Detoxification of T-2 toxin and feeding behavior changes associated with T-2 toxin

by

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A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of
MASTER OF SCIENCE

Department: Veterinary Pathology
Major: Veterinary Pathology
(Veterinary Toxicology)

Iowa State University
Ames, Iowa

1988

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INTRODUCTION

Vast quantities of grain are required to feed the world population. Grain supplies are at risk of spoilage prior to harvest, especially during damp environmental conditions. Feedstuffs contaminated with trichothecene mycotoxins often result in reduced animal performance. Little scientific information is available for practical application of decontamination of trichothecene contaminated feedstuffs. Also, sparse information is available concerning mechanisms by which trichothecene mycotoxins cause reduced feed intake, or why in some circumstances reduced feed intake persists after removal of trichothecene contaminated feedstuffs.

The objective of this project was to evaluate hydrogen peroxide as a decontaminating agent for feedstuffs contaminated with T-2 toxin. The second aspect of the project was to investigate changes in feeding behavior associated with T-2 toxin contaminated feed, and to evaluate development of taste aversion associated with T-2 toxin.

REVIEW OF LITERATURE

Trichothecene Mycotoxins

The trichothecene mycotoxins comprise a large class of fungal metabolites that occur naturally in various foodstuffs. Trichothecene mycotoxins are characterized by chemical structures consisting of a tetracyclic 12,13-epoxytrichothec-9-ene nucleus. At present, at least 45 naturally occurring trichothecene mycotoxins have been identified, of which only a few appear to be of economic or health-related interest (Ueno et al., 1973a). Most of the trichothecenes are produced by Fusarium spp. of fungi, which are common throughout the world. Environmental conditions appear to dictate whether or not a particular Fusarium spp. will produce a trichothecene mycotoxin.

Several diseases have been associated with molded foodstuffs in past history, from which Fusarium spp. were isolated. Trichothecene mycotoxins were associated with many of the diseases. Due to the lack of analytical methodology developed for specific trichothecenes, diagnoses were often made according to clinical signs or symptoms, combined with isolation of particular Fusarium spp. from the foodstuffs. Since many of the Fusaria spp. have the ability to produce many different mycotoxins, it was difficult to determine the specific mycotoxin that was responsible for the syndrome in question (Pfeiffer, 1986).

Trichothecene mycotoxins that have received the most attention are deoxynivalenol (DON, vomitoxin), T-2 toxin, diacetoxyscirpenol (DAS), and nivalenol.

Deoxynivalenol was isolated from corn associated with poor feed consumption (Vesonder et al., 1973). Deoxynivalenol is not uncommon in feed grains in the United States (Cote et al., 1984). Fusarium graminearum and Fusarium culmorum are most often responsible for producing deoxynivalenol in feed grains, however Fusarium tricinctum and Fusarium nivale also have been shown to produce deoxynivalenol (Vesonder et al., 1981b).

Nivalenol was first detected in barley infested with Fusarium roseum (Morooka et al., 1972). Nivalenol was also found to be produced by Fusarium nivale (Tatsuno et al., 1968).

Fusarium spp. that produce diacetoxyscirpenol include Fusarium roseum (Mirocha et al., 1976) and Fusarium moniliforme (Rukmini and Bhat, 1978).

T-2 toxin is produced by Fusarium tricinctum (Hsu et al., 1972), Fusarium sporotrichioides, Fusarium poae, Fusarium solani (Joffe and Palti, 1975), Fusarium semitectum, Fusarium roseum (Burmeister et al., 1972), and Fusarium moniliforme (Vesonder et al., 1981b).

Toxicity

The general toxic effects of trichothecene mycotoxins

include oral epithelial necrosis, vomiting, food refusal, hemorrhaging, neural disturbances, and immunosuppression (Joffe, 1986).

Chemistry and mechanism of action

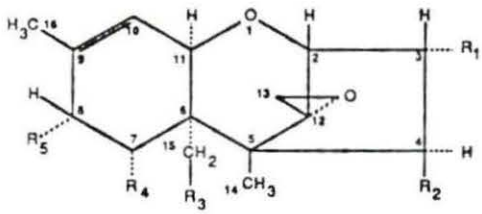
The trichothecenes have been subdivided by chemical structure into four types: A, B, C, and D (Ueno et al., 1973a). Type A trichothecenes include T-2 toxin, diacetoxyscirpenol, neosolaniol, HT-2 toxin, and others. Type A toxins contain an H, OH, or extra ester at the eighth carbon. Figure 1 illustrates chemical structures of the four types of trichothecenes.

Type B trichothecenes are characterized by a ketone function at the eighth carbon, rather than an H, OH, or ester as is present in Type A trichothecenes. The rest of the chemical structure is very similar to the Type A trichothecene nucleus. Deoxynivalenol is an example of a trichothecene that belongs to the Type B group.

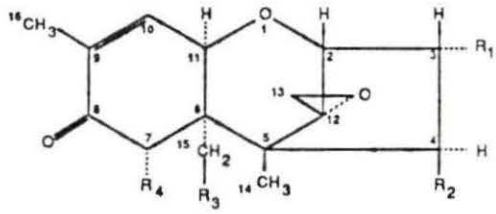
Type C trichothecene mycotoxins contain an oxirame ring at the 7-8 carbon position, and includes only one compound, crotocin, which is produced by Cephalosporum crotocenigenum (Ishii, 1983, cited by Joffe, 1986).

The fourth type, Type D, includes the macrocyclic derivatives of verrucarins and verrucarol. This type contains a macrocyclic ring at the fourth and fifteenth carbons, and includes various compounds produced by several

Figure 1. Chemical structures of the classes of trichothecene mycotoxins



Type A



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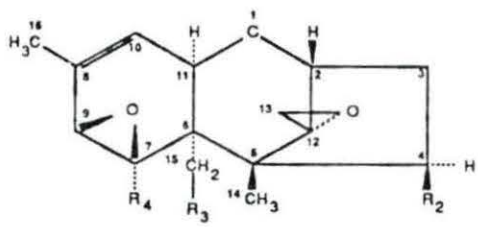

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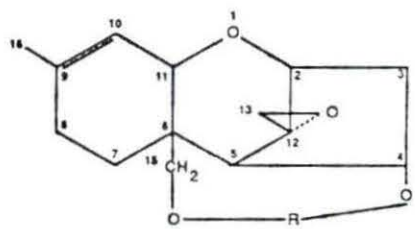

Type D

fungi. Type D trichothecenes are not produced by Fusarium spp. (Joffe, 1986).

Type A trichothecene toxins caused stronger vomiting, feed refusal, dermal necrotizing effects, and hemorrhages in the gastrointestinal tract than Type B toxins. Leukopenia, reduction in weight gains, infertility, abortion, and inhibition of protein synthesis were also more pronounced with Type A trichothecene toxins (Ueno, 1977).

The C-12 - C-13 epoxide ring of the trichothecene molecule appears to impart the biological effects of the substance, as opening the C-12 - C-13 epoxide abolishes toxicity of the trichothecene (Ueno, 1977). Derivatives of the basic trichothecene nucleus with a hydroxyl or acetate group at C-3 or C-4 have about the same toxicity, and reducing the C-9 - C-10 double bond only slightly decreases toxicity (Ueno, 1977).

The mechanism of inhibition of protein synthesis has been investigated. T-2 toxin was found to bind to a receptor site on eukaryotic ribosomes, and was proposed to inhibit peptide bond formation on ribosomes (Cannon et al., 1976). Trichothecene mycotoxins have also been shown to inhibit DNA synthesis (Cannon et al., 1976).

The mode of administration of a trichothecene toxin also has an effect on the degree of toxicity. The LD_{50} for

fusarenon-X in mice was found to be 3.4 mg/kg when administered intravenously, 4.2-4.6 mg/kg when administered subcutaneously, 3.4 mg/kg when given intraperitoneally, and 4.5 mg/kg with oral dosing (Ueno et al., 1971).

The effects of trichothecenes on poultry

Chickens. Reports of decreased feed consumption and reduced growth rates in chickens caused by T-2 toxin exist. Growth rate in chickens is inhibited significantly by levels of T-2 toxin as low as 4 mg/kg in feedstuffs (Wyatt et al., 1972b). Chickens orally gavaged with T-2 toxin at levels of 1.5 to 3.0 mg/kg of body weight for 14 daily doses also lost weight (Hoerr et al., 1982b).

The 72 hour single oral LD_{50} for T-2 toxin in the chicken is 4.0 mg/kg of body weight, and the 14 daily oral LD_{50} is 2.9 mg/kg of body weight (Hoerr and Carlton, 1981).

An obvious sign of dietary T-2 toxicosis in broiler chicks and laying hens are oral lesions (Chi et al., 1977). Detailed descriptions of the oral lesions have been published. Oral lesions caused by T-2 toxin first appear on the hard palate and along the margin of the tongue (Wyatt et al., 1972b). Gross lesions are described as being raised, yellowish to white, and caseous in nature. Lesions may progress to the underside of the tongue, and on the inside of the lower beak (Hoerr et al., 1982a). In some chickens, the lesions become so severe that they were unable

to completely close their mouths (Wyatt et al., 1972b). Lesions also occur on the tip of the tongue, floor of the buccal cavity, and beak near the commissures (Hoerr et al., 1982a). Oral lesions occur in chickens consuming feedstuffs with T-2 toxin as low as 1 mg/kg in the feedstuff (Wyatt et al., 1972b). Oral lesions were reported in chickens consuming feed containing 0.5 mg/kg of T-2 toxin, although the trichothecene neosolaniol was also present in the feed at 0.05 mg/kg (Hoerr et al., 1982a). Oral lesions are reported to be present as early as 4 days after T-2 toxin contaminated feeds are offered to chickens (Hoerr et al., 1982a).

Field reports of oral lesions in poultry have been documented in the United States. Lesions resembled those caused by T-2 toxin, although no feed analyses were performed to confirm the presence of T-2 toxin or other trichothecene mycotoxins. Lesions were also present on the shanks, feet, and around the eyes (Wyatt et al., 1972a).

Histopathologic examination of oral mucosal lesions revealed intense inflammatory reactions, coupled with localized necrosis of the oral mucosa. The outer layer of the lesions consisted mainly of fibrinous material. Underlying epithelium was heavily infiltrated with granular leukocytes (Wyatt et al., 1972b). Within the outer layer of fibrinous material were feed particles, keratinized debris,

and aggregates of bacteria. Necrosis was reported to spread from the epithelium to the maxillary salivary glands, mandibles, and cartilagenous extensions of the basihyoid bones. In this study, oral lesions were reported to decrease in time, even though chickens were continually fed the toxin (Hoerr et al., 1982a).

Other histopathologic lesions that occur in chickens orally gavaged with large doses of T-2 toxin or diacetoxyscirpenol include necrosis with depletion of lymphocytes in the bursa of Fabricius, thymus, spleen, cecal tonsil and ectopic lymphoid tissue. Necrosis with depletion of hematopoietic tissue in the bone marrow occurred. Necrosis was present in the intestinal villus and crypt epithelium, and lymphocytes in the lamina propria. Other tissues with necrotizing lesions included liver and kidney (Hoerr et al., 1982b).

Microscopic lesions in chickens that were allowed to eat feedstuffs contaminated with T-2 toxin and neosolaniol in combination included severe necrosis of lymphocytes within lymphoid and hematopoietic tissues, and necrotizing lesions of the alimentary tract mucosa, liver and gall bladder, feathers, and thyroid glands (Hoerr et al., 1982a).

T-2 toxin significantly reduced spleen and bursal weights when chicks were orally gavaged with high doses of the toxin (Hoerr et al., 1982b). When allowed to orally

ingest feed contaminated with T-2 toxin and neosolaniol, spleen and bursal weights were also decreased (Hoerr et al., 1982a). In another study, T-2 toxin fed to chicks at dietary concentrations up to 10 mg/kg did not reduce spleen, bursal, or thymus weights (Richard et al., 1978).

Feather abnormalities in growing chickens have resulted from ingestion of T-2 toxin. Feather changes were present at doses of T-2 toxin in the feed as low as 4 mg/kg, and severity of the feather abnormalities was dose related. Chickens were sparsely covered with feathers, which protruded at odd angles, and tail feathers were short (Wyatt et al., 1975). Feather changes were also reported in chickens orally gavaged with T-2 toxin (Hoerr et al., 1982b).

Neurological signs in chicks fed a combination of T-2 toxin and neosolaniol consisted of an abnormal righting reflex. The author stated that this clinical sign was not associated with anemia or microscopic lesions in the central nervous system (Hoerr et al., 1982a).

T-2 toxin has also been incriminated in hemorrhagic anemia syndrome of chickens. T-2 toxin has been shown to increase prothrombin times in chickens fed growth inhibitory doses of T-2 toxin, however blood clotting times were not significantly prolonged (Doerr et al., 1974). The

coagulopathy of T-2 toxin has been attributed to a primary defect in Factor VII activity and secondary effects on prothrombin and fibrinogen (Doerr et al., 1981).

T-2 toxin and diacetoxyscirpenol both resulted in significant reductions in hematocrit values of chickens which were orally gavaged with relatively high doses of the mycotoxins (Hoerr et al., 1982b).

The effect of deoxynivalenol on chickens appears to be similar to the other trichothecenes that have been studied, although a higher dosage is required for equivalent effects. The single oral dose LD_{50} for the chicken for deoxynivalenol is approximately 140 mg/kg (Huff et al., 1981). Feed containing up to 1.87 mg/kg deoxynivalenol had no significant effect on body weight gain in one-day old broiler chicks (Hulan and Proudfoot, 1982). Reduced feed intake caused by deoxynivalenol fed to chickens did not occur until a level of approximately 116 mg/kg in feed was reached (Moran et al., 1982).

Other clinical findings of chickens intoxicated with deoxynivalenol include neural disturbances and diarrhea. Postmortem findings included ecchymotic hemorrhages throughout the intestinal tract, liver, and musculature (Huff et al., 1981).

Oral mucosal lesions were produced in chickens fed deoxynivalenol. Chicks offered feed contaminated with 116.1

mg/kg deoxynivalenol at 6 days of age developed lesions of the oral mucous membranes, whereas chicks receiving 49.4 mg/kg in feed did not develop necrotizing oral lesions (Moran et al., 1982).

Turkeys. The effect of trichothecene mycotoxins on turkeys is similar to the effect on chickens. Decreased weight gains are manifested at T-2 toxin concentrations as low as 2 mg/kg in the diet (Richard et al., 1978). Necrotizing oral lesions were produced at dietary concentrations of 10 mg/kg in the diet (Richard et al., 1978), and 5 mg/kg in the diet (Dziuk et al., 1979). Thymus weights of poults offered 10 mg/kg of T-2 toxin in the feed were significantly reduced, but bursal and spleen weights of poults were not affected (Richard et al., 1978).

The effects of trichothecenes on rodents

Trichothecene mycotoxins have been found to cause feed refusal in rats. T-2 toxin in rats' diets at 40 mg/kg resulted in 75% food refusal, diacetoxyscirpenol in the diet at 40 mg/kg resulted in 74% food refusal, and deoxynivalenol at 40 mg/kg in the food resulted in 54% refusal (Vesonder et al., 1979).

T-2 toxin in the food of mice at 20 mg/kg resulted in decreased consumption, Control mice ate 2 to 3 times as much food as mice on the T-2 toxin diet (Hayes and Schiefer, 1980).

Trichothecenes instilled into the water supply of mice caused reduced water intake. T-2 toxin in the water at 2 mg/L reduced water consumption in mice, as did diacetoxyscirpenol also at 2 mg/L. Deoxynivalenol concentrations in water were required to be 5 mg/L before significant depression of water consumption was recognized in mice (Burmeister et al., 1980).

Reduced body weight gain is also an effect of T-2 toxin when rats' diets are contaminated with the toxin. Rats fed 5 mg/kg T-2 toxin in the diet for 3 weeks had only a slight depression in weight gains, but when the dietary concentration was increased to 15 mg/kg in the diet, marked reduction in body weight gain was present. Reduced growth rate was attributed only to the reduction in food intake (Marasas et al., 1969).

A single dose oral LD_{50} value reported for T-2 toxin in rats is 3.8 mg/kg (Kosuri et al., 1971).

Hematologic changes in rodents have been reported due to the effects of T-2 toxin. Mice fed a diet containing 20 mg/kg T-2 toxin became anemic and reticulocytopenic. Erythropoiesis in the spleen and bone marrow of the mice was diminished (Hayes and Schiefer, 1980). Rats dosed with 2 mg/kg T-2 toxin had prothrombin times which were nearly doubled over control rats (Kosuri et al., 1971).

Organ lesions described in rats dosed intraperitoneally

with T-2 toxin at relatively high doses included myocardial lesions. Lesions were not present in rats necropsied at 6 hours post-exposure, but were present when rats were necropsied at 24 or more hours post-exposure (Yarom et al., 1983).

Detoxification of trichothecenes

Attempts have been made to alleviate the effects of trichothecene mycotoxins in feedstuffs. Diets containing 20 mg/kg T-2 toxin were formulated to contain three levels of protein, which were then fed to mice to determine if higher levels of dietary protein would lessen the toxic effects of the trichothecene. All mice in the study that received the toxin developed atrophy of hematopoietic tissue in bone marrow and spleen. However, the degree of myeloid and megakaryocyte proliferation in the splenic red pulp was greater in mice receiving the highest protein level. The author postulated that the higher dietary protein level may have resulted in increased hepatic mixed function oxidase enzyme levels, which may be responsible for biotransformation of the toxin (Hayes and Schiefer, 1980).

In another study, rats were fed diets with 3 mg/kg T-2 toxin, with the addition of 20% alfalfa in the diets of the test animals. Rats fed alfalfa had a higher level of feed consumption than the controls whose diets had no added alfalfa. The effect of alfalfa in the diet was attributed

to the ability of increased fiber to bind and increase fecal excretion of the toxin (Carson and Smith, 1983).

Copper was added to swine diets contaminated with deoxynivalenol, in an attempt to relieve effects of reduced feed intake. The addition of 250 mg/kg copper to feed containing deoxynivalenol at levels up to 3.6 mg/kg did not result in significantly increased feed intake or average daily gain as compared to no added copper (Carlson et al., 1983).

An experiment was done to evaluate the effect of water washing corn contaminated with Fusarium graminearum. Mycotoxin identification was not mentioned by the author. Washing the corn for 10 hours with water did not improve the decreased feed intake in pigs which was thought to be caused by mold comtamination, but washing for 48 hours with rinses every 2 hours removed the toxic factor from the corn. Pigs fed the corn washed for 48 hours had feed intakes and weight gains equivalent to those fed control corn (Forsyth et al., 1976).

A study was done to determine the effect of physical treatment on deoxynivalenol in wheat. Wheat naturally contaminated with deoxynivalenol at 0.45 mg/kg was milled into flour. It was found that most of the deoxynivalenol was isolated in the bran portion of the milled products, and lower levels were present in the flour. Baking products

from the contaminated flour resulted in no reduction to a 35% decline in deoxynivalenol concentrations (Young et al., 1984).

Oxidizing agents have been used to destroy aflatoxins, which are mycotoxins produced by Aspergillus spp. Hydrogen peroxide (HPO) is an example of an oxidizing agent which is acceptable to use in certain food processing. A combination of riboflavin and HPO were used with pasteurization to inactivate up to 98% of aflatoxin M_1 in milk (Applebaum and Marth, 1980, cited by Doyle et al., 1982). The citing author stated that the amount of HPO and riboflavin required are excessive and would probably not be suitable for practical application.

Wheat naturally contaminated with deoxynivalenol was treated with aqueous solutions of HPO in an effort to reduce deoxynivalenol levels (Young et al., 1986). Applications of 6% HPO at 33 ml/kg of wheat (low volume) did not reduce deoxynivalenol level, however 6% HPO at 750 ml/kg of wheat (high volume) reduced deoxynivalenol concentration to 34% of the value prior to treatment. The procedure in this study used aqueous solutions which were applied to wheat for 24 hours then milled (low volume) or allowed to air dry (large volume) prior to milling. In this study, sodium bisulfite was found to be very effective in reducing deoxynivalenol in wheat, reducing levels to less than 2% of concentration

prior to treatment.

In another study, autoclaving deoxynivalenol contaminated corn after application of sodium bisulfite reduced deoxynivalenol levels from 7.21 mg/kg to 0.79 mg/kg (Young et al., 1987). Pigs were fed treated and untreated corn, and the sodium bisulfite treatment in combination with autoclaving removed the toxic effect of deoxynivalenol in the pigs.

MATERIALS AND METHODS

Chick Trial I

Preparation of T-2 toxin

An agar slant containing a live culture of Fusarium sporotrichioides was blended with 50 ml of sterile water. Twenty ml of this solution was added to 50 grams of sterile corn grits in a sterile container, which was then incubated at room temperature (25°) for 7 days, at which time visible growth was present. This culture was placed in an incubator for 5 weeks at 12° C. The culture was then air-dried, and forced through a sieve to reduce the particle size to a more homogeneous consistency. The prepared T-2 toxin concentrate was then analyzed for T-2 toxin concentration and stored in a freezer until mixed into feed.

The T-2 toxin concentrate contained 4744 mg/kg T-2 toxin.

Preparation of feedstuffs

Control feed. The control feed (C feed) used was a commercial broiler-starter-finisher feed[1] which contained not less than 22% crude protein and not less than 5% fat. It also contained 0.0125% amprolium and 100 grams per ton of chlortetracycline. The feed was ground to a fine

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[1]Protein Blenders, Inc., Iowa City, IA.

consistency, using a laboratory mill[2] with a screen diameter of 2 mm.

T-2 toxin feed. The T-2 toxin feed (T-2 feed) was formulated by using the control feed as a base. The T-2 toxin concentrate containing 4744 mg/kg T-2 toxin was diluted to 474 mg/kg by blending 400 grams of the T-2 toxin concentrate with 3.6 kg of control feed. The 474 mg/kg T-2 toxin concentrate was then blended with control feed to a final calculated level of 19 mg/kg (see p. 21 for mixing details).

Peroxide-treated T-2 toxin feed. The peroxide-treated T-2 toxin feed (P-T-2 feed) was made using the T-2 feed listed above as a base. This feed was mixed with 15% hydrogen peroxide (HPO) at a rate of 1.3 liters of HPO per 2 kg of feed. This mixture was then allowed to dry in plastic and galvanized metal pans in an outdoor, sunlit location, with an ambient temperature ranging from 25-35^{o} C. Drying required approximately 4-6 hours. Prior to feeding, the feed was reground through a laboratory hand mill to a consistency similar to the control feed.

Peroxide-treated T-2 toxin concentrate feed. The peroxide-treated T-2 toxin concentrate feed (P-T-2 Conc

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[2]Thomas Wiley Model 4, Thomas Scientific, Swedesboro, NJ.

feed) was prepared by treating the T-2 concentrate directly as follows. Two hundred grams of the concentrate was mixed with 1600 ml of 15% HPO and blended in a laboratory blender slowly for approximately 15 minutes. This mixture was then filtered, saving the treated concentrate. The concentrate was then rinsed with 500 ml of water and filtered again. The above process was repeated two more times, then the concentrate material was dried in a drying oven at 40° C. The treated T-2 toxin concentrate material was then mixed with control feed in an amount to provide 19 mg/kg T-2 toxin based on the original level of T-2 toxin in the concentrate.

Peroxide-treated control feed. The peroxide-treated control feed (P-C feed) was formulated by using the control feed as a base. The control feed was treated with 15% HPO at a rate of 2 liters of HPO per kilogram of control feed. After allowing this mixture to stand for a few minutes, the HPO was decanted off, and the feed was rinsed three times with water. After each rinsing, the feed was strained using cheesecloth. After the final rinse, the feed was allowed to air dry on plastic or galvanized metal pans in the outside air, in a sunlit location, with an ambient temperature of 25-35° C. Prior to feeding, the feed was reground through a laboratory hand mill to a consistency similar to the control feed.

Mixing of feedstuffs. A modified cement mixer slowed

to a rate of 6 revolutions per minute was used to mix the feedstuffs. To mix the 474 mg/kg concentrate, the ingredients were placed in an enclosed plastic container with approximately 16 liters capacity. It was then placed in the mixer (Figure 2), which revolved the container end to end. The concentrate was mixed for 3 hours.

To mix the T-2 feed as fed, 2 kg of the 474 mg/kg concentrate was placed in the drum of the mixer, along with 50 kg of control feed. Mixing was allowed to take place for 2 to 3 hours. This same procedure was used to mix the P-T-2 Conc feed, first mixing 200 grams of the peroxide-treated concentrate with 1.8 kg of control feed, then mixing 2 kg of this mixture with 50 kg of control feed.

Analysis of feedstuffs

Nutrient analysis. Control, P-T-2, and P-C feeds were analyzed for nutrient levels[3]. The results of this analysis are shown in Table 1. The protein and energy constituents of the feedstuffs were not markedly altered by the peroxide treatment process, nor were macromineral values altered greatly. The major change in the nutritional analysis was the great increase in zinc and iron content of the peroxide-treated feeds. Sodium and potassium were also decreased in the peroxide-treated feeds.

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[3]Livestock Nutrition Laboratory Service, Columbia, MO.

Figure 2. Mixer that was used to mix feedstuffs

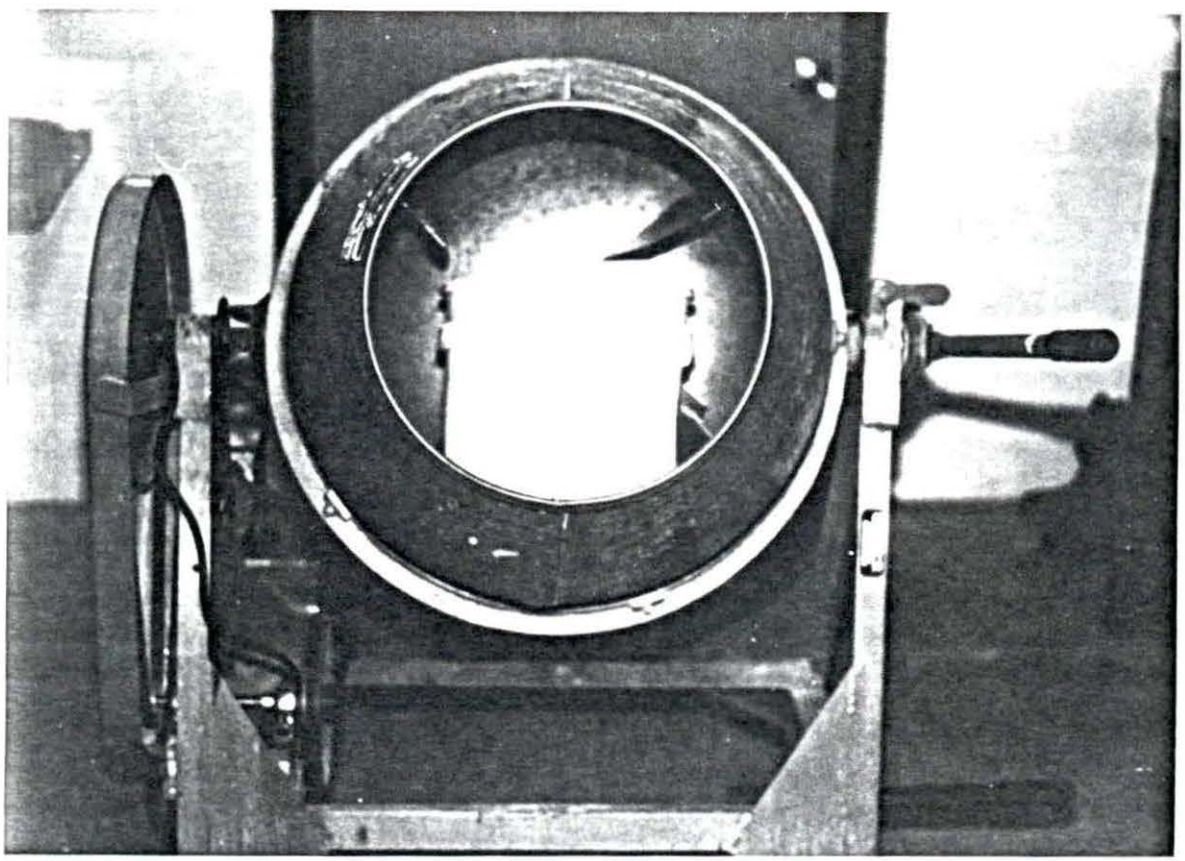

Table 1. Nutrient analysis of feedstuffs used in CHICK TRIAL I

Analysis[a]	Feed		
	Control[b]	P-C[c]	P-T-2[d]
Moisture	11.7	9.1	7.7
Crude protein	25.6	28.5	23.4
Fiber	5.3	6.5	6.8
TDN	77.8	79.0	79.9
Calcium	1.1	1.6	0.9
Phosphorous	0.8	0.9	0.7
Magnesium	0.18	0.13	0.18
Sodium	0.19	0.09	0.12
Potassium	0.80	0.39	0.75
Sulphur	0.22	0.22	0.22
Iron	252.0	666.7	339.2
Copper	6.0	8.9	6.0
Manganese	85.3	92.8	98.2
Zinc	89.3	790.2	971.9

[a]Values are expressed as % except iron, copper, manganese, and zinc, which are expressed as mg/kg. All values are represent on an as-fed basis.

[b]Control feed.

[c]Peroxide-treated control feed.

[d]Peroxide-treated T-2 toxin feed.

T-2 toxin analysis. The feedstuffs were analyzed for T-2 toxin quantitatively. The analyses were performed by first extracting 20 gram samples of feed with a 90% acetonitrile, 10% water solution. Extracts were then defatted with petroleum ether, and a ferric gel solution was used for cleanup. Samples were then dried under nitrogen, and derivatized with acetic acid and pyridine. Analyses were performed by gas chromatography[4], using a 1% OV-17, 100 - 120 mesh Gas-Chrome Q column[5]. The temperature used was 230° C., with a flame ionization detector.

A sample of the peroxide-treated 4744 mg/kg T-2 toxin concentrate contained 18 mg/kg T-2 toxin. Three samples of T-2 feed and two samples of P-T-2 feed were taken from the mixer after the mixing process. The T-2 feed samples contained 19.1, 18.9, and 25.0 mg/kg T-2 toxin (21 +/- 3.5). Both samples of P-T-2 feed contained no detectable amount of T-2 toxin (less than 1 mg/kg). Three samples of P-T-2 feed taken after grinding with the hand laboratory mill contained 15.7, 15.9, and 14.6 mg/kg T-2 toxin (15.4 +/- 0.7). One sample each of control and P-C contained no detectable amount of T-2 toxin (less than 1 mg/kg).

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[4]Bendix, Model 2500, Romcevert,WV.

[5]Ohio Valley Specialty Chemical, Inc. Marietta, OH.

Chicks

Chicks used were Heavy White Rock broiler-type chicks[6], received at one day of age. After receipt, chicks were acclimated to their surroundings and given access to control feed for the first 6 days.

Experimental design

Pen design. Pens used for the chicks were located in an enclosed room that was divided into 6 areas, using plywood partitions. Pine wood sawdust was used for litter. Each pen was approximately 2 m by 3 m in size, and held a single treatment group. A single heat lamp was located approximately one meter above the litter in each pen. The room temperature was maintained at 27-30° C. A single gravity flow metal watering container was located in each pen.

Randomization of chicks. The chicks were randomly divided into treatment groups (Cochran and Cox, 1957), and aluminum wing bands were applied, which allowed identification of individuals. Twenty-one chicks were allotted to each treatment.

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[6]WELP, Inc., Bancroft, IA.

Treatment groups. The treatment groups used in the experiment were:

Group	Code
Control	C
Peroxide-treated control	P-C
Peroxide-treated T-2 toxin concentrate	P-T-2 Conc
Peroxide-treated T-2 toxin	P-T-2
Inanition control	I-C
T-2 toxin	T-2

Feeding techniques. The respective feeds were offered to treatment groups on the first day of the experiment through day 21 (chicks were 7 days of age on day 1). The 21 day feeding period was divided into 3 weekly feeding periods which were designated Periods 1, 2, and 3. Feeders were galvanized metal with reels to prevent the chicks from standing in the feeders. As the chicks increased in size, larger feeders were used. Feeds were offered in the morning, and feed remaining in the feeders prior to re-feeding was weighed and subtracted from the amount offered on the previous day. The I-C group was offered only the amount consumed by the T-2 group on the previous day. Feeds were weighed using an electronic balance[7].

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[7]Ohaus Scale Corporation, Florham Park, NJ.

Weighing of chicks. Chicks were individually weighed on an electronic balance[7] at the beginning of the experiment (day 0), and again on days 8, 15, and 22. Chicks found dead in the pens were weighed prior to necropsy, and all chicks euthanatized were weighed prior to necropsy.

Scoring oral lesions. Chicks were observed for oral mucosal lesions on the days they were weighed. Mouths were manually opened and the presence of gross lesions was noted. Oral lesions were scored at necropsy according to location and severity. Four locations used were 1) epithelium of the hard palate, 2) epithelium of the lower beak, 3) commissures of the mouth, and 4) mucosa of the tongue. Severity of the lesions was estimated subjectively by the area of the lesion and the approximate thickness of the lesion, with scoring from 0 (no lesion apparent) to 4 (most severe lesion).

Necropsy. All chicks were necropsied after dying in the pens or immediately after euthanasia. Euthanasia was accomplished by cervical dislocation. Tissues were observed grossly, and sections of tissues were fixed in buffered neutral formalin for histopathologic examination. Tissues collected for histopathology included oral mucosal lesions, thymus, esophagus, lung, liver, proventriculus, ventriculus, small intestine, colon, cecum, bursa of Fabricius, spleen,

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[7]Ohaus Scale Corporation, Florham Park, NJ.

and kidney.

Spleen weights. Spleens were weighed on an electronic balance[8] immediately after necropsy.

Histopathology. Tissues were examined by light microscopy on two chicks from each group. Tissues were embedded in paraffin blocks and sectioned at a thickness of 5 microns, and stained with hematoxylin and eosin.

Statistical methods. Data analyzed were feed consumption, weight gains, and spleen weights. Individual chicks were the experimental units. Analysis of variance techniques[9] were used along with least significant differences to compare group means for significant differences at $p < 0.05$.

Chick Trial II

Preparation of feedstuffs

The T-2 toxin concentrate and control feed were the same as described previously in Chick Trial I (Trial I). Control and T-2 toxin feed (T-2 feed) were prepared and mixed as described in Trial I.

Peroxide-treated T-2 toxin feed. To prepare the peroxide treated T-2 toxin feed (P-T-2 feed), T-2 toxin feed was treated with 1.3 liters of 15% HPO per 2 kg of feed.

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[8]Mettler PC 2200, Mettler Instrument Corp, Highstown, NJ.

[9]Statistical Analysis System, SAS, Inc., Cary, NC.

This mixture was dried indoors on plastic pans at an ambient temperature of approximately 30° C. Drying was accomplished within 24 hours. Feed was ground for feeding using a food mill, to make the particle size similar to the control feed.

Peroxide-treated control feed. The peroxide-treated control feed (P-C) was prepared by treating control feed with HPO in the same manner as the P-T-2 feed.

Analysis of feedstuffs

T-2 toxin analysis. Feedstuffs were analyzed quantitatively for T-2 toxin (see p. 26). Four 20-gram samples of T-2 feed were taken at random for analysis as the feed was removed from the mixer. These samples contained 12.5, 19.0, 14.0, and 8.5 mg/kg T-2 toxin (13.5 +/- 4.3). Three representative 20-gram samples of P-T-2 feed contained 15.7, 15.9, and 14.6 mg/kg T-2 toxin (15.4 +/- 0.7). One sample each of C and P-C feed contained no detectable amount of T-2 toxin (< 1 mg/kg).

Chicks

Chicks used were Cornish-cross broiler-type chicks, straight run[10]. One day old chicks were acclimated to surroundings and given free choice access to control feed for the first 6 days after being received.

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[10]Hoover Hatchery, Rudd, IA.

Experimental design

Randomization of chicks, environment and location of chicks, weighing of spleens, histopathologic techniques, and statistical methods were accomplished as described in Trial I.

Treatment groups. The treatment groups and number of individuals per group were:

Group	Code	N
Control	C	20
Peroxide treated control	P-C	20
Inanition peroxide treated control	I-P-C	10
Inanition control	I-C	10
Peroxide treated T-2 toxin	P-T-2	20
T-2 toxin	T-2	20

The experiment originally had two groups of controls, however on day 4 one of the control groups was used to form another peroxide-treated control group and a counterpart inanition control group. The control group of 20 chicks was divided randomly into two groups of 10 chicks. The inanition peroxide-treated control group was fed P-C feed, and the inanition control group was pairfed with control feed to match the amount of feed consumed by the inanition peroxide-treated control group on the previous day.

Feeding techniques. Treatment feeds were offered to chicks each morning, beginning at 8 days of age. Feeders used were the same as Trial I.

After 14 days on treatment feeds, chicks were placed on control feed for the remainder of the experiment (14 days). Feeding periods were designated as periods 1, 2, 3, and 4, the first two periods being periods when treatment feeds were offered. Feeding periods were 7 days duration except period 4, which was 8 days.

The I-P-C and I-C groups were assigned feeding periods separately from the remainder of the groups. Periods 1 and 3 were 3 days duration, period 2 was 4 days, and period 4 was 5 days duration.

Weighing of chicks. Chicks were weighed at the beginning of the experiment (day 0), and also on days 3, 7, 11, 14, 17, 21, 24, and 29. On days when chicks were euthanatized, weights were taken just prior to necropsy.

Scoring oral lesions. Oral cavity lesions were scored as described in Trial I. Scoring was done on days 7, 11, 14, 15, 17, 21, 23, 24, and 29.

Necropsy techniques. Five chicks were removed at random from each group on days 7, 14, 21, and 29. They were euthanatized by cervical dislocation and necropsied. Organs were examined grossly for lesions, and tissues were fixed in 10% buffered neutral formalin for histopathology. Tissues

collected were oral mucosae, esophagus, crop, spleen, proventriculus, stomach, small intestine, pancreas, colon, bursa of Fabricius, thymus, liver, and kidney.

Rat Taste Aversion Trial

Preparation of feedstuffs

Feed. Commercial laboratory rodent feed[11] was the control feed, and was a base for the toxin feed groups. The feed contained not less than 24% protein, not less than 6% fat, and not more than 4.5% fiber. Feed was ground using a laboratory mill (see p. 20) with a 2 mm diameter screen size. After grinding, novel diets were formulated by adding 22 grams of sodium saccharin[12] per kilogram of feed, along with T-2 toxin concentrate which contained approximately 474 mg/kg of T-2 toxin (formulated as described on page 17). Novel diets were formulated to contain approximately 0, 10, and 20 mg/kg of T-2 toxin. The control diet had no saccharin and no T-2 toxin added. Diet ingredients were placed in plastic containers of approximately 16 liters capacity and thoroughly mixed by revolving in a cement mixer as described earlier (see p. 21).

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[11]Rat/Mouse Diet 1526, Simonsen Mill, Inc., Quimby, IA.

[12]Sprinkle Sweet, The Pillsbury Company, Minneapolis, MN.

Feeds were then labeled as follows:

Feed	Code
Control	C
Novel + 0 mg/kg T-2 toxin	N+0
Novel + 10 mg/kg T-2 toxin	N+10
Novel + 20 mg/kg T-2 toxin	N+20

Analysis of feedstuffs

T-2 toxin analysis. Feedstuffs were analyzed quantitatively for T-2 toxin content (see p. 26). Eight 2.5-gram samples each of N+0, N+10, and N+20 feed were taken directly from feeders during the period when these feeds were offered. All samples of N+0 contained no detectable amount of T-2 toxin. The N+10 samples contained a mean of 8.1 mg/kg T-2 toxin, +/- 1.1, and N+20 samples contained a mean of 13.2 mg/kg, +/- 3.4.

Rats

Twenty-four male Sprague-Dawley rats[13] weighing approximately 150 to 175 grams each were placed in individual cages, offered standard laboratory rodent feed, and started on the trials within 24 to 48 hours of being received. The experiment was executed in two time periods with 12 rats in each period. Rats were randomly assigned to cages (Cochran and Cox, 1957).

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[13]Bio-Lab Corp., St. Paul, MN.

Experimental design

Cages. A bank of stainless steel laboratory animal cages[14] with wire mesh floors was used to house the rats. The cage design is shown in Figure 3. Backs and sides of the cages were solid stainless steel, and the fronts were wire mesh. Sawdust was used under cages to absorb urine and collect feces. Nipple waterers were used.

Feeders. Two feeders were fastened inside the front of each cage. Figure 4 demonstrates a feeder that has been opened to show internal details. Feeders were fabricated using aluminum sheeting, and dimensions were 10 by 10 by 5 cm. A square opening in the front of the feeder was sized at 5.75 by 5.75 cm to allow feeding but prevent rats from climbing into the feeder. Small pans were placed under each feeder to collect spilled feed, which was placed back into the respective feeder prior to being weighed.

The feeding activity detection system in the feeder consisted of a Plexiglas framework to which an infrared light emitting diode[15] was attached on one side and a light detector photodarlington[16] was placed on the opposite side. This is also shown in Figure 4. The pathway of the

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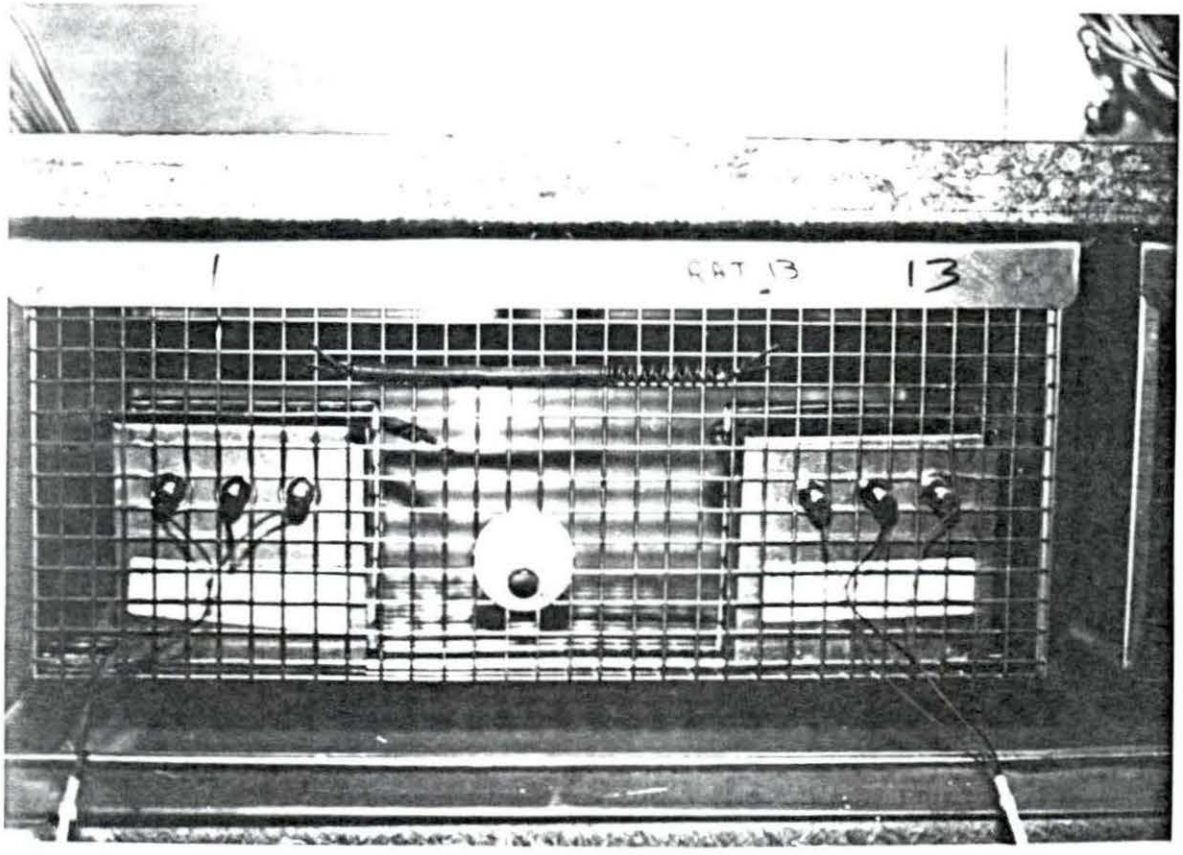
[14]Waumann (Lab Products, Maywood, NJ).

[15]ECG 3017, ICS Computer Products, San Diego, CA.

[16]ECG 3036, ICS Computer Products, San Diego, CA.

Figure 3. Rat cage with feeders present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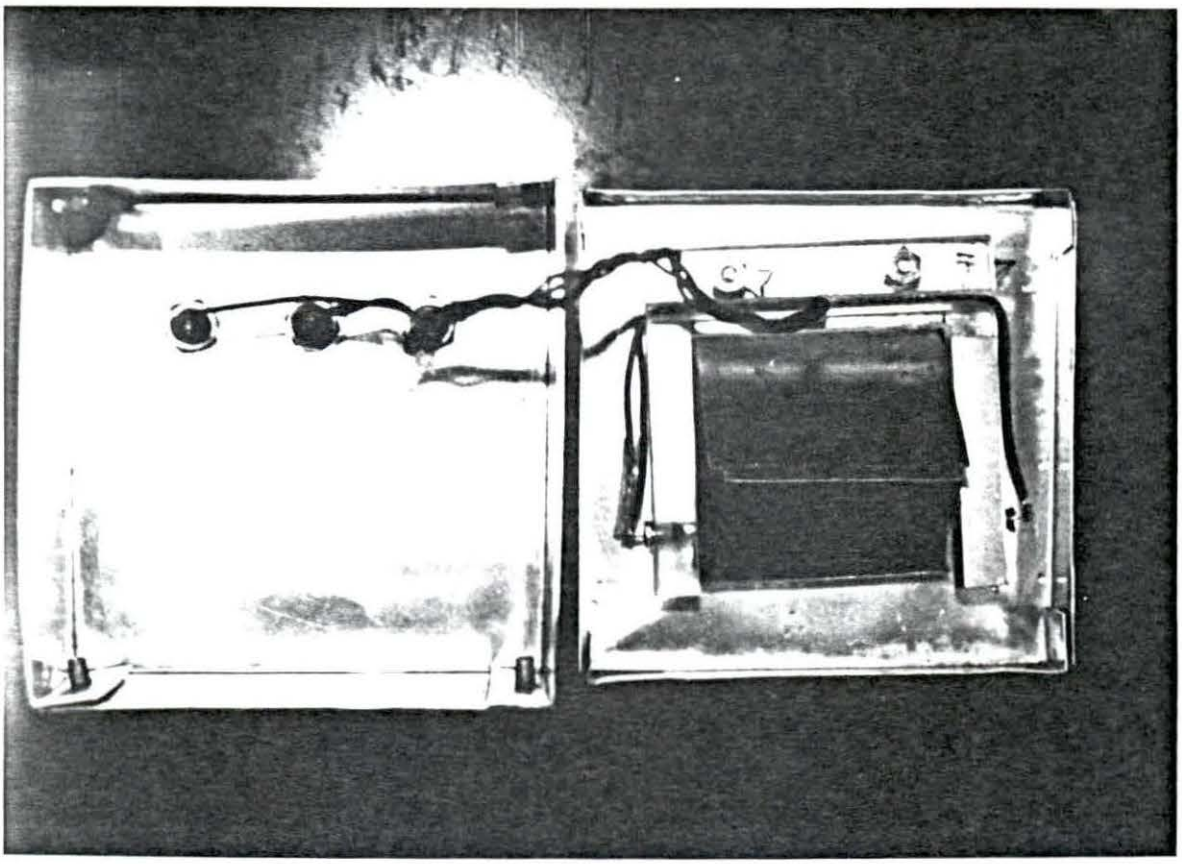
CAT 13
13

Figure 4. A rat feeder that has been opened to demonstrate internal parts



infrared light beam was therefore located in a position that required that it would be interrupted upon feeding attempts by a rat.

Feeding activity monitoring system. A microcomputer[17] was used to collect feeding activity data. An interface card[18] was connected to the computer, to which individual leads were distributed to feeders. Leads from feeders were connected to a 0.01 microfarad capacitor and a hex invertor[19]. Individual wires to each feeder were fitted with banana plugs to allow easy disconnection of wires from feeders for removal of the feeders for weighing the feed consumed each day.

Room lighting. Room lighting was supplied by 3 high intensity incandescent flood lamps mounted approximately 1 m above the top row of cages. The lights were directed at the fronts of the cages. An automatic timer was used to turn the lights on at 0800 hours. Lights remained on during daylight hours, and were automatically turned off at 1700 hours. The room housing the rats was not entered by personnel during the period when lights were out.

Weighing of rats. Rats were weighed individually on an

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[17]IBM PC AT, IBM Corp., Charlotte, NC.

[18]Model DI024, ICS Computer Products, San Diego, CA.

[19]ECG 74LS04, ICS Computer Products, San Diego, CA.

electronic balance (see p. 28) on the initial day of the trial, and on days 7, 10, 16, 19, and 26.

Feeding schedule. After the electronic monitoring of feeding activity was completed for each day at 1200 hours, feeders were removed from the cages. Feeders were weighed using the balance for weighing rats, and the feeder weights before and after the feeding period were recorded. The feeders were re-filled with feed to a weight of 165 grams, then returned to the cages between 1630 and 1700 hours.

Feeding behavior was monitored from 1700 hours one day through 1200 hours the following day, for all test days.

Watering. Water consumption was measured at approximately 1230 hours daily by weighing the water bottles on the electronic balance used to weigh feed.

Treatment groups. Rats were randomly assigned to treatment groups (Cochran and Cox, 1957). Groups were identified as 0 ppm (control), 10 ppm, and 20 ppm.

Feeding paradigm. The feeding paradigm is summarized in Table 2. For the first 7 days of the trial, all rats were offered control feed. Feed was placed in both feeders in each rat's cage. This feeding period was labeled ACCLIM. Days within periods are also referred to by period names.

On days 8 through 10 of the experiment, rats in each group were offered novel feed for their respective group (8

Table 2. Feeding protocol for RAT TASTE AVERSION TRIAL

Day of trial	Group	Feed offered
Days 1-7 (ACCLIMATION)	All	Control (both feeders)
Days 8-10 (NOVEL)	0 ppm	N+0[a] (both feeders)
	10 ppm	N+10[b] (both feeders)
	20 ppm	N+20[c] (both feeders)
Days 11-15 (RECOVERY 1)	All	Control (both feeders)
Day 16 (NO FOOD)	All	No feed
Days 17-18 (CHOICE 1)	All	Control[d] (one feeder)
		N+0 (one feeder)
Days 19-25 (RECOVERY 2)	All	Control (both feeders)
Day 26 (CHOICE 2)	All	Control (one feeder)
		N+0 (one feeder)

[a]Novel feed containing 0 ppm T-2 toxin.

[b]Novel feed containing 10 ppm T-2 toxin.

[c]Novel feed containing 20 ppm T-2 toxin.

[d]Feeders were placed at random in cages during CHOICE days.

rats in the 0 ppm group received N+0 feed, 8 rats in the 10 ppm group received N+10 feed, and 8 rats in the 20 ppm group received N+20 feed). Novel feed was placed in both feeders of each cage. This period was labeled NOVEL.

Days 11-15 of the experiment were a recovery period in which rats were offered the control diet, again in both feeders. This period was labeled REC 1.

On Day 16, no food was offered to the rats. Feeders were removed from the cages on the previous day at 1200 hours as usual, but were not placed back in the cages with the rats. Only water consumption was monitored on day 16, which was designated NO FOOD.

Days 17 and 18 were the first choice test. This period was labeled CHOICE 1, and rats were offered N+0 feed in one feeder, and control feed in the other feeder in the cage. The position of the feeders in each rat's cage was assigned randomly to reduce the effects of previous feeder preferences or a preference of feeder position by the rats.

Days 19-25 of the experiment were a second recovery period, during which control feed was offered to all rats in both feeders. This period was named REC 2.

During day 26 rats again received a choice of control versus N+0 feed, and this day was labeled CHOICE 2. The feed choice was placed at the same random position that was used during the CHOICE 1 period.

Necropsy. Rats were euthanatized by carbon dioxide asphyxiation within 24 hours of termination of the second choice test, and necropsied immediately after euthanasia. Internal organs were observed grossly for lesions, and tissue sections were fixed in 10% buffered neutral formalin for histopathologic examination. Tissues fixed included lung, heart, stomach, small intestine, colon, liver, kidney, adrenal, pancreas, and mesenteric lymph node.

Histopathology. Tissues from the rats were cast in paraffin blocks, sectioned at 5 microns, stained with hematoxylin and eosin, and examined by light microscopy. Tissues were examined from all rats in the experiment.

Statistical analysis. Data collected during the experiment were daily food consumption per rat per feeder, daily water consumption per rat, and body weights. The daily feeding activity collected included the number of times the rat's body interrupted the path of the infrared beam for greater than 0.50 seconds (number of feeding attempts), and the duration of each feeding attempt, in seconds. Feeding attempts with durations greater than 30 minutes were assumed to be nonsense values, and were excluded from data.

Data were analyzed by analysis of variance methods (see p. 30). Least significant difference techniques were used to compare group means for significant differences. A

group by feed interaction was used to evaluate differences during CHOICE periods. Data analyzed included feed consumption, weight gains, water consumption, daily feeding attempts, and duration of feeding attempts.

RESULTS

Chick Trial I

Observations

All chicks appeared to be acting and feeding normally by direct observation for the first 4 days after being placed on their respective treatment feeds. The control and P-T-2 Conc groups had less feed wasted around their feeders than the other 3 groups. The inanition control group displayed much greater hunger behavior than the other groups, by moving quickly to the feeder when their pen was entered.

By day 6 of the feeding period, chicks in the P-T-2 group and the P-C group were listless and were not eating. They did not move to the feeders when feed was offered, as chicks in the other groups did. On day 10, one chick in the P-C group was found in a moribund condition, and all of the chicks in this group and in the P-T-2 group were very depressed and inactive. Severely debilitated and weakened chicks were removed from the pens, euthanatized, and necropsied. All chicks were removed from the P-T-2 group by day 14, due to their debilitated condition. Chicks in the P-C group followed a similar pattern, however the course of their condition was somewhat delayed as compared to the P-T-2 group. All chicks in the P-C group were euthanatized by day 19, due to emaciation.

Chicks in the T-2 group displayed behavior patterns similar to those in the C and P-T-2 Conc groups. However, when feeding, they scratched more litter away from around the feeders.

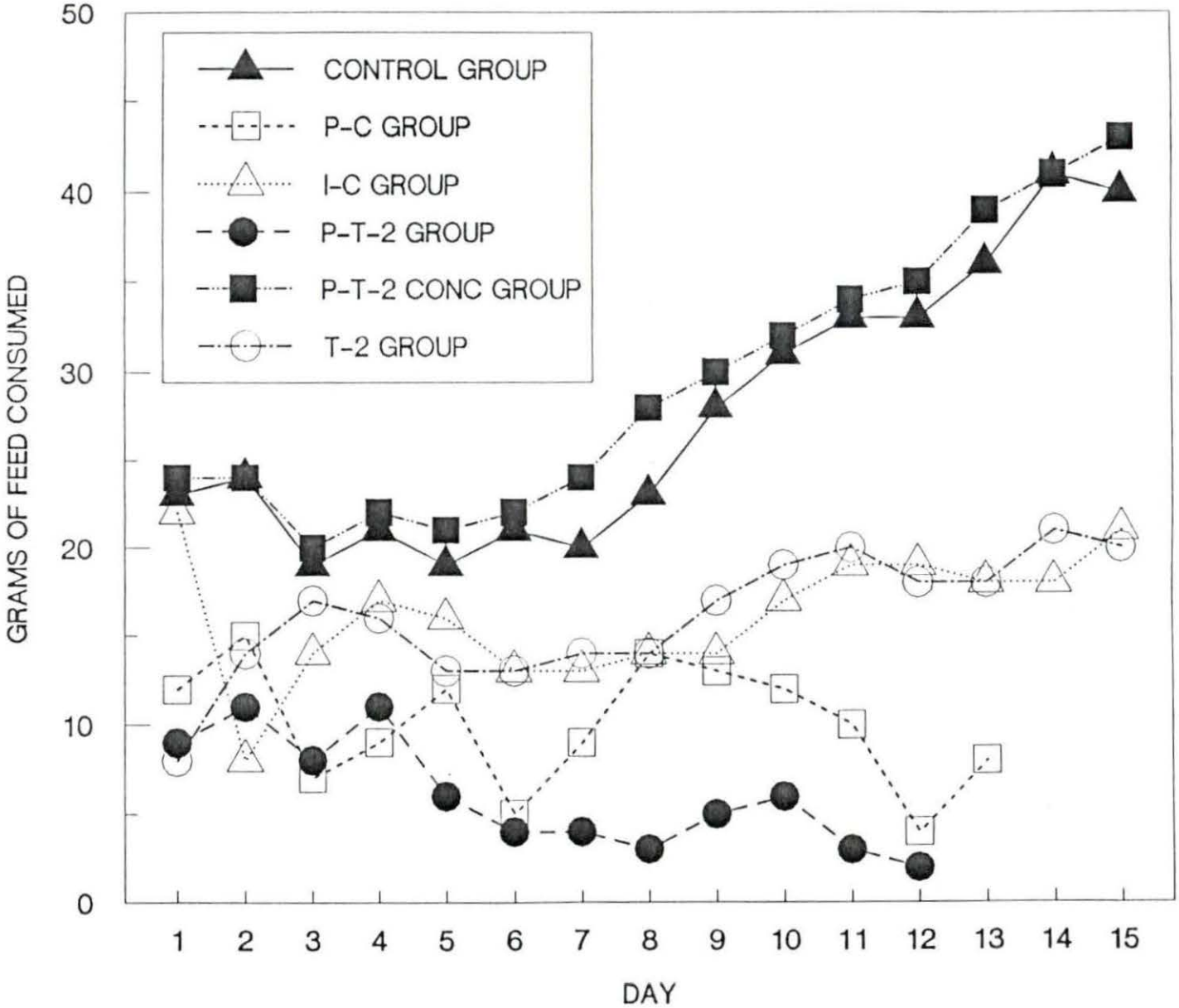
Oral lesions were first noticed on day 8, and were found only in the T-2 group. Ten of 21 chicks in this group were affected by day 8, and all chicks in the T-2 group eventually developed necrotizing oral mucosal lesions. Lesions were most obvious on the mucosa of the hard palate, and appeared as a thickened, yellow to white pseudomembrane. Feed particles and litter adhered to lesions in some of the chicks. As a lesion progressed, it affected the tip of the tongue, and the commissures of the mouth.

Even though all chicks in the T-2 group developed oral mucosa lesions, only one individual in the group became severely debilitated requiring euthanasia. Three chicks in this group developed distorted beaks due to adherent litter and feed particles in their oral cavities. Oral mucosal lesions were not detected in chicks in treatment groups other than the T-2 group.

Feed consumption. Feed consumption by treatment group is shown in Figure 5. P-T-2 and P-C groups consumed very little feed. Since feed consumption was not measured on an individual chick basis, statistical analyses could not be used to compare group differences.

Figure 5. Mean feed consumption per chick in CHICK TRIAL I during days of the experiment. All groups were offered their respective treatment feed

P-C group=Peroxide-treated control
I-C group=Inanition control
P-T-2 group=Peroxide-treated T-2 toxin
P-T-2-Conc group=Peroxide-treated T-2 toxin concentrate
T-2 group=T-2 toxin


CONTROL GROUP
P-C GROUP
I-C GROUP
P-T-2 GROUP
P-T-2 CONC GROUP
T-2 GROUP
GRAMS OF FEED CONSUMED
0
10
20
30
40
50
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
DAY

Weight gains. Weight gains in the various treatment groups followed feed consumption. Group means are shown in Table 3. In this table and others following, lower case letters positioned to the right of mean values are used to indicate significant differences ($p<0.05$) between treatment groups. Different lower case letters among mean values designate significantly different values. For example, a versus ab indicates values that are not significantly different, whereas a versus b indicates significantly different values. Weight gains were divided into 3 weekly periods. The P-T-2 Conc group weight gain was not significantly different from the controls during any of the 3 weeks. The T-2 group gained significantly less than controls during all 3 weeks of the experiment. The P-T-2 group and P-C group gained significantly less than the control, T-2, and I-C groups during the first week of the test. Due to loss of chicks from the P-T-2 and P-C groups, comparisons with these groups were made only during the first week. The P-T-2 group actually lost weight, and the P-C group gained very little weight during this period.

Inanition controls gained significantly more weight than the T-2 group during the first two weeks of the experiment, however there was not a significant difference during the third week. There was not a significant difference in weight gains between the I-C group and the T-2

Table 3. Mean weight gains by group during CHICK TRIAL I by feeding period (values in grams per chick per day)

Group	Period 1	Period 2	Period 3	Overall[1]
Control	13.9a[2]	18.2a	30.2a	20.3a
P-T-2 Conc[3]	13.7a	17.9a	28.9a	20.2a
I-C[4]	9.1b	9.8b	14.3b	11.1b
T-2[5]	4.9c	7.5c	15.7b	9.0b
P-C[6]	1.4d	-	-	-
P-T-2[7]	-1.4e	-	-	-
SEM[8]	0.4	0.6	1.1	0.8
LSD[9]	1.1	1.8	3.1	2.3

[1]Values averaged over all 3 feeding periods.

[2]Different lower case letters within a given period denotes significance ($p<0.05$).

[3]Peroxide-treated T-2 toxin concentrate group.

[4]Inanition controls.

[5]T-2 toxin group.

[6]Peroxide-treated control group.

[7]Peroxide-treated T-2 toxin group.

[8]Standard error of treatment mean.

[9]Least significant difference ($p<0.05$, df= 5,125 for Period 1, and df=3,83 for other periods).

group when the weight gains over the entire feeding period were compared.

Oral lesion scores. Oral mucosal lesions were scored in the T-2 group at necropsy. The mean lesion score was 5.5, +/- 3.3. Oral lesions were not present in other groups for scoring.

Spleen weights. Spleen weights of the chicks were expressed as a percentage of body weight (SWP), since there was great variability in weight of the chicks in the different groups at necropsy. SWP values were significantly decreased in the T-2, I-C, P-C, and P-T-2 groups as compared to controls (Table 4). The SWP value for the T-2 group was not significantly less than the I-C group, however it was significantly lower in the P-T-2 group as compared to the P-C group.

Necropsy. Gross necropsy findings in the P-T-2 and the P-C groups consisted of emaciated carcasses. Thymus glands in these two groups were also small and difficult to locate. No other lesions were observed in chicks of these groups.

All chicks in the T-2 group had oral mucosal lesions when necropsied. Lesions consisted of caseous necrotic exudate on the mucosa of the hard palate, necrosis of the tip of the tongue, and crusting lesions on the commissures of the mouth and beak margins. Lesions were not observed grossly in other organs of chicks in the T-2 group. Gross

Table 4. Mean spleen weight values (SWP)[1] of chicks by group in CHICK TRIAL I

Group	SWP
Control	0.154a[2]
P-T-2 Conc[3]	0.155a
T-2[4]	0.095b
I-C[5]	0.102b
P-C[6]	0.071c
P-T-2[7]	0.041d
SEM[8]	0.008
LSD[9]	0.022

[1]Spleen weight expressed as a percentage of body weight.

[2]Different lower case letters denotes significance ($p<0.05$).

[3]Peroxide-treated T-2 toxin concentrate group.

[4]T-2 toxin group.

[5]Inanition controls.

[6]Peroxide-treated control group.

[7]Peroxide-treated T-2 toxin group.

[8]Standard error of treatment mean.

[9]Least significant difference ($p<0.05$, df=5,125).

lesions were not present in chicks of the I-C group.

Histopathology. Oral mucosal lesions were present only in chicks in the T-2 group. These lesions were characterized by diffuse hyperkeratosis and multifocal erosions of the epidermis. Inflammatory cells and bacteria were adhering to the eroded surface of the epidermis. Mild epidermal hyperplasia was present, along with mild infiltrates of inflammatory cells in subepidermal areas.

Thymic atrophy was present in the P-C and P-T-2 groups. Thymic lobules were much smaller in these groups as compared to those in control, P-T-2 Conc, T-2, and I-C groups. The atrophic thymic lobules had poorly defined cortical and medullary areas, and foci of degenerating cells.

Chick Trial II

Observations

By day 5 of the experiment, chicks in the P-T-2 and P-C groups were huddled up, depressed, and inactive. They did not move to the feeders when feed was offered, as chicks in other groups did. Poor weight gains of chicks in the P-T-2 and P-C groups as compared to controls was apparent by day 4 of the experiment, and became more obvious as the experiment proceeded. On day 13, one of the chicks from the P-T-2 group was in lateral recumbency, due to cachexia. All chicks in the P-T-2 and P-C groups were emaciated by day 13.

Oral mucosal lesions were present in the T-2 group by

day 3 of the experiment. The lesions at this point were minor fibrinous exudates on the mucosa of the hard palate of 14 of the chicks. By day 7 of the experiment, all chicks in the T-2 group had obvious oral mucosal lesions. Lesions were present on the mucosa of the hard palate, tip of the tongue, and commissures of the mouth, and were very similar to those described previously in Trial I. Chicks in the T-2 group displayed normal feeding behavior, except the litter surrounding the feeder in the T-2 group was more disturbed than in other groups.

Four chicks in the P-T-2 group developed mild necrotizing oral mucosal lesions, which were present by day 11. Lesions were not scored in this group, since the lesions were very mild, and only a few chicks were affected. Oral cavity lesions were not observed in the other 11 chicks in the P-T-2 group, or in the C, P-C, I-C, or I-P-C groups.

Feather abnormalities were observed in the T-2 group on day 14. Chicks in this group had areas on their backs which were not covered with feathers, and feathers on the wings were shorter and more sparse as compared to control chicks.

The remaining chicks in all groups were placed on control feed on day 16. Chicks in the P-T-2 and P-C groups began feeding ravenously within one hour of receiving control feed. The second day after being changed to control feed, 2 chicks in the P-C group and 6 chicks in the P-T-2

had locomotor difficulties. Affected chicks could walk only short distances, and appeared to have weakness of the legs. Wings of affected chicks were carried in an abnormal position, and were dragged along the litter as they attempted to walk. Chicks with locomotion disturbances all recovered within 5 days of onset of the signs, except for two that were euthanatized and necropsied.

After chicks in the T-2 group were given control feed, their oral mucosal lesions regressed. Lesions were noticeably improved after 7 days on control feed. Within 14 days of being placed on control feed, oral mucosal lesions could not be observed grossly in chicks in the T-2 group.

Feed consumption. Feed consumption values are shown by graph in Figure 6. Feed consumption by groups consuming peroxide treated feeds was markedly reduced (on days 1-14).

Weight gains. Weight gains of the different groups during the first two feeding periods are shown in Table 5. Weight gains in period 1 are means for 20 chicks in each group which received their respective feed for 7 days, and weight gains shown in period 2 are the for the 15 remaining chicks in each group from period 1. Period 2 was also 7 days in duration.

During period 1, chicks in the T-2 group gained significantly less than controls. The P-C and P-T-2 groups both gained significantly less than controls. There was not

Figure 6. Mean feed consumption per chick in CHICK TRIAL II during days of the experiment. All groups were offered their respective treatment feed on days 1-14. Control feed was offered to all groups on days 15-28

P-C group=Peroxide-treated control
P-T-2 group=Peroxide-treated T-2 toxin
T-2 group=T-2 toxin

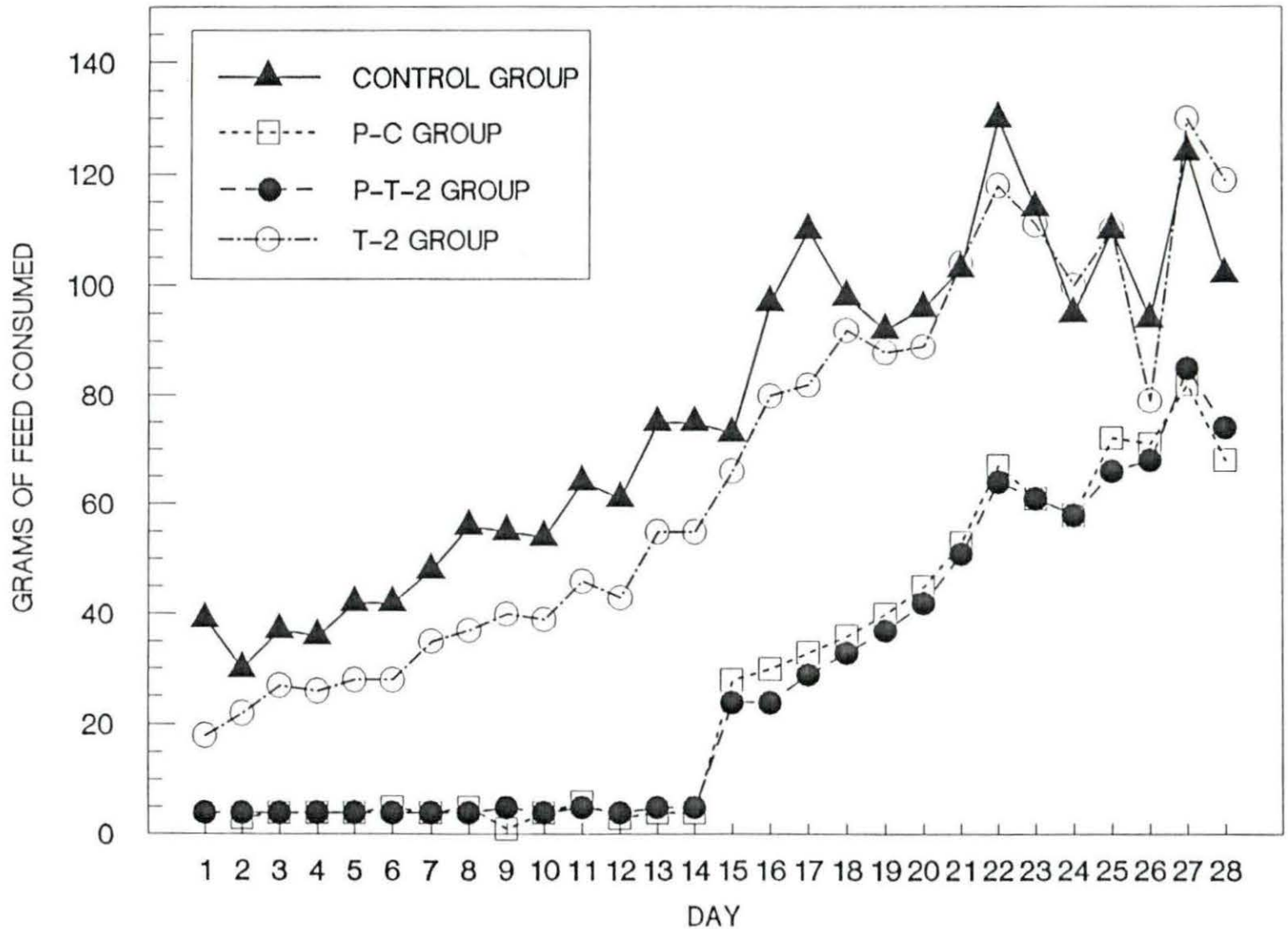

CONTROL GROUP
P-C GROUP
P-T-2 GROUP
T-2 GROUP
GRAMS OF FEED CONSUMED
0
20
40
60
80
100
120
140
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
DAY

Table 5. Mean weight gains in CHICK TRIAL II by group during first two feeding periods (weight gain per chick per day in grams)

Group[1]	Period 1	Period 2
Control	28.1a[2]	37.2a
T-2[3]	17.5b	25.7b
P-T-2[4]	-4.4c	-1.3c
P-C[5]	-3.9c	-2.5c
SEM[6]	0.7	0.9
LSD[7]	2.0	2.6

[1]All groups were offered their respective treatment feed during Periods 1 and 2.

[2]Different lower case letters within a given period denotes significance ($p<0.05$).

[3]T-2 toxin group.

[4]Peroxide-treated T-2 toxin group.

[5]Peroxide-treated control group.

[6]Standard error of treatment mean.

[7]Least significant difference ($p<0.05$, df=3,79 for Period 1, df=3,59 for Period 2).

a significant difference in weight loss between the P-C and the P-T-2 groups during this period.

During period 2, all groups gained significantly less than controls. Again, there was not a significant difference between weight loss in the P-C and the P-T-2 groups.

Weight gains for the inanition controls as compared to the I-P-C group are shown in Table 6. On the second day of the second period of the experiment, it was discovered that the I-P-C group was wasting a significant amount of feed, which resulted in the I-C group being fed more feed than the I-P-C group actually consumed. Therefore, the weight gains during Periods 1 and 2 were inaccurate, and were not used. During Periods 3 and 4, there were not significant differences in weight loss between the I-C and P-C groups.

Weight gains during the recovery period in which all groups received control feed are shown in Table 7. Chicks in the P-T-2 and the P-C groups gained significantly less than controls and the T-2 group during Period 3, however during the period 4, there were not significant differences in weight gain between groups. Chicks being compared in the groups were different in body weights when started in the recovery period, due to the effect of the various treatment feeds offered previously.

Feed conversion. Table 8 demonstrates feed efficiency

Table 6. Mean weight gains for inanition control group and inanition peroxide control group by period in CHICK TRIAL II (grams per chick per day)

Group	Period 3[a]	Period 4[b]
I-P-C[c]	-1.8	-1.4
I-C[d]	-3.6	-3.5
SEM[e]	0.7	0.8
LSD[f]	2.2	2.4

[a]Period 3 was 3 days duration.

[b]Period 4 was 5 days duration.

[c]Peroxide-treated control group, which received peroxide-treated control feed.

[d]Inanition control group, which received control feed.

[e]Standard error of treatment mean.

[f]Least significant difference ($p<0.05$, df=1,19).

Table 7. Mean weight gains by group for CHICK TRIAL II during Periods 3 and 4 (grams per chick per day)

Group[1]	Period 3	Period 4
Control	32.1a[2]	26.0a
T-2[3]	31.2a	23.5a
P-C[4]	19.3b	20.7a
P-T-2[5]	19.1b	21.7a
SEM[6]	1.7	2.2
LSD[7]	4.8	6.7

[1]All groups were offered control feed during Periods 3 and 4.

[2]Different lower case letters within a given period denotes significance ($p<0.05$).

[3]T-2 toxin group.

[4]Peroxide-treated control group.

[5]Peroxide-treated T-2 toxin group.

[6]Standard error of treatment mean.

[7]Least significant difference ($p<0.05$, df=3,39 for Period 3, df=3,19 for Period 4).

Table 8. Feed conversion of chicks in CHICK TRIAL II by group during periods (grams of feed consumed per gram of weight gained)

Group[a]	Period 1[b]	Period 2	Period 3	Period 4
Control	1.6	1.5	1.7	2.0
P-T-2[c]	-0.9[d]	-1.7	1.1	1.4
P-C[e]	-1.1	-4.0	0.9	1.3
T-2[f]	1.4	1.6	1.5	2.0

[a]Groups received their respective treatment feed during Periods 1 and 2, and all groups received control feed during Periods 3 and 4.

[b]Periods were all 7 days duration.

[c]Peroxide-treated T-2 toxin group.

[d]Negative values indicate weight loss.

[e]Peroxide-treated control group.

[f]T-2 toxin group.

values for groups, by weekly period. Negative values for P-C and P-T-2 groups during the first two periods are due to weight losses during these periods. A slight difference was present between control and T-2 groups. During periods 3 and 4 in which all chicks consumed control feed, P-C and P-T-2 groups were also quite similar, and feed efficiencies during these periods were similar to controls during the first two periods.

Spleen weights. Spleen weight was expressed as a percentage of body weight (SWP value). SWP values were not significantly decreased in the T-2 group as compared to controls, but were decreased in the P-T-2 and P-C groups (Table 9). P-T-2 and P-C groups did not have a significant difference in SWP values. SWP values for the I-C group could not be included because of an error which resulted in a mixup of spleens from this group.

Oral lesion scores. The method of scoring oral lesions was described previously (see p. 29). A total score was then given to each chick, by summing the score for each location. Mean values were then calculated for each period the oral cavities were examined for lesions. Lesion scores in the T-2 toxin group increased between days 7 and 11 (Table 10). This group was placed on control feed on day 15, and the lesion scores declined steadily following the change in feed.

Table 9. Mean spleen weight values (SWP)[1] of chicks by group in CHICK TRIAL II

Group	SWP
Control	0.099a[2]
T-2[3]	0.084a
P-T-2[4]	0.064c
P-C[5]	0.053c
SEM[6]	0.005
LSD[7]	0.016

[1]Spleen weight expressed as a percentage of body weight. Values were calculated from chicks necropsied at the ends of Period 1 and 2.

[2]Different lower case letters denotes significance ($p<0.05$).

[3]T-2 toxin group.

[4]Peroxide-treated T-2 toxin group.

[5]Peroxide-treated control group.

[6]Standard error of treatment mean.

[7]Least significant difference ($p< 0.05$, df=3,39).

Table 10. Mean oral mucosal lesion scores for chicks in in the T-2 toxin group during CHICK TRIAL II

Day[a]	Score	SEM[b]
0	0.0	-
7	3.1	1.7
11	5.7	3.0
15	4.7	2.6
17	3.6	1.6
21	1.4	1.1
23	0.8	0.8
24	0.2	0.4
28	0.0	-

[a]Chicks were offered feed containing T-2 toxin on days 1-14, and control feed on days 15-28.

[b]Standard error of mean.

Necropsy. Necropsy of chicks in the P-T-2 and P-C groups revealed emaciated carcasses and small thymus glands. No other lesions were noticed grossly in these groups. Lesions were found on the oral mucosa of all chicks in the T-2 group, which consisted of fibrinous plaques on the mucosa of the hard palate, and necrotizing lesions on the tip of the tongue (Figure 7). Crusts were present at commissures of the mouth and on the external margin of beaks of many of the chicks in the T-2 group. Gross lesions were not detected in other organs of the chicks in the T-2 group.

Histopathology. Microscopic lesions were present only in the oral mucosa of chicks in the T-2 group (Figure 8). These lesions were similar to those described previously in Trial I.

Thymic atrophy occurred in chicks in the P-C, P-T-2, I-P-C, and I-C groups. Microscopically, the lesions were very similar to those described in Trial I. Thymus glands from the P-C, P-T-2, I-P-C, and I-C groups were very similar in appearance. Thymus glands from control chicks were very similar in appearance to those from chicks in the T-2 group (Figures 9 and 10).

Rat Taste Aversion Trial

Feed consumption

ACCLIM period. During the 7 day acclimation period

Figure 7. Gross appearance of a lesion involving the mucosa of the palate of a chick that was offered feed containing 13.5 mg/kg T-2 toxin

Figure 8. Microscopic appearance of a lesion involving the mucosa of the palate of a chick that was offered feed containing 13.5 mg/kg T-2 toxin. H&E stain

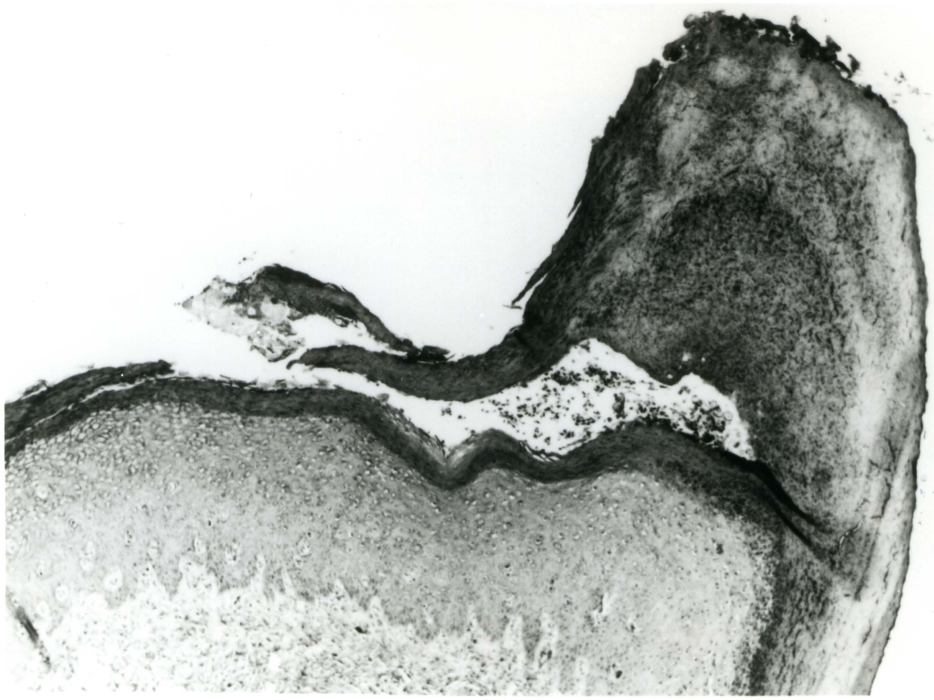
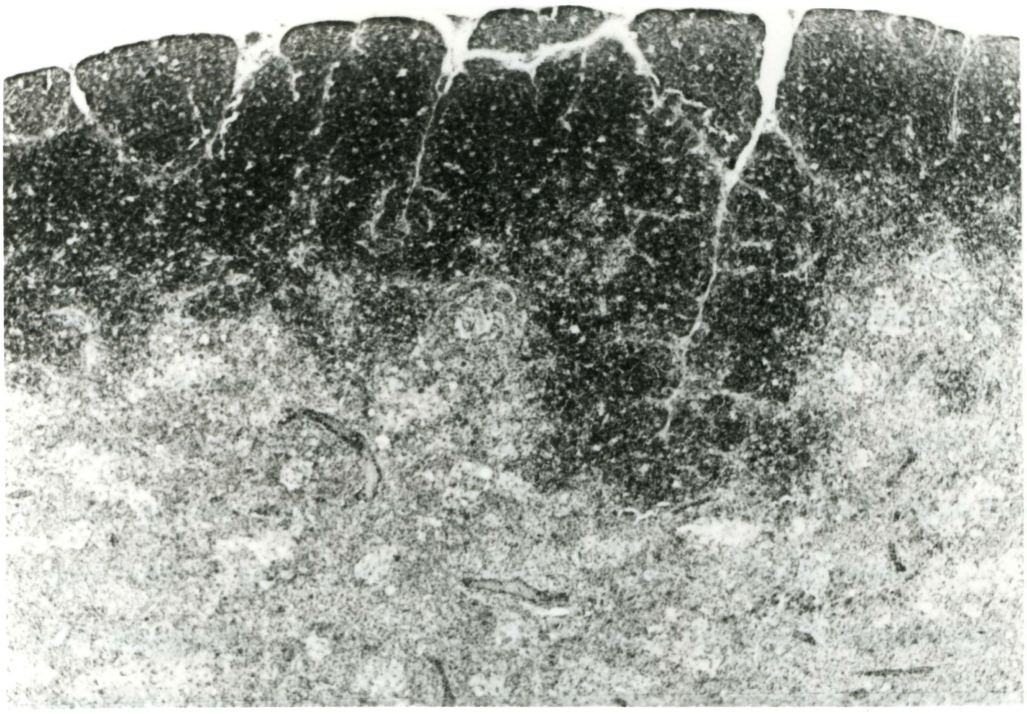


Figure 9. Microscopic appearance of a thymus gland from a control chick. H&E stain

Figure 10. Microscopic appearance of a thymus gland from a chick that was offered peroxide-treated control feed. Thymic atrophy is present, with no definition between cortical and medullary areas. H&E stain



there were no significant differences in feed consumption by group. Feed consumption is shown in Figure 11 and Table 11.

NOVEL period. The novel period was the only period during which rats were exposed to T-2 toxin. Significant differences in feed consumption between groups were observed during this period. On all 3 NOVEL days, the 10 ppm group had reduced feed intake as compared to controls. Rats in the 20 ppm group consumed significantly less feed than those in 10 ppm and 0 ppm groups on all 3 NOVEL days also.

REC periods. There were no significant differences in feed consumption between groups during days of either REC period.

CHOICE periods. During CHOICE days, rats had a choice of control versus N+0 feed to consume. Based on total feed consumed, there were no significant differences between groups during any of the CHOICE days. A group by feed interaction was evaluated to determine if a group preferred N+0 feed or control feed, as compared to other groups. Significant differences were not present (Table 12).

Weight gains

Weight gains by group are shown in Table 13.

ACCLIM period. There were no significant differences in weight gain between groups during the ACCLIM period in which rats received control feed in both feeders.

Figure 11. Mean feed consumption per rat in grams by group during days of the feeding paradigm. Rats were offered control feed on days 1-7 (ACCLIM period), days 11-15 (REC 1 period), and days 19-25 (REC 2 period). On days 8-10 (NOVEL period) rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective group. No feed was offered on day 16. On days 17-18 (CHOICE 1 period and day 26 (CHOICE 2 day), rats were offered a choice of control feed versus novel feed with no T-2 toxin (N+0 feed)

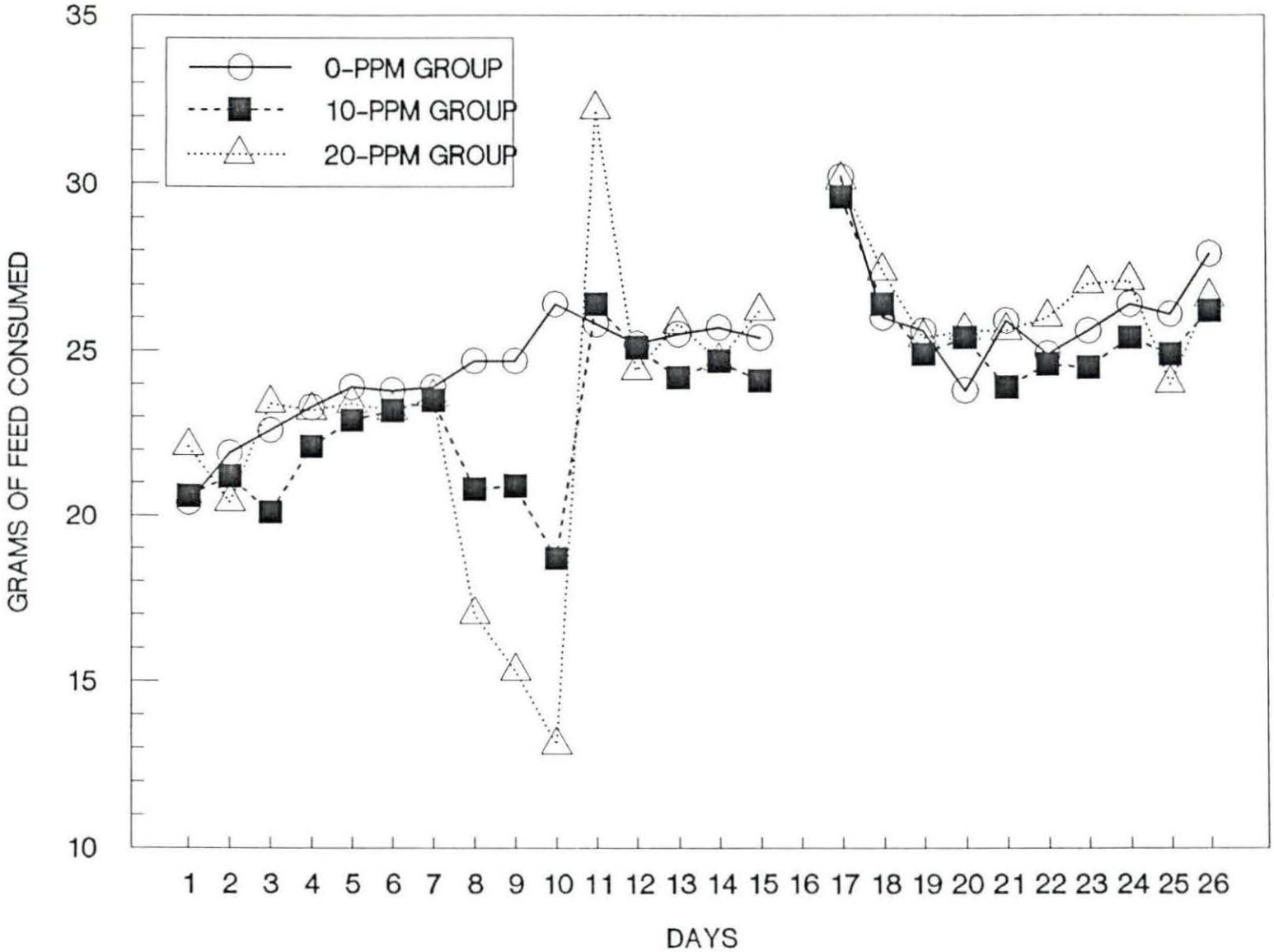

0-PPM GROUP
10-PPM GROUP
20-PPM GROUP
GRAMS OF FEED CONSUMED
35
30
25
20
15
10
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAYS

Table 11. Mean feed consumption per rat per day in grams by group during days of the feeding paradigm

Day	Code[1]	Group 0 ppm	10 ppm	20 ppm	SEM[2]	LSD[3]
1	ACCLIM 1	20.4	20.6	22.1	1.3	3.8
2	ACCLIM 2	21.9	21.2	20.4	1.6	4.7
3	ACCLIM 3	22.6	20.1	23.4	2.0	3.8
4	ACCLIM 4	23.3	22.1	23.2	0.7	2.1
5	ACCLIM 5	23.9	22.9	23.4	1.0	3.0
6	ACCLIM 6	23.8	23.2	23.2	0.7	2.0
7	ACCLIM 7	23.9	23.5	23.6	0.7	2.2
8	NOVEL 1	24.7a[4]	20.8b	17.0c	0.8	2.2
9	NOVEL 2	24.7a	20.9b	15.3c	0.9	2.8
10	NOVEL 3	26.4a	18.7b	13.1c	0.7	2.2
11	REC 1.1	25.8	26.4	32.2	2.0	6.0
12	REC 1.2	25.2	25.1	24.4	0.9	2.8
13	REC 1.3	25.5	24.2	25.8	1.2	3.5
14	REC 1.4	25.7	24.7	24.7	1.0	3.0
15	REC 1.5	25.4	24.1	26.2	1.2	3.6
16	NO FOOD	-	-	-	-	-
17	CHOICE 1.1	30.2	29.6	30.1	1.1	3.3
18	CHOICE 1.2	26.0	26.4	27.4	1.0	3.0
19	REC 2.1	25.6	24.9	25.4	1.0	3.0
20	REC 2.2	23.8	25.4	25.6	1.1	3.2
21	REC 2.3	25.9	23.9	25.6	1.1	3.2
22	REC 2.4	24.9	24.6	26.0	1.2	3.6
23	REC 2.5	25.6	24.5	27.0	1.4	4.2
24	REC 2.6	26.4	25.4	27.1	1.4	4.1
25	REC 2.7	26.1	24.9	24.0	1.5	4.3
26	CHOICE 2	27.9	26.2	26.6	1.5	4.3

[1]Rats received control feed during ACCLIM and REC periods. During NOVEL period, rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective treatment group. During CHOICE periods, rats received a choice of control feed versus novel feed with no T-2 toxin (N+0 feed).

[2]Standard error of treatment mean.

[3]Least significant difference ($p<0.05$, df=3,23).

[4]Different lower case letters within a given day denotes significance ($p<0.05$).

Table 12. Mean feed consumption per rat[a] per day in grams by group and feed during CHOICE (CH) days of the feeding paradigm

	Group							
	0 ppm		10 ppm		20 ppm			
	Feed							
Day	C	N+0	C	N+0	C	N+0	SEM[b]	P[c]
CH 1.1	12.4	17.8	13.8	15.8	13.0	17.1	2.8	0.83
CH 1.2	9.1	16.9	11.7	14.7	5.5	21.9	3.7	0.20
CH 2	7.1	20.6	8.7	17.5	7.6	19.0	2.8	0.69

[a]Rats were offered a choice of Control (C) feed versus novel feed with no T-2 toxin (N+0) during CHOICE periods. Feeders were placed at random in rats' cages.

[b]Standard error of treatment mean.

[c]Probability of a greater value of F (df=2,47).

Table 13. Mean weight gains per rat per day in grams by group during periods of the feeding paradigm

Period[1]	Group			SEM[2]	LSD[3]
	0 ppm	10 ppm	20 ppm		
ACCLIM	9.8	9.4	9.3	0.4	1.2
NOVEL	9.4a[4]	2.6b	-2.2c	0.6	1.9
REC 1	3.1a	4.9b	6.8c	0.5	1.5
REC 2	8.5	7.9	8.9	0.6	1.7

[1]Periods varied in length. ACCLIM=7 days, NOVEL=3 days, REC 1=5 days, and REC 2=7 days. Rats received control feed during ACCLIM, REC1, and REC2 periods, and novel feed during the NOVEL period. Novel feed contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective treatment group.

[2]Standard error of treatment mean.

[3]Least significant difference ($p<0.05$, df=3, 23).

[3]Different lower case letters within a given period denotes significance ($p<0.05$).

NOVEL period. During the NOVEL period, there were significant differences between groups in the weight gain. Both the 10 ppm and 20 ppm groups gained significantly less weight than the 0 ppm group. The 20 ppm group also gained significantly less weight than the 10 ppm group.

REC 1 period. During the REC 1 period, rats in the 10 ppm and 20 ppm groups gained significantly more weight than those in the 0 ppm group. The 20 ppm group also gained significantly more than the 10 ppm group during this period.

REC 2 period. During the REC 2 period, there were no significant differences in weight gains between groups.

Water consumption

Water consumption was significantly reduced during all 3 NOVEL days in the 10 ppm and 20 ppm groups as compared to controls (Table 14). The 20 ppm group did not consume less water than the 10 ppm group during these days. The 10 ppm group consumed less water than controls during ACCLIM 4, ACCLIM 7, REC 1.5, and REC 2.7 days, although the 20 ppm group did not consume less than controls during these days. The 20 ppm group consumed less water than controls on NO FOOD day.

Feeding behavior

Number of feeding attempts. Significant differences in number of feeding attempts per day by group were present

Table 14. Mean water consumption per rat per day in ml by group during days of the feeding paradigm.

Day[1]	Code	Group 0 ppm	10 ppm	20 ppm	SEM[2]	LSD[3]
1	ACCLIM 1	61	56	60	1.8	5.4
2	ACCLIM 2	35	35	30	3.8	11.4
3	ACCLIM 3	41	34	39	2.4	7.3
4	ACCLIM 4	39a[4]	32b	36ab	2.1	6.0
5	ACCLIM 5	41	35	36	2.5	7.5
6	ACCLIM 6	42	37	38	2.0	6.0
7	ACCLIM 7	45a	37b	39ab	2.5	7.5
8	NOVEL 1	46a	35b	33b	2.7	8.0
9	NOVEL 2	42a	33b	30b	2.7	8.1
10	NOVEL 3	43a	32b	29b	2.8	8.4
11	REC 1.1	43	40	46	2.3	6.9
12	REC 1.2	42	38	39	2.5	7.4
13	REC 1.3	41	35	40	2.8	8.4
14	REC 1.4	41	37	40	2.0	5.9
15	REC 1.5	45a	37b	40ab	2.3	7.0
16	NO FOOD	32a	22ab	18b	4.1	12.1
17	CHOICE 1.1	61	54	58	2.9	8.9
18	CHOICE 1.2	42	38	41	1.9	5.9
19	REC 2.1	41	39	39	2.7	8.1
20	REC 2.2	42	36	41	2.2	6.6
21	REC 2.3	42	37	39	2.5	7.6
22	REC 2.4	43	37	41	2.3	6.9
23	REC 2.5	45	38	41	2.4	7.3
24	REC 2.6	47	39	42	2.8	8.4
25	REC 2.7	43a	35b	40ab	2.4	7.2
26	CHOICE 2	45	39	42	2.7	8.2

[1]Rats received control feed during ACCLIM and REC periods. During NOVEL period, rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective treatment group. During CHOICE periods, rats were offered a choice of control feed versus novel feed that contained no T-2 toxin (N+0 feed).

[2]Standard error of treatment mean.

[3]Least significant difference ($p<0.05$, df=3,23).

[4]Different lower case letters within a given day denotes significance ($p<0.05$).

during ACCLIM 6, ACCLIM 7, and REC 1.5 days (Figure 12 and Table 15). During these days, the 10 ppm group had fewer feeding attempts than controls, although the 20 ppm group was not different from controls.

A group by feed interaction was evaluated for all 3 CHOICE days, to determine if a feed choice difference was present among groups. Significant differences were present during CHOICE 2 day (Table 16). On this day, rats in the 10 ppm and 20 ppm groups had more feeding attempts at feeders containing control feed than at feeders containing N+0 feed, as compared to control rats, which had comparatively more attempts at feeders containing N+0 feed than at feeders with control feed. Significant differences were not observed in the 20 ppm group as compared to the 10 ppm group during the CHOICE 2 day.

Data were analyzed during the first two hours after which rats received feed during CHOICE 1.1 day, in comparison to the first two hours of CHOICE 1.2 and CHOICE 2 days. The group by feed by day interactions that were used to evaluate this parameter did not disclose differences.

Duration of feeding attempts. Significant differences among groups in mean duration of feeding attempts were present during NOVEL 1 and REC 2.7 days (Figure 13 and Table 17). On NOVEL 1 day, the 20 ppm group had a reduction in duration of feeding attempts as compared to controls. The

Figure 12. Mean number of feeding attempts per rat per day by group during days of the feeding paradigm. Rats were offered control feed on days 1-7 (ACCLIM period), days 11-15 (REC 1 period), and days 19-25 (REC 2 period). On days 8-10 (NOVEL period) rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective group. No feed was offered on day 16. On days 17-18 (CHOICE 1 period) and day 26 (CHOICE 2 day), rats were offered a choice of control feed versus novel feed with no T-2 toxin (N+0 feed)

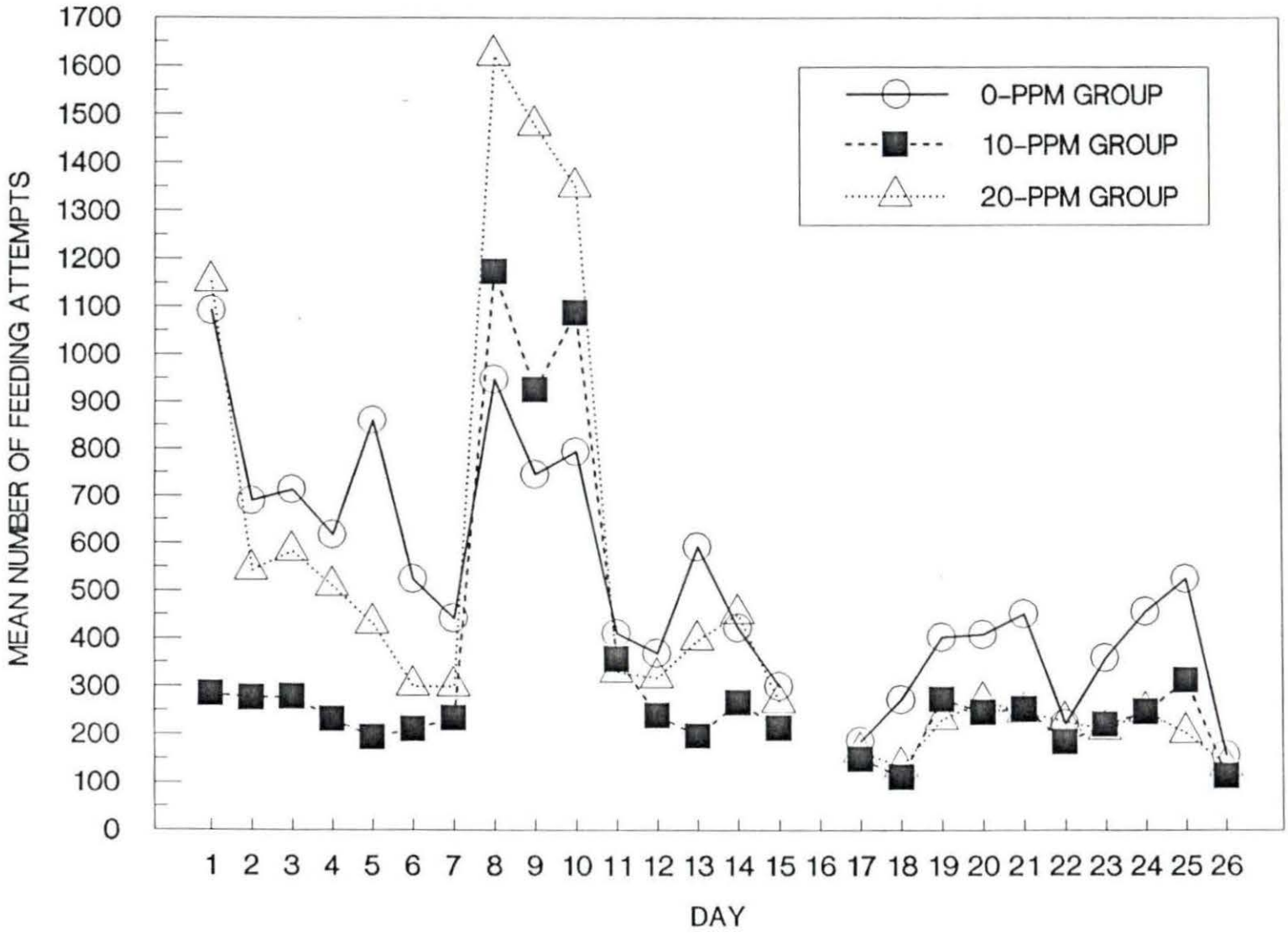

MEAN NUMBER OF FEEDING ATTEMPTS
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAY
0-PPM GROUP
10-PPM GROUP
20-PPM GROUP

Table 15. Mean number of feeding attempts per rat per day by group during days of the feeding paradigm

Day[1]	Code	Group 0 ppm	10 ppm	20 ppm	SEM[2]	LSD[3]
1	ACCLIM 1*[4]	1092	285	1152	419	1340
2	ACCLIM 2	690	277	543	186	549
3	ACCLIM 3	714	279	583	200	591
4	ACCLIM 4	618	232	509	146	429
5	ACCLIM 5	861	194	431	232	683
6	ACCLIM 6	525a[5]	211b	301ab	93	274
7	ACCLIM 7	443a	234b	300ab	62	182
8	NOVEL 1	948	1174	1622	291	860
9	NOVEL 2	746	926	1477	250	738
10	NOVEL 3	794	1088	1349	266	784
11	REC 1.1	411	358	330	101	297
12	REC 1.2	370	240	319	67	198
13	REC 1.3	593	197	397	210	619
14	REC 1.4	423	267	452	128	368
15	REC 1.5	301a	214b	267ab	29	86
16	NO FOOD	-	-	-	-	-
17	CHOICE 1.1	186	149	163	25	72
18	CHOICE 1.2	273	112	133	64	189
19	REC 2.1	403	274	232	78	230
20	REC 2.2	409	247	269	75	222
21	REC 2.3	452	254	246	80	235
22	REC 2.4*	223	185	228	31	98
23	REC 2.5	360	221	210	62	183
24	REC 2.6	457	248	248	89	263
25	REC 2.7*	526	314	203	122	389
26	CHOICE 2	157	114	134	24	73

[1]Rats received control feed during ACCLIM and REC periods. During NOVEL period, rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective treatment group. During CHOICE periods, rats received a choice of control feed versus novel feed with no T-2 toxin (N+0 feed).

[2]Standard error of treatment mean.

[3]Least significant difference ($p<0.05$, df=3,23).

[4]On days marked by *, n=12.

[5]Different lower case letters within a given day denotes significance ($p < 0.05$).

Table 16. Mean number of feeding attempts[a] per rat per day by group and feed during CHOICE (CH) days

	Group							
	0 ppm		10 ppm		20 ppm			
	Feed							
Day	C	N+0	C	N+0	C	N+0	SEM[b]	P[c]
CH 1.1	115	258	138	161	158	168	35	0.13
CH 1.2	73	472	106	117	79	188	90	0.10
CH 2	42	272	87	142	100	168	34	0.03

[a]Rats were offered a choice of control (C) feed versus novel feed with no T-2 toxin (N+0) on CHOICE days. Feeders were placed at random in rats' cages.

[b]Standard error of treatment mean.

[c]Probability of a greater value of F (df=2,47).

10 ppm group was not significantly different from controls during this day. During REC 2.7 day, the 10 ppm group had significantly shorter feeding attempts than controls, although the 20 ppm group was not different from controls on that day.

When groups were analyzed for differences in duration of feeding attempts by choice of feed during CHOICE days, significant differences were not present (Table 18).

Figure 13. Mean duration of feeding attempts per rat per day by group during days of the feeding paradigm. Rats were offered control feed on days 1-7 (ACCLIM period), days 11-15 (REC 1 period), and days 19-25 (REC 2 period). On days 8-10 (NOVEL period) rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective group. No feed was offered on day 16. On days 17-18 (CHOICE 1 period) and day 26 (CHOICE 2 day), rats were offered a choice of control feed versus novel feed with no T-2 toxin (N+0 feed)

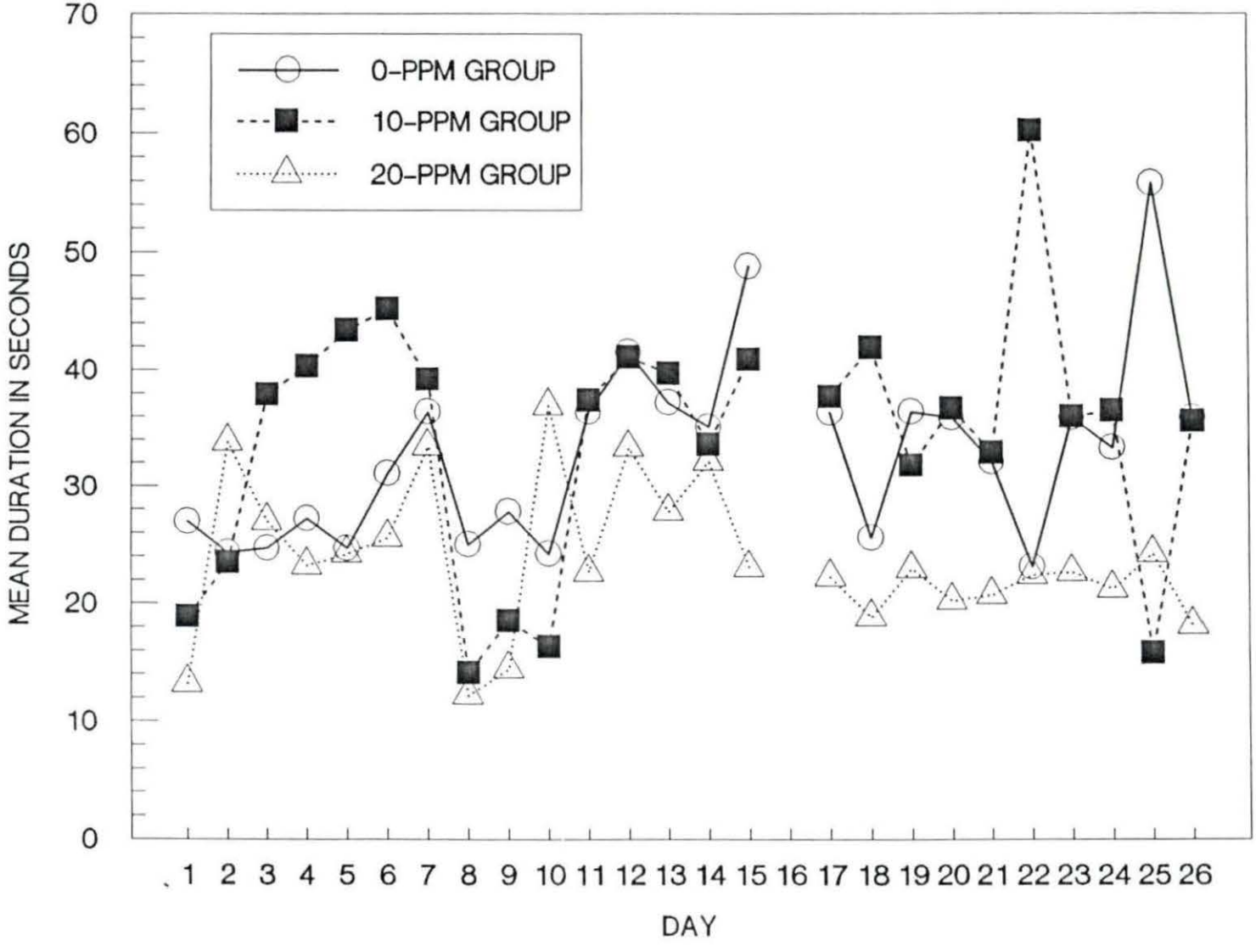

0-PPM GROUP
10-PPM GROUP
20-PPM GROUP
MEAN DURATION IN SECONDS
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
DAY

Table 17. Mean duration of feeding attempts of rats by group during days of the pararadigm (seconds)

Day[1]	Code	Group 0ppm	10ppm	20ppm	SEM[2]	LSD[3]
1	ACCLIM 1*[4]	27.0	18.9	13.2	4.9	15.8
2	ACCLIM 2	24.3	23.5	33.8	8.7	25.9
3	ACCLIM 3	24.7	37.9	27.0	8.6	25.4
4	ACCLIM 4	27.2	40.3	23.2	8.2	24.1
5	ACCLIM 5	24.7	43.4	24.2	8.4	24.8
6	ACCLIM 6	31.1	45.2	25.6	9.9	29.1
7	ACCLIM 7	36.4	39.2	33.4	10.8	31.7
8	NOVEL 1	25.0a[5]	14.1ab	12.2b	3.9	11.4
9	NOVEL 2	27.8	18.5	14.4	5.4	16.1
10	NOVEL 3	24.2	16.3	36.9	17.0	50.2
11	REC 1.1	36.4	37.4	22.6	9.9	29.3
12	REC 1.2	41.5	41.1	33.3	11.3	33.5
13	REC 1.3	37.2	39.7	27.8	10.2	31.4
14	REC 1.4	35.1	33.6	32.1	10.3	31.6
15	REC 1.5	48.8	40.9	23.0	15.1	44.4
16	NO FOOD	-	-	-	-	-
17	CHOICE 1.1	36.3	37.7	22.2	11.4	33.8
18	CHOICE 1.2	25.6	41.9	18.8	12.1	36.1
19	REC 2.1	36.4	31.8	22.9	6.8	20.1
20	REC 2.2	35.9	36.7	20.2	9.5	28.1
21	REC 2.3	32.1	32.9	20.7	8.2	24.1
22	REC 2.4*	23.1	60.2	22.4	18.6	59.4
23	REC 2.5	35.9	36.0	22.6	9.5	28.2
24	REC 2.6	33.3	36.5	21.2	8.8	25.8
25	REC 2.7*	55.8a	15.8b	24.4b	9.6	30.6
26	CHOICE 2	35.9	35.6	18.1	10.7	32.3

[1]Rats received control feed during ACCLIM and REC periods. During NOVEL period, rats were offered novel feed, which contained saccharin and T-2 toxin at 0, 10, or 20 mg/kg for the respective treatment group. During CHOICE periods, rats were offered a choice of control feed versus novel feed with no T-2 toxin (N+0 feed).

[2]Standard error of treatment mean.

[3]Least significant difference ($p<0.05$, df=3,23).

[4]On days marked by *, n=12.

[5]Different lower case letters within a given day denotes significance ($p<0.05$).

Table 18. Mean duration of feeding attempts of rats[a] by group and feed during CHOICE (CH) days (in seconds)

	Group							
	0 ppm		10 ppm		20 ppm			
	Feed							
Day	C	N+0	C	N+0	C	N+0	SEM[b]	P[c]
CH 1.1	23.8	48.8	19.8	55.6	22.9	21.4	16	0.13
CH 1.2	22.2	29.0	21.0	62.8	11.8	25.8	17	0.57
CH 2	22.1	45.6	16.7	54.5	13.6	22.7	15	0.65

[a]Rats were offered a choice of control (C) feed versus novel feed with no T-2 toxin (N+0) on CHOICE days. Feeders were placed at random in rats' cages.

[b]Standard error of treatment mean.

[c]Probability of a greater value of F (df=2,47).

DISCUSSION

Chick Trials I and II

Effects of T-2 toxin on chicks

The most striking effect of T-2 feed on chicks was development of oral mucosal lesions. Lesions developed in all chicks of T-2 groups in both Trials I and II. Lesions were not present in control groups. Oral lesions developed in patterns very similar to those described previously (Wyatt et al., 1972a; Wyatt et al., 1972b; Hoerr et al., 1982a). Histopathologic lesions were also very similar to previous microscopic descriptions (Hoerr, 1982a).

Oral lesions in chicks in the T-2 group in Trial II quickly regressed after control feed replaced T-2 feed on day 15. Gross lesions in this group were not visible after day 28. Previous reports have not evaluated regression of lesions after uncontaminated feed was offered to affected chickens.

Lesions were not present grossly or microscopically in tissues other than oral epithelium in chicks in T-2 groups. Necrotizing lesions caused by T-2 toxin have been described in salivary glands, mandibles, and basihyoid bones (Hoerr et al., 1982a). Necrosis in lymphoid tissues, liver, and thyroids has also been described previously (Hoerr et al., 1982b). Gastrointestinal mucosae, kidneys, livers, spleens, thymus glands, and bursae of Fabricius were examined

microscopically in chicks in this study for such lesions.

Although T-2 toxin was present in P-T-2 feeds in Trial I and II, oral mucosal lesions were not present in chicks in the P-T-2 group in Trial I, and were minimal in only 4 chicks in the P-T-2 group in Trial II. The most plausible reason for this finding is due to lack of intake of feed, resulting in lack of intake of T-2 toxin sufficient to induce lesions as were present in T-2 groups.

Feather changes were noted in chicks in the T-2 group of Trial II. These changes, which consisted of sparseness and shortening of feathers as compared to controls, were similar to those previously described (Wyatt et al., 1975).

Spleen weight values expressed as percentage of body weight (SWP) were decreased in chicks in the T-2 group as compared to controls in Trial I. SWP values were not significantly different between T-2 and I-C groups in Trial I. These findings indicate that reduction in the SWP value in the T-2 group was caused by lack of feed intake rather than direct toxic effects of T-2 toxin on this lymphoid organ. Previous reports of reduction in spleen weights (Hoerr et al., 1982b) suggest that reduction in spleen weight was due to T-2 toxin, but does not propose mechanisms by which this occurred. Another study (Richard et al., 1978) determined that spleen weight values expressed as percentage of body weight in chickens fed T-2 toxin at 10

mg/kg in feed were not depressed as compared to controls. Findings in this experiment are in agreement with those reported by Richard et al.

Feed intake of the T-2 group was 92% of control values during period 1 of Trial I, 99.7% during period 2, and 98% during period 3. In Trial II, intake of T-2 feed by the T-2 group was 82% of controls during period 1, and 96.9% during period 2. These values were determined by calculating feed consumption as a percentage of average body weight during the respective period. The most likely reason for increasing values as the experiment progressed is due to the fact that as body weight increased, feed intake as a percentage of body weight declined. Chicks in the control group were considerably larger than those in the T-2 group, and feed consumption as a percentage of body weight declined in this group as compared to the T-2 group, as a function of larger body size. Values of percentage of feed consumed by T-2 groups compared to controls during later periods may be overestimated because of this effect.

Effects of hydrogen peroxide treatment on feed

Treatment of the 4744 mg/kg T-2 toxin concentrate with hydrogen peroxide (HPO) in preparing the P-T-2 Conc feed greatly reduced T-2 toxin concentration. When this feed was mixed for feeding, there was no detectable amount of T-2 toxin present (< 1 ppm). Further evidence of removal of T-2

toxin from this feed was substantiated by performance of chicks in the P-T-2 Conc group in Trial I. Weight gains in this group were not significantly different from controls, nor were oral mucosal lesions present in this group. Spleen weights also were not affected as compared to controls.

Treatment of T-2 feed with HPO as done by methods for the P-T-2 groups in Trials I and II did not effectively remove T-2 toxin. T-2 toxin concentration was reduced from 21 +/- 3.5 mg/kg to 15.4 +/- 0.7 mg/kg by HPO in P-T-2 feed in Trial I, however in Trial II, HPO treatment did not reduce T-2 toxin concentration (13.5 mg/kg +/- before, 15.4 +/- 0.7 mg/kg after HPO).

Oral lesions were not found in the P-T-2 group in Trial I, but minor lesions were present in 4 chicks in this group in Trial II. This indicates that the biological effect of T-2 toxin in P-T-2 feed in Trial II was still present. Reduction of lesions in chicks in P-T-2 groups as compared to chicks in T-2 groups is most likely due to lack of sufficient intake of T-2 toxin to induce oral lesions.

Feed consumption and weight gains were drastically reduced in chicks consuming P-T-2 feeds. Chicks in P-T-2 groups did not consume enough feed to maintain body weight in either Trial I or II. Chicks in the P-C groups in both Trials I and II also had severely reduced feed intake with subsequent lack of body weight gains as compared to

controls. Chicks in the P-T-2 group in Trial I had significantly lower weight gains as compared to chicks in the P-C group, however weight gains by chicks in P-T-2 and P-C groups in Trial II were not significantly different. Methods used on P-C feeds were different in Trial I versus Trial II, which may explain the difference among these groups in the different trials. P-C feed used in Trial I was rinsed with water after HPO treatment, while P-C feed in Trial II was not rinsed with water after HPO treatment. This finding suggests that rinsing of feed after HPO treatment may limit damage that is done to feed causing reduction in feed intake. Regardless of method, HPO treatment of feed in these trials reduced feed intake to levels that did not allow chicks to maintain body weight.

SWP values in chicks in the P-T-2 group were significantly lower than those of the P-C group in Trial I, however SWP values were not significantly different between the P-T-2 and P-C groups in Trial II. This again suggests that rinsing of P-C feed after HPO treatment limited damage caused by HPO in Trial I.

Oral mucosal lesions were not present in P-C groups, which indicates that T-2 toxin was the factor that caused minor oral lesions in 4 chicks of the P-T-2 group in Trial II.

Inanition controls in Trial II did not have

significantly different weight gains from those in the I-P-C group. This indicates that poor weight gains in chicks of the P-C group was due to lack of feed intake, and there were no additional toxic effects of HPO causing lack of weight gain beyond that of reduced feed intake.

The proposed reason that chicks in the P-T-2 Conc group performed much better than those in the P-T-2 group in Trial I is that T-2 toxin was subjected to much more rigorous treatment in the P-T-2 Conc feed preparation method than in the method used to prepare the P-T-2 feed. The T-2 toxin concentrate used to prepare P-T-2 Conc feed was mechanically agitated with large volumes of HPO and water and filtered 6 times. This is in comparison to simply applying HPO to feed in preparation of the P-T-2 feed. The treatment method used in preparing P-T-2 Conc feed, if attempted on large quantities of feedstuff, would be impractical due to amounts of HPO required, and difficulties encountered in liquids from feedstuffs after application of HPO.

Even though methods used in treating P-T-2 feed in Trials I and II were similar, the method used in Trial I reduced T-2 toxin more effectively. The major difference in methods was drying of HPO treated feed outdoors in sunlight in Trial I versus indoors in Trial II. Exposure to sunlight of T-2 feed containing HPO may have resulted in an interaction, reducing T-2 toxin more effectively than drying

indoors. However, neither method satisfactorily removed T-2 toxin.

HPO treatment of P-T-2 and P-C feeds used in Trial I did not markedly alter protein and energy constituents of the feedstuff. Phosphorous, sodium, and potassium were decreased in P-C feed as compared to C and P-T-2 feeds, which suggests rinsing out of these nutrients. The markedly increased values of iron and zinc in P-C and P-T-2 feeds in Trial I resulted from drying HPO treated feedstuffs on galvanized metal pans. This was corrected when preparing feedstuffs for Trial II by using plastic pans to dry feedstuffs. Even though zinc and iron levels were greatly elevated in Trial I, it did not appear to affect chicks in the P-T-2 and P-C groups differently from Trial II. Had chicks in these groups consumed more feed, toxic effects from zinc or iron may have been present in Trial I.

Thymic atrophy was present in chicks in P-T-2 and P-C groups in Trial I, and chicks in P-T-2, P-C, I-P-C, and I-C groups in Trial II. Thymic atrophy in these chicks appears to be due to lack of feed intake rather than due to direct toxic effects of T-2 toxin or HPO treatment of feed on this tissue.

The cause of reduced feed intake in P-C and P-T-2 groups was due to HPO treatment of feed. The mechanism for this phenomenon was not fully disclosed. HPO treated feeds

were blanched in appearance and lacked the normal odor of control feed. Factors that normally cause prehension and consumption of feed by chicks were apparently destroyed by HPO treatment of these feeds. The effect does not appear to persist after offering normal feed, as chicks in P-T-2 and P-C groups in Trial II were eating ravenously within one hour of being offered control feed on day 15. Further investigation into this area is necessary to disclose mechanisms by which HPO treatment of feed causes reduced intake.

Eight chicks out of 20 from P-C and P-T-2 groups had locomotor difficulties less than 2 days after receiving control feed on day 15. Clinical signs of leg weakness and partial paresis gradually improved until chicks were normal by day 20. Chicks in these groups were emaciated on day 15, but all chicks appeared to consume control feed readily. Lack of intake of essential nutrients or cofactors in HPO treated feeds may have existed, with sudden onset of neural disturbance after sudden growth which occurred when control feed was offered. Intake of essential nutrients in control feed may not have met demands quickly enough in the first few days of return to control feed.

The deleterious effects of T-2 feed and HPO treated feed did not appear to cause residual effects on chicks after replacing these feeds with control feed on day 15.

This was substantiated in Trial II, when chicks were offered control feed after receiving treatment feeds for 2 weeks. Marked increases in feed consumption was evident in P-T-2 and P-C groups. Feed intake also increased in the T-2 group after receiving control feed. Weight gains were significantly lower in P-C and P-T-2 groups compared to controls and T-2 groups during period 3, however weight gain differences cannot be evenly compared during this period, due to a great difference in weight of chicks between groups. During period 4 there were no weight gain differences between groups, even though chicks in P-T-2 and P-C groups had not attained body weights equivalent to those in C and T-2 groups. This suggests no residual effects of HPO treated feeds on chicks from P-T-2 and P-C groups.

Rat Taste Aversion Trial

Effects of T-2 toxin feed on rats

Feed intake was significantly reduced when feed containing T-2 toxin was offered to rats during days 8-10 (NOVEL days). A dose-response effect was also present, as feed consumption values differed significantly as the level of T-2 toxin increased from 10 mg/kg to 20 mg/kg in novel feeds. Rats receiving N+10 feed consumed 79.7% of controls (N+0 group) during novel periods, while rats receiving N+20 feed consumed only 59.8% of controls. Control rats received control feed with saccharin (N+0 feed) during NOVEL days.

Reduced intake of feed containing T-2 toxin has been previously reported (Vesonder et al., 1979). Rats in that study had feed intake of 75% of normal when T-2 toxin was present in feed at 40 mg/kg, which is consistent with results of the present study.

Weight gains were reduced as expected when reduced feed intake resulted from ingestion of T-2 toxin during the NOVEL period. Weight gains were significantly lower in the 10 ppm group compared to controls, and also in the 20 ppm group compared to the 10 ppm group.

On day 11 (REC 1.1), rats in N+10 and N+20 groups compensated for reduced intake during the NOVEL period by increasing feed intake. Rats in the 20 ppm group consumed significantly more control feed than control rats on day 11. This suggests that residual effects of lowering of feed consumption by T-2 toxin were not present for more than 24 hours.

Water consumption was also reduced in 10 ppm and 20 ppm groups compared to controls during days 8-10. Water intake declined as feed intake decreased in these rats.

When feed containing T-2 toxin was offered to rats, the number of feeding attempts increased in 10 ppm and 20 ppm groups compared to controls. Although the increase in number of feeding attempts was not significantly different at $p<0.05$, a definite trend was apparent. This suggests that

T-2 toxin caused rats to search for a more preferable feed.

Durations of feeding attempts were significantly shorter in the 20 ppm group compared to controls on day 8 (NOVEL 1), and values approached significance ($p<0.09$) on day 9 (NOVEL 2). This finding indicates that when rats search for a more preferable feed, the duration of feeding attempts declined.

Aversive effects associated with novel feed

The major purpose of this experiment was to determine if rats in 10 ppm and 20 ppm groups would associate the novel taste of saccharin with T-2 toxin when novel feeds were offered on days 8-10, and later display taste aversion to N+0 feed. This was accomplished by offering rats a choice of N+0 feed versus control feed on CHOICE days, after rats had consumed novel feeds.

A group by feed interaction was used to evaluate taste aversion on CHOICE days. Feed consumption was not significantly different by group and feed choice during these days. This suggests that rats previously exposed to novel feed with T-2 toxin did not show aversion to N+0 feed based on feed consumption.

Differences in the number of feeding attempts were also evaluated to determine if taste aversion to N+0 feed occurred during CHOICE days. On CHOICE 1.1 and 1.2 days, a significant interaction was not present, although a trend was present. On CHOICE 2 day, a significant interaction was

present. This suggests a mild aversion to N+0 feed by rats in the 10 and 20 ppm groups as compared to controls during the first two choice days, and a definite effect during CHOICE 2 day, based on number of feeding attempts. The proposed reason for this effect is that rats which received novel feeds containing T-2 toxin during the NOVEL period recognized saccharin in the N+0 feed during CHOICE days. This theory would be greatly supported if feed consumption data had agreed with the number of feeding attempts on these days, days, but this was not the case.

Differences in the duration of feeding attempts between groups by feed choice were not observed on CHOICE days. This finding agrees with feed consumption on these days. When a choice of feed was present, the duration of feeding attempts at a feeder was directly proportion to the amount of feed consumed from that feeder.

If rats developed feeder preferences during days prior to CHOICE days, they may have eaten from feeders based on preference of feeder rather than feed in the feeder during the CHOICE days. Feeder preference was determined on day 15 by identifying rats that consumed more than 75% of their feed from one feeder. Feeder preference was observed in 13 of the 24 rats. Of the 13 rats that developed a feeder preference, 7 rats were offered control feed in the preferred feeder on day CHOICE 1.1. Feeder preference was

maintained in the previously preferred feeder in only one of the 7 rats during day CHOICE 1.1. This suggests that feeder preferences were overcome by the N+0 feed offered on day CHOICE 1.1.

The results of this experiment appear contradictory. Evaluation of feed consumption and duration of feeding attempts do not suggest that taste aversion to N+0 feed occurred in rats previously exposed to novel feeds containing T-2 toxin. However, behavioral data during CHOICE days indicate possible aversion, based upon the number of feeding attempts. Since the amount of feed consumed will determine performance of weight gains in food producing animals, perhaps the best parameter to evaluate feed aversion would be feed consumption.

CONCLUSIONS

In Chick Trials I and II, hydrogen peroxide was used to detoxify feed contaminated with T-2 toxin. Hydrogen peroxide was successful in removing T-2 toxin from the T-2 toxin concentrate by methods that employed large volumes of hydrogen peroxide and water rinses. Weight gain of chicks that consumed feed mixed with the detoxified T-2 toxin concentrate was not different from controls.

Application of hydrogen peroxide directly to feed containing T-2 toxin did not reduce toxin levels to acceptable concentrations. Furthermore, treatment of feed with hydrogen peroxide caused a reduction in feed intake in chicks to a degree that body weights were not maintained.

In conclusion, applicable methods of detoxifying animal feedstuffs containing T-2 toxin were not afforded. Although T-2 toxin could be reduced to a concentration that did not hinder growth, the methods necessary to accomplish the task were beyond the realm of present feed processing potential.

Rats were used in a separate experiment to evaluate taste aversion associated with T-2 toxin. Taste aversion based on the amount of feed consumed by rats was not discovered. Based on feeding attempt frequencies, recognition of saccharin in novel feed with no T-2 toxin (on CHOICE days) resulted in mild behavior changes that were probably associated with taste aversion. Since the amount

of feed consumed dictates animal performance in healthy animals, perhaps feed consumption is the more reliable parameter to use for evaluation of taste aversion.

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ACKNOWLEDGEMENTS

To my wife, Mary, I wish to offer my sincere thanks for patience, help, and understanding which allowed me to complete this task. Also to my son, John, whose presence helps to make it worthwhile.

To Dr. Gary Osweiler, chairman of my graduate committee, I would like to express my gratitude for the assistance and encouragement you have given me.

I would like to thank Drs. John Andrews, Lorraine Hoffman, David Hopper, and Darrell Trampel for participation on my graduate committee.

Appreciation is expressed to Dr. Vaughn Seaton for allowing me the opportunity for invaluable experience at the Iowa State University Veterinary Diagnostic Laboratory.

To Nancy Goetsch, great thanks are offered for your immeasurable technical assistance. Also to the staff of the ISU VDL Analytical Chemistry Laboratory, thanks are extended for mycotoxin analyses. I am also grateful to Jim Wright for your technical assistance.