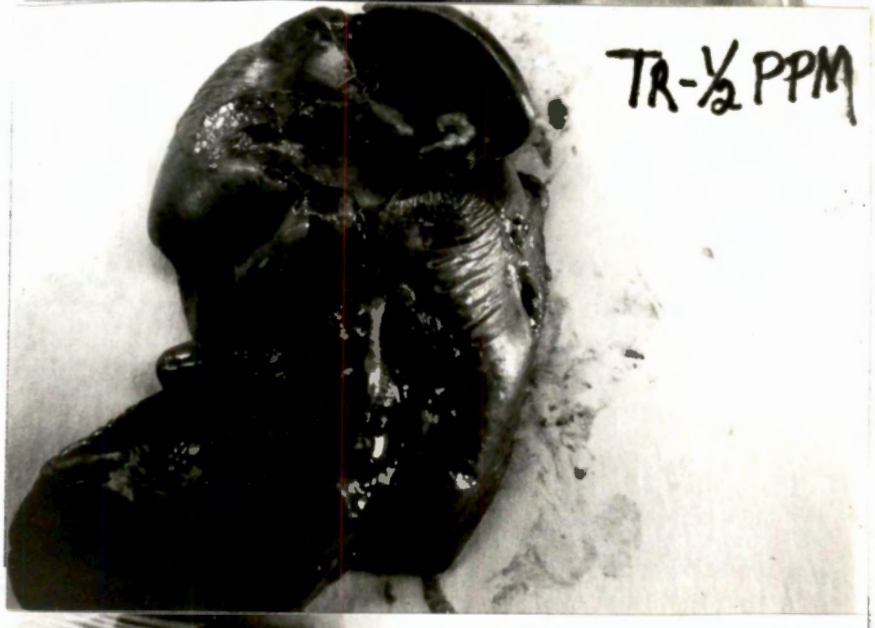
[b]Livers partly yellow in color with few hemorrhages were classified as slightly abnormal.

[c]Livers totally yellow with hemorrhages were classified as abnormal.

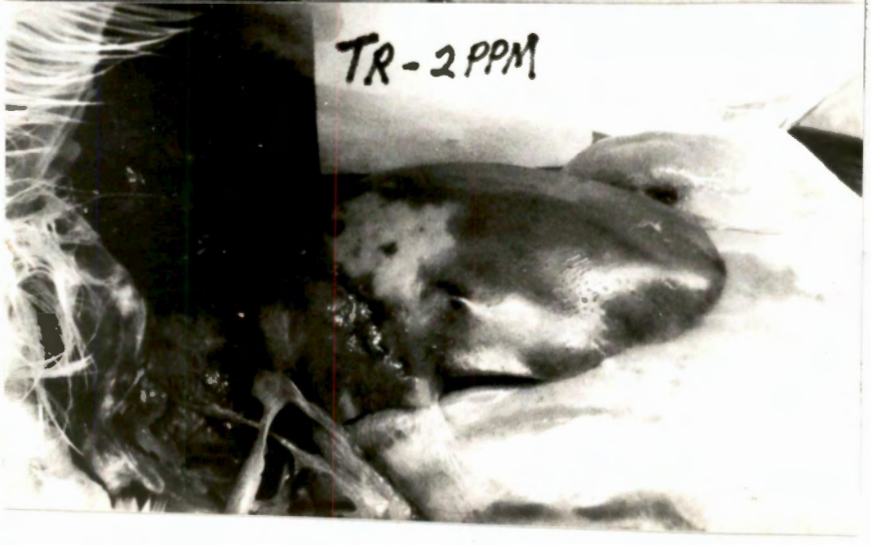
*Significantly different from control group ($P \leq .05$).



3A. Normal Liver



3B. Slightly Abnormal Liver



3C. Abnormal Liver

Figure 3. The three classifications of the liver.

Kidney Changes

The biological target organ of the ochratoxin appears to be the kidney. Many investigators such as Elling et al. (1975), Peckham et al. (1971), Krough et al. (1976), and Page et al. (1980) have found that renal disease characterized by atrophy and degeneration of the tubular epithelium of the kidney was due to ochratoxin administration. In this study, microscopical examination did not show clear histopathologic alterations in the kidney although macroscopic changes were observed.

Five birds out of 12 had pale colored kidneys for both high level groups (1 and 2 ppm ochratoxin), while only one bird out of 12 had pale colored kidneys for the control group (Table 12). These results agree with most of the results obtained by many investigators such as Elling et al. (1975), Huff et al. (1974), Galtier et al. (1976), and Page et al. (1980) who described the affected kidneys from ochratoxin administration as enlarged and pale.

TABLE 12

THE EFFECTS OF OCHRATOXIN ON KIDNEYS[a]

Treatment No.	Ochratoxin PPM (mg/kg)	Normal No.	Normal %	Pale No.	Pale %
1 (control)	0	11	91.66	1	8.33
2	0.25	8	66.66	4	33.33
3	0.5	9	75.00	3	25.00
4	1	7	58.33	5	41.66
5	2	7	58.33	5	41.66

[a] n = 12 hens per treatment level.

CHAPTER V

SUMMARY AND CONCLUSIONS

Ochratoxin at levels of 0, 0.25, 0.5, 1, and 2 ppm (mg per kg feed) was fed to 280 S. C. W. L. hens for 12 weeks. Economic traits such as egg production, egg weight, egg quality, body weight, feed consumption, water consumption, urine and urate excretion, comb color, and mortality were studied. At the end of the test, 12 birds from each treatment were killed to observe macroscopical renal and liver changes. Liver weights were taken and kidney slides were made for histological examination. Analysis of variance was conducted for each trait and comparisons between treatment one, the control, and each of the other treatments were made.

All of the treated groups were lower than the control group in percent hen-day egg production, but differences were not statistically significant. Egg weights were reduced significantly ($P \leq .05$) for the 2 ppm group during weeks 8 through 12 and for the 1 ppm group during weeks 11 and 12. The 0.5, 1, and 2 ppm level of ochratoxin increased significantly ($P \leq .05$) the percentages of egg blood and meat spots at 8 weeks of the experiment. All levels of ochratoxin reduced significantly the egg specific gravity score at the end of the experiment and the extent of reduction was proportional to the level of ochratoxin. The 0.5, 1, and 2 ppm levels of ochratoxin significantly reduced body weight at 4 weeks, but the highest body weight loss occurred at 12 weeks for the 1 and 2 ppm groups.

Water consumption, mortality rate, and average liver weight were significantly increased ($P \leq .05$) only in the hens receiving 2 ppm of ochratoxin.

There was no significant effect of any level of ochratoxin on egg Haugh units, feed consumption, or urine and urate excretion.

The 1 and 2 ppm ochratoxin groups were significantly lower than the control group in the percent of hens with red comb color ($P \leq .05$) and had a lower number of birds with normal livers and kidneys. They were significantly higher than the control group in the percent of hens with pale comb color ($P \leq .05$) and had a higher number of birds with abnormal livers and pale kidneys. Microscopical examination did not show clear histopathologic alterations in the kidneys.

Generally, it can be concluded that each trait studied was affected by some level of ochratoxin. Some traits were affected by 0.25 ppm ochratoxin and above, while other traits were not affected by even 2 ppm ochratoxin. Some symptoms of ochratoxicosis appeared early in the experiment, while other symptoms appeared only toward the end of the experiment. Some of the effects were proportional to the level of ochratoxin or became significant as the duration of exposure increased.

The effects of ochratoxin at levels used in this study on laying hens were generally not severe. These levels can be considered lower than the level required to produce acute ochratoxicosis.

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APPENDIX

TABLE A-1

EXAMPLE OF THE ANOVA TABLE USED TO ANALYZE EACH TRAIT

	ANOVA			
S.O.V.	Degrees of Freedom	Sum of Squares	Mean Squares	F
Block	1			
Treatment	4			
$T_1V_sT_2$	1			
$T_1V_sT_3$	1			
$T_1V_sT_4$	1			
$T_1V_sT_5$	1			
Error	14			
Total	19			

TABLE A-2

PERCENT HEN-DAY EGG PRODUCTION FOR EACH LOT
FIRST 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	74.23	84.43	83.41	84.43	326.50	81.62
T_2	77.29	76.27	83.16	81.37	318.09	79.52
T_3	77.04	85.05	78.06	75.00	315.15	78.78
T_4	82.14	77.29	83.16	75.00	317.59	79.39
T_5	80.27	83.16	74.23	73.97	311.63	77.90
Y.j.	797.17		791.79		1588.96	

TABLE A-3

PERCENT HEN-DAY EGG PRODUCTION FOR EACH LOT
SECOND 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	77.04	82.65	80.10	82.14	321.93	80.48
T_2	77.55	78.31	81.63	76.27	313.76	78.44
T_3	77.04	83.24	80.61	75.76	316.65	79.16
T_4	81.37	77.80	82.39	75.51	317.07	79.26
T_5	79.39	79.84	72.70	73.21	305.14	76.28
Y.j.	794.23		780.32		1574.55	

TABLE A-4

PERCENT HEN-DAY EGG PRODUCTION FOR EACH LOT
THIRD 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	75.51	81.12	79.33	81.63	317.59	79.39
T_2	72.95	75.51	85.20	81.37	315.03	78.75
T_3	79.33	82.41	79.08	74.23	315.05	78.76
T_4	81.63	76.42	81.63	76.53	316.21	79.05
T_5	80.22	79.08	72.82	74.06	306.18	76.54
Y.j.	784.18		785.88		1570.06	

TABLE A-5

PERCENT HEN-DAY EGG PRODUCTION FOR EACH LOT
ENTIRE PERIOD

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	75.59	82.73	80.95	82.75	322.00	80.50
T_2	75.93	76.70	83.33	79.67	315.63	78.90
T_3	77.80	83.57	79.25	75.00	315.62	78.90
T_4	81.71	77.18	82.39	75.68	316.96	79.24
T_5	79.96	80.69	73.25	73.74	307.64	76.91
Y.j.	791.86		785.99		1577.85	

TABLE A-6

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
FIRST WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	63.44	62.33	61.53	63.00	250.30	62.57
T_2	62.60	62.50	61.00	61.58	247.68	61.92
T_3	60.25	61.40	63.41	65.60	250.66	62.66
T_4	60.83	62.63	62.85	61.16	247.47	61.86
T_5	61.42	62.09	63.10	61.27	247.88	61.97
Y.j.	619.49		624.50		1243.99	

TABLE A-7

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
SECOND WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	62.70	61.90	61.30	63.54	249.44	62.36
T_2	64.18	63.50	61.30	62.53	251.51	62.87
T_3	61.08	62.50	60.44	64.58	248.60	62.15
T_4	61.63	63.75	61.25	61.90	248.53	62.13
T_5	61.63	61.81	63.63	61.50	248.57	62.14
Y.j.	624.68		621.97		1246.65	

TABLE A-8

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
THIRD WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	63.00	63.00	63.75	63.46	253.21	63.30
T_2	62.41	62.66	62.15	63.07	250.29	62.57
T_3	59.07	62.50	61.54	63.45	246.56	61.64
T_4	61.83	63.70	61.21	63.11	249.85	62.46
T_5	62.27	60.66	63.41	61.90	248.24	62.06
Y.j.	621.10		627.05		1248.15	

TABLE A-9

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
FOURTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	65.25	63.58	64.41	62.23	255.47	63.86
T_2	65.16	64.16	65.00	61.41	255.73	63.93
T_3	59.42	63.63	62.58	64.50	250.13	62.53
T_4	63.50	61.50	60.90	61.61	247.51	61.87
T_5	63.23	61.50	62.30	63.20	250.23	62.55
Y.j.	630.93		628.14		1259.07	

TABLE A-10

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
FIFTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	63.00	62.83	63.00	63.33	252.16	63.04
T_2	62.62	63.81	65.40	62.85	254.68	63.67
T_3	60.00	60.85	63.09	64.81	248.75	62.18
T_4	62.42	63.77	61.75	63.44	251.38	62.84
T_5	63.00	62.18	63.63	61.90	250.71	62.67
Y.j.	624.48		633.20		1257.68	

TABLE A-11

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
SIXTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	64.87	63.22	62.07	65.20	255.36	63.84
T_2	63.72	64.00	65.25	64.00	256.97	64.24
T_3	62.00	63.57	64.83	64.90	255.30	63.82
T_4	63.72	64.37	63.00	62.45	253.54	63.38
T_5	62.63	61.75	63.66	63.70	251.74	62.93
Y.j.	633.85		639.06		1272.91	

TABLE A-12

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
SEVENTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	64.90	64.72	62.50	62.71	254.83	63.70
T_2	65.28	65.70	64.58	63.62	259.18	64.79
T_3	59.37	63.00	65.27	65.62	253.26	63.31
T_4	63.00	63.54	62.70	63.90	253.14	63.28
T_5	62.87	63.40	62.66	61.25	250.18	62.54
Y.j.	635.78		634.81		1270.59	

TABLE A-13

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
EIGHTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	64.69	62.69	64.58	62.92	254.88	63.72
T_2	63.00	63.50	62.92	63.50	252.92	63.23
T_3	60.15	63.23	64.08	64.41	251.87	62.96
T_4	61.61	63.18	61.33	62.72	248.84	62.21
T_5	62.27	61.83	62.66	61.33	248.09	62.02
Y.j.	626.15		630.45		1256.60	

TABLE A-14

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
NINTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	64.36	63.41	64.83	62.60	255.20	63.80
T_2	64.20	62.90	64.18	62.20	253.48	63.37
T_3	61.33	61.00	64.30	65.81	252.44	63.11
T_4	63.30	61.45	62.00	63.75	250.50	62.62
T_5	61.91	63.30	63.40	60.63	249.24	62.31
Y.j.	627.16		633.70		1260.86	

TABLE A-15

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
TENTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	64.75	63.18	63.44	64.37	255.74	63.93
T_2	65.57	64.66	62.84	63.54	256.61	64.15
T_3	62.60	63.27	65.42	64.00	255.29	63.82
T_4	62.16	63.54	63.75	63.66	253.11	63.27
T_5	62.00	60.50	63.11	62.85	248.46	62.11
Y.j.	632.23		636.98		1269.21	

TABLE A-16

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
ELEVENTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	64.07	64.25	63.81	64.42	256.55	64.13
T_2	65.66	64.81	64.00	64.15	258.62	64.65
T_3	62.84	63.60	64.92	65.88	257.24	64.31
T_4	62.80	62.66	62.57	62.00	250.03	62.50
T_5	62.64	61.53	63.83	62.25	250.25	62.56
Y.j.	634.86		637.83		1272.69	

TABLE A-17

AVERAGE EGG WEIGHT (GRAMS) FOR EACH LOT
TWELFTH WEEK

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	65.41	65.33	63.64	63.53	257.91	64.47
T_2	64.83	64.27	65.81	65.64	260.55	65.13
T_3	61.69	64.50	64.54	65.35	256.08	64.02
T_4	63.07	63.08	63.13	62.13	251.41	62.85
T_5	62.66	62.53	62.64	62.64	250.47	62.61
Y.j.	637.37		639.05		1276.42	

TABLE A-18

PERCENT OF EGG BLOOD AND MEAT SPOTS FOR EACH LOT AT 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	20	10	0	10	40	10.00
T_2	20	0	0	10	30	7.50
T_3	10	20	10	20	60	15.00
T_4	20	20	10	20	70	17.50
T_5	30	20	20	20	90	22.50
Y.j.	170		120		290	

TABLE A-19

PERCENT OF EGG BLOOD AND MEAT SPOTS FOR EACH LOT AT 8 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	10	10	0	0	20	5.00
T_2	10	10	0	20	40	10.00
T_3	10	20	10	20	60	15.00
T_4	20	10	20	10	60	15.00
T_5	10	20	20	20	70	17.50
Y.j.	130		120		250	

TABLE A-20

PERCENT OF EGG BLOOD AND MEAT SPOTS FOR EACH LOT AT 12 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	10	10	0	10	30	7.50
T_2	10	0	20	10	40	10.00
T_3	10	40	20	10	80	20.00
T_4	20	20	20	10	70	17.50
T_5	10	30	20	30	90	22.50
Y.j.	160		150		310	

TABLE A-21

AVERAGE EGG HAUGH UNITS FOR EACH LOT AT 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	73	75	71	74	293	73.25
T_2	74	71	73	73	291	72.75
T_3	72	76	72	71	291	72.75
T_4	75	74	72	74	295	73.75
T_5	69	74	74	75	292	73.00
Y.j.	73.3		72.9		1462	

TABLE A-22

AVERAGE EGG HAUGH UNITS FOR EACH LOT
AT 8 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	75	75	69	71	290	72.50
T_2	70	67	71	75	283	70.75
T_3	76	77	71	73	297	74.25
T_4	70	72	72	73	287	71.75
T_5	75	72	74	72	293	73.25
Y.j.	729		721		1450	

TABLE A-23

AVERAGE EGG HAUGH UNITS FOR EACH LOT
AT 12 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	75	71	72	74	292	73.00
T_2	73	72	71	73	289	72.25
T_3	75	76	67	75	293	73.25
T_4	74	74	72	74	294	73.50
T_5	73	72	72	71	288	72.00
Y.j.	735		721		1456	

TABLE A-24

AVERAGE EGG SPECIFIC GRAVITY SCORES FOR EACH LOT AT 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	3.1	3.2	4.4	2.9	13.6	3.40
T_2	2.5	3.0	3.9	3.9	13.3	3.32
T_3	2.8	2.3	2.3	2.4	9.8	2.45
T_4	3.1	3.9	3.4	1.8	12.2	3.05
T_5	2.1	2.8	2.8	2.0	9.7	2.42
Y.j.	28.8		29.8		58.6	

TABLE A-25

AVERAGE EGG SPECIFIC GRAVITY SCORES FOR EACH LOT AT 8 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	3.2	2.8	2.7	3.7	12.4	3.10
T_2	2.4	2.1	2.3	2.8	9.6	2.40
T_3	2.9	2.0	2.0	2.1	9.0	2.25
T_4	2.9	2.6	2.5	1.4	9.4	2.35
T_5	1.2	2.5	2.2	1.9	7.8	1.95
Y.j.	24.6		23.6		48.2	

TABLE A-26

AVERAGE EGG SPECIFIC GRAVITY SCORES FOR EACH LOT AT 12 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	3.5	2.6	2.6	3.2	11.9	2.97
T_2	2.5	1.9	2.0	2.9	9.3	2.32
T_3	2.6	2.0	1.9	1.9	8.4	2.10
T_4	1.6	2.0	2.1	2.3	8.0	2.00
T_5	2.2	2.2	2.1	1.8	8.3	2.07
Y.j.	23.1		22.8		45.9	

TABLE A-27

AVERAGE BODY WEIGHT GAIN OR LOSS (GRAMS) FOR EACH LOT AT 4 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	13.43	6.43	15.43	19.14	54.43	13.60
T_2	56.43	-48.57	17.00	- 5.57	19.29	4.82
T_3	-14.28	-58.15	-11.14	-28.43	-112.00	-28.00
T_4	-55.15	-29.57	- 2.00	-13.15	- 99.87	-24.96
T_5	-52.00	-36.14	-28.85	-48.29	-165.28	-41.32
Y.j.	-217.57		-85.86		-303.43	

TABLE A-28

AVERAGE BODY WEIGHT GAIN OR LOSS (GRAMS) FOR EACH LOT AT 8 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	22.71	3.71	16.71	15.28	58.41	14.60
T_2	66.86	-23.72	- 3.43	0	39.71	9.92
T_3	-29.71	- 4.86	42.86	31.72	40.01	10.00
T_4	10.57	- 8.57	17.29	- 5.43	13.86	3.46
T_5	-39.29	-39.14	-27.85	-83.86	-190.14	-47.53
Y.j.	-41.44		3.29		- 38.15	

TABLE A-29

AVERAGE BODY WEIGHT GAIN OR LESS (GRAMS) FOR EACH LOT AT 12 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	59.57	92.71	56.00	29.57	237.85	59.46
T_2	69.15	- 36.57	- 7.00	23.43	49.01	12.25
T_3	1.57	10.43	22.57	45.43	80.00	20.00
T_4	-16.29	-101.69	-21.00	-22.43	-161.41	-40.35
T_5	-56.38	- 85.72	-64.85	-93.86	-300.81	-75.20
Y.j.	-63.22		-32.14		- 95.36	

TABLE A-30

AVERAGE AMOUNT OF FEED CONSUMED (GM/DAY/BIRD) FOR EACH LOT
FIRST 2 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	108	111	106	108	433	108.25
T_2	106	106	113	108	433	108.25
T_3	111	113	113	111	448	112.00
T_4	106	106	115	108	435	108.75
T_5	113	108	111	111	443	110.75
Y.j.	1088		1104		2192	

TABLE A-31

AVERAGE AMOUNT OF FEED CONSUMED (GM/DAY/BIRD) FOR EACH LOT
SECOND 2 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	106	106	111	104	427	106.75
T_2	111	106	111	115	443	110.75
T_3	104	112	102	111	429	107.25
T_4	106	106	106	115	433	108.25
T_5	112	106	115	111	444	111.00
Y.j.	1075		1101		2176	

TABLE A-32

AVERAGE AMOUNT OF FEED CONSUMED (GM/DAY/BIRD) FOR EACH LOT
THIRD 2 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	115	113	111	102	441	110.25
T_2	106	104	111	113	434	108.50
T_3	115	124	104	108	451	112.75
T_4	115	115	111	113	454	113.50
T_5	109	113	113	113	448	112.00
Y.j.	1129		1099		2228	

TABLE A-33

AVERAGE AMOUNT OF FEED CONSUMED (GM/DAY/BIRD) FOR EACH LOT
FOURTH 2 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	111	111	108	104	434	108.50
T_2	104	104	113	113	434	108.50
T_3	115	117	108	104	444	111.00
T_4	106	113	111	104	434	108.50
T_5	117	115	111	111	454	113.50
Y.j.	1113		1087		2200	

TABLE A-34

AVERAGE AMOUNT OF FEED CONSUMED (GM/DAY/BIRD) FOR EACH LOT
FIFTH 2 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	108	102	102	102	414	103.50
T_2	102	111	102	104	419	104.75
T_3	106	114	102	102	424	106.00
T_4	99	105	104	106	414	103.50
T_5	114	104	106	113	437	109.25
Y.j.	1065		1043		2108	

TABLE A-35

AVERAGE AMOUNT OF FEED CONSUMED (GM/DAY/BIRD) FOR EACH LOT
SIXTH 2 WEEKS

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	108	106	104	108	426	106.50
T_2	104	97	111	106	418	104.50
T_3	102	109	106	104	421	105.25
T_4	104	117	108	106	435	108.75
T_5	116	102	110	114	442	110.50
Y.j.	1065		1077		2142	

TABLE A-36

AVERAGE AMOUNT OF WATER CONSUMED (ML/DAY/BIRD) FOR EACH LOT

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	175.74	190.83	198.08	162.24	726.89	181.72
T_2	188.49	184.83	192.58	177.75	743.65	185.91
T_3	198.33	192.74	172.58	165.24	728.89	182.22
T_4	187.66	171.16	197.00	173.74	729.56	182.39
T_5	198.24	207.16	205.58	197.74	808.72	202.18
Y.j.	1895.18		1842.53		3737.71	

TABLE A-37

PERCENT OF HENS WITH WET REAR END FOR EACH LOT

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	28.57	14.28	21.42	14.28	78.55	19.63
T_2	28.57	7.14	7.14	28.57	71.42	17.85
T_3	21.42	15.38	28.57	14.28	79.65	19.91
T_4	21.42	15.38	21.42	28.57	86.79	21.69
T_5	25.00	21.42	46.15	30.76	123.33	30.83
Y.j.	198.58		241.16		439.74	

TABLE A-38

PERCENT OF HENS WITH RED COMB COLOR FOR EACH LOT

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	42.85	14.28	14.28	28.57	99.98	24.99
T_2	28.57	35.71	28.57	28.57	121.42	30.35
T_3	7.14	30.76	7.14	21.42	66.46	16.61
T_4	14.28	15.38	7.14	7.14	43.94	10.98
T_5	16.66	7.14	15.38	7.69	46.87	11.71
Y.j.	212.77		165.90		378.67	

TABLE A-39

PERCENT OF HENS WITH PALE COMB COLOR FOR EACH LOT

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	7.14	7.14	7.14	7.14	28.56	7.14
T_2	14.28	14.28	7.14	14.28	49.98	12.49
T_3	14.28	7.69	14.28	14.28	50.53	12.63
T_4	14.28	23.07	7.14	21.42	65.91	16.47
T_5	16.66	21.42	15.38	7.69	61.15	15.28
Y.j.	140.24		115.89		256.13	

TABLE A-40

PERCENT OF MORTALITY FOR EACH LOT

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	0	0	0	0	0	0
T_2	0	0	0	0	0	0
T_3	0	7.14	0	0	7.14	1.78
T_4	0	7.14	0	0	7.14	1.78
T_5	14.28	0	7.14	7.14	28.56	7.14
Y.j.	28.56		14.28		42.84	

TABLE A-41

AVERAGE LIVER WEIGHT (GRAMS) FOR EACH LOT

	B_1		B_2			
	R_1	R_2	R_1	R_2	$Y_{i..}$	$\bar{y}$
T_1	44.00	42.00	39.66	39.00	164.66	41.16
T_2	40.00	41.00	40.33	36.33	157.66	39.41
T_3	41.00	36.66	38.33	38.66	154.65	38.66
T_4	40.00	47.00	42.66	40.00	169.66	42.41
T_5	47.33	42.33	42.00	49.00	180.66	45.16
Y.j.	421.32		405.97		827.29	

VITA

Saad Hussain Tohala was born in Mosul, Iraq, on December 5, 1948. He was educated in the elementary and secondary schools of that city and was graduated from Al-Markazia High School in June 1965. The following September he entered the College of Agriculture, University of Mosul, receiving a Bachelor's degree in Animal Husbandry in June 1969. In September 1969, he was employed as a demonstrator by the University of Mosul, College of Agriculture, in the Animal Husbandry Department. In 1973, he joined the post graduate courses at the same department. In 1976, he was granted a Master's degree in Animal Science and worked as an assistant teacher in the department until December 1978, when he was awarded a study leave from the University of Mosul to study at The University of Tennessee, Knoxville. In March 1983 he was granted the Ph. D. degree with a major in Animal Science, with emphasis on Poultry Physiology.