Egg Production, Shell Thickness, and Other Physiological Parameters of Laying Hens Affected by T-2 Toxin[1]

R. D. WYATT, J. A. DOERR, P. B. HAMILTON,* AND H. R. BURMEISTER

Departments of Poultry Science and Microbiology, North Carolina State University, Raleigh, North Carolina 27607, and Northern Regional Research Laboratory, Peoria, Illinois 61604*

T-2 toxin has been reported to cause severe oral lesions and neural disturbances in young broiler chickens. T-2 toxin, when added at a level of 20 µg per g of feed, caused oral lesions but no abnormal neural disturbances in young broiler chickens. T-2 toxin, when added at a level of 20 µg per g of feed, caused oral lesions but no abnormal neural symptoms in laying hens. T-2 toxin had no effect on either hemoglobin, hematocrit values, erythrocyte count, plasma glucose, prothrombin times, or the sizes of the liver, spleen, pancreas, and heart. Lipid content of the liver was not altered. Feed consumption, however, was reduced, as were the total plasma protein and lipid concentrations and the total leukocyte count. Most important economically was the lowered egg production and a thinner egg shell. The timing and severity of the symptoms suggest that T-2 toxin causes primary oral lesions that reduce feed consumption with a consequent reduction in serum proteins and lipids, which culminate in decreased egg production. The leucopenia and thinner egg shell may be independent systemic effects of T-2 toxin in laying hens.

T-2 toxin [4,15-diacetoxy-8-(3-methyl-butyryloxy) - 12,13 - epoxy - Δ^9 - tricothecene-3-ol] is a mycotoxin originally isolated from a strain of *Fusarium tricinctum* (1). Both the toxin and the fungus have been implicated in moldy corn toxicosis of farm animals (7, 11, 13, 22). The toxin induces severe inflammatory reactions in humans, rodents, and trout (17). In young broiler chickens the dose-related oral lesions are similar to the third or necrotic angina stage of alimentary toxic aleukia (ATA), a toxicosis of humans caused by grains infested with *Fusarium* (30). These lesions appear to be characteristic enough of the toxicosis in chickens to aid in the diagnosis of field cases (29). T-2 toxin consumption also provokes neural disturbances in young chickens suggestive of the profound neural abnormalities underlying ATA (28).

The effect of T-2 toxin in adult chickens does not appear to have been studied previously. Our interest in the effect of T-2 toxin on laying hens was stimulated by a field case referred to us which was suggestive of T-2 toxicosis. In this paper we give a general description of T-2 toxicosis in laying hens.

MATERIALS AND METHODS

Production of T-2 toxin. T-2 toxin was produced by the method of Burmeister (6) to give a crystalline product melting at 148 to 150 C.

Animal husbandry. Mature laying hens (Dekalb 131), 30 weeks old, were assigned randomly to individual laying cages. They were housed in an environmentally controlled room with a temperature of 25 C and relative humidity of 20%. The birds were exposed to 15 h of light per day with a 15-min increase weekly. Feed consisted of a commercial layer ration free of all medications. T-2 toxicosis was induced by incorporating known amounts of crystalline T-2 toxin dissolved in 50% (vol/vol) aqueous ethanol into small portions of the diet which were dried before mixing into the remainder of the feed. Individual egg production was recorded daily, and individual body weights and feed consumption were recorded weekly.

Experimental design. Twenty hens were selected on the basis of uniform egg production. Ten birds received the control diet and ten received the diet containing 20 µg of T-2 toxin per g of feed for the same period. The experimental design was completely randomized. The data from the experimental and control birds were compared statistically by Student's *t* test (5).

Blood and tissue analyses. A 5-ml blood sample was taken from the brachial vein of all birds at weekly intervals. The 5-ml sample was immediately mixed with 0.6 ml of 0.18 M sodium citrate and kept on ice until the analyses were performed. Plasma protein was measured by the biuret method (27), plasma glucose by the method of Dubowski (8), plasma lipid by the method of Friedman (10), and the prothrombin times were obtained with a Fibrometer (Baltimore Biological Laboratories, Cockeysville, Md.) using ho-

mologous thromboplastin. At the end of the experiment hematocrit, hemoglobin, and erythrocyte and leukocyte counts were made. Hemoglobin was estimated according to Sunderman et al. (24), and the erythrocyte and leukocyte numbers were counted by standard hemocytometer procedures after staining with Natt-Herrick (21) stain modified to have brilliant cresol blue and methyl red instead of methyl violet (F. Craig, personal communication). Liver lipid was determined as outlined by Smith and Hamilton (23).

Determination of egg parameters. Eggs were collected eight times daily and weighed immediately to negative water evaporation. Egg quality was evaluated by comparing the egg weight to the height of the thick white on a Haugh unit conversion chart (12). Egg shell thickness was measured at three points equidistant along the equator of the shell after drying the shells at 100 C for 2 h.

Neurological examination. At weekly intervals all birds were subjected to a series of manipulations to observe whether the dietary T-2 toxin altered normal neural responses. Corneal reflexes were checked, the birds were manually placed on their backs to test their righting reflex, and the birds were manipulated in an effort to provoke the hysteroid seizures seen during T-2 toxicosis in young birds (28).

Microbiological examination of the liver. Immediately after the birds were killed, the visceral organs were exposed. An exposed area of the liver was seared with a hot spatula and a sterile loop was inserted through the seared area. The material thus obtained was streaked on brain heart infusion agar plates (Difco) and incubated at 37 C for 7 days. The plates were examined at daily intervals for bacterial and fungal growth.

Examination of the internal organs. At the end of the experiment the birds were killed by cervical dislocation. The internal organs were excised, trimmed of extraneous tissue, blotted, and weighed. Liver was frozen immediately for subsequent lipid analysis.

RESULTS

Oral lesions characteristic of T-2 toxicosis (30) appeared during the first week of toxin administration. The lesions appeared first on the sublingual and palatine areas and then on the tongue and in the corners of the mouth. The raised lesions were yellowish-white to gray in color, and they began as small necrotic foci that progressed to larger lesions about 1 cm in diameter. Although lesions appeared at three main areas of the mouth, the lesions in the corners of the mouth appeared more severe and occasionally impaired normal mouth movement.

Among the first signs of T-2 toxicosis in laying hens was the drop in feed consumption; within 1 week consumption of the toxic diet was 25% less than the control (Fig. 1). Feed intake

stabilized by the second and third weeks (Fig. 1).

There was a significant ($P < 0.05$) weight loss after 1 week in birds receiving 20 μg of T-2 toxin per g of diet (Table 1). Moreover, by the third week the group fed T-2 toxin lost approximately 10% of their initial body weight, whereas the control group maintained their body weight.

Concomitant with the appearance of oral lesions and reduction in feed consumption was a significant decline in plasma protein (Fig. 2). After the first week of the experiment the plasma protein concentration was 15% lower and by the third week was 20% lower than plasma protein concentrations of control birds. Plasma lipid was decreased about 60% by the end of the third week (Table 2), but plasma glucose was not altered.
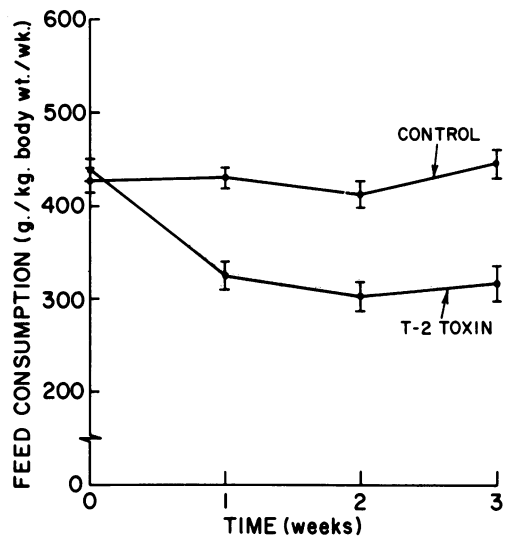
Responses of various hematological parame-

FIG. 1. *Effect of dietary T-2 toxin (20 µg/g) on feed consumption of laying hens. Each data point represents the mean of 10 birds, and vertical bars on the points are standard errors of the means.*

TABLE 1. *Dietary T-2 toxin and body weights of laying hens*

Time (weeks)	Mean body wt (kg) after dose of:	
	0 µg/g	20 µg/g
0	1.69 ± 0.11[a]	1.81 ± 0.05
1	1.72 ± 0.12	1.71 ± 0.05[b]
2	1.70 ± 0.10	1.67 ± 0.05[b]
3	1.72 ± 0.12	1.63 ± 0.05[b]

[a] Standard error of the mean.
[b] These values differ significantly ($P < 0.05$) from the control value for the particular column.
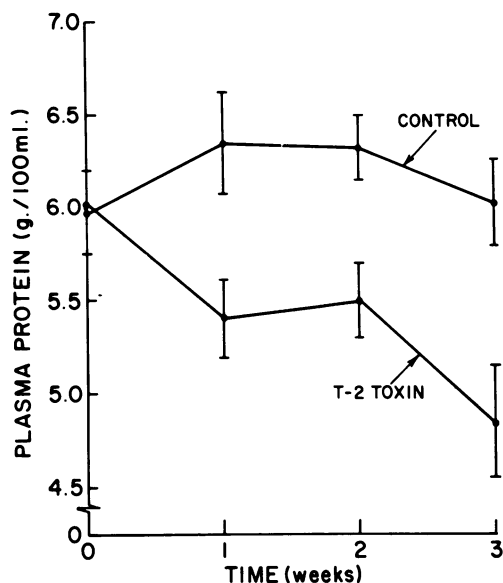
FIG. 2. Plasma protein response of laying hens to dietary T-2 toxin (20 µg/g). Each data point represents the mean of 10 birds, and vertical bars on the data points are standard errors of the means.

TABLE 2. Response of various blood parameters to dietary T-2 toxin

Parameter	Determination after dose[a] of:	
	0 µg/g	20 µg/g
Hemoglobin (g/100 ml)	7.9 ± 0.3	7.7 ± 0.2
Hematocrit (%)	29.3 ± 0.7	28.0 ± 0.5
Erythrocyte count (× 16⁶/mm³)	2.68 ± 0.14	2.61 ± 0.9
Leukocyte count (× 10³/mm³)	30.7 ± 2.1	21.6 ± 1.6[b]
Prothrombin time (s)	8.4 ± 0.3	8.9 ± 0.2
Plasma lipid (g/100 ml)	3.27 ± 0.34	1.40 ± 0.19[b]
Plasma glucose (mg/100 ml)	212 ± 6	205 ± 5

[a] Each dose was fed to 10 birds for 3 weeks, and each experimental value is the mean ± standard error of the mean.
[b] These values differ significantly (P < 0.05) from their corresponding control value.

ters to T-2 toxin, i.e., hematocrit values, hemoglobin concentration, and erythrocyte numbers, were not significantly affected (Table 2). The leukocyte number was significantly (P < 0.05) lowered by about 30% in birds receiving T-2 toxin. Prothrombin times were not altered significantly. These data suggest that T-2 toxin

caused a leucopenia without anemia or impaired hemostasis in the laying hens.

No significant change in any of the internal organs in response to dietary T-2 toxin was noted (Table 3). There was no effect on the lipid content of the liver, and microbiological examination of this organ revealed no bacterial or fungal contamination. Neurological examination of the hens revealed no neural disturbances like those seen in young broiler chickens.

During the first week, no significant change in egg production was observed between laying hens receiving the dietary T-2 toxin and the control diet (Fig. 3). During the next 2 weeks egg production declined by about 20% in birds fed toxic feed. The egg weight and Haugh units were not significantly altered by the T-2 toxin-contaminated feed, but the shell thickness was

TABLE 3. Dietary T-2 toxin and the relative organ weights of laying hens

Organ	Wt after dose[a] of:	
	0 µg/g	20 µg/g
Liver	2.09 ± 0.08	2.31 ± 0.12
Pancreas	0.20 ± 0.01	0.18 ± 0.01
Spleen	0.093 ± 0.006	0.084 ± 0.010
Heart	0.43 ± 0.01	0.40 ± 0.01

[a] Each dose was fed to 10 birds for 3 weeks, and each experimental value is the mean ± the standard error of the mean. Weights are expressed as grams/100 g of body weight.
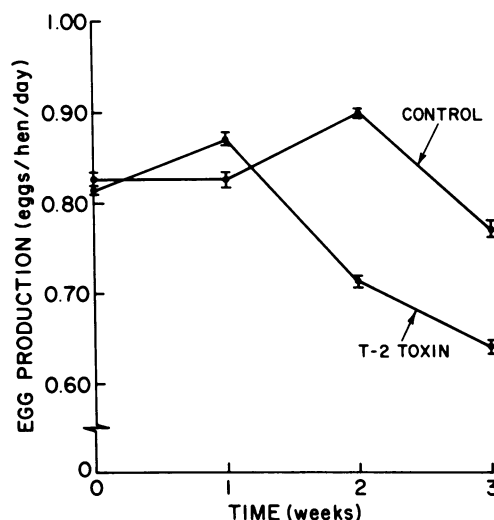

FIG. 3. Alteration of egg production by laying hens by dietary T-2 toxin (20 µg/g). Each data point represents the mean of 10 birds, and vertical bars on the points are standard errors of the means.

significantly ($P < 0.05$) decreased (Table 4). The shells were so fragile that the pressure from a grease pencil used to mark the eggs for identification often caused cracks to occur.

DISCUSSION

The primary effect of T-2 toxicosis in laying hens appears to be an inflammatory reaction in the mouths of affected birds. This inflammatory reaction was first noted about 4 to 5 days after initiation of treatment. Lesions first manifested themselves as small gray to cream-colored areas on the upper beak and progressed to the distal portions of the tongue and corners of the mouth. The lesions appeared quite similar to those described in broiler chickens by Wyatt et al. (30) except that the lesions on the inside of the beaks were oval shaped and did not assume the V-shaped pattern seen in broilers. The oral lesions appear to reduce feed intake with a subsequent weight loss. Proteinemia and lipemia observed during T-2 toxicosis are probably a reflection of the lowered feed consumption without a concomitant immediate decrease in egg production, which draws down the hen's reserves of proteins and lipids. The thin-shelled eggs could result from lower consumption of calcium and phosphorous, although there might be an independent effect on egg shells. Certainly a mechanism independent of nutritional inadequacy would appear to be needed for the leucopenia observed. Lack of any significant effect on internal organs also supports the hypothesis that the primary effect of T-2 toxin is to cause an oral inflammation and not to act specifically on any target organ.

Decrease in egg production and the occurrence of thin-shelled eggs suggest that T-2 toxin poses a threat to poultry health and the industry. Wyatt et al. (28) described outbreaks of disease in avians that were characterized by

TABLE 4. *Influence of dietary T-2 toxin on various egg quality parameters*

Parameter	Determination after dose[a] of:	
	0 µg/g	20 µg/g
Egg weight (g) ...	58.0 ± 1.3	61.4 ± 1.2
Shell thickness (mm)	0.360 ± 0.007	0.335 ± 0.008[b]
Egg white (Haugh units)	84.0 ± 1.4	85.7 ± 1.4

[a] Each dose was fed to 10 birds for 3 weeks, and each experimental value is the mean ± the standard error of the mean.

[b] This differs significantly ($P < 0.05$) from the corresponding control value.

irritation and necrosis of the mouth, head, and feet. Subsequently we have encountered several apparent outbreaks of T-2 toxicosis in laying hens. One such outbreak involved 20,000 commercial layers. This disease was characterized by the sudden appearance of oral lesions like those observed in this study in a significant number of the birds, a 25% drop in egg production, and a high percentage of thin-shelled eggs that would not reach their normal markets. Unfortunately the feed had been consumed when the case was referred to us; hence no chemical analysis of the feed was possible. The birds returned to normal once a new supply of feed was obtained.

These findings of decreased egg production and egg shell thickness may have considerable ecological importance if they can be extrapolated to wild birds. Much public and scientific concern has resulted from data linking certain commerically important pesticides to the production of thin-shelled eggs in some desirable avian species that are approaching extinction (4, 15, 16). Certainly grains and seeds become infected with molds under natural conditions, and T-2 toxin is one of many toxins likely produced under such conditions (3). It would be surprising if mycotoxicoses did not occur in wild birds, and these diseases may account for otherwise inexplicable variations in wild bird populations. A study of wildlife for signs of mycotoxicoses appears to be warranted.

ATA was a major health problem in Russia for almost two decades (18). The toxic principle(s) responsible for the disease apparently was not isolated at the time of the disease outbreaks (3, 19). More recent chemical and toxicological studies (2, 20, 30) have suggested that T-2 toxin may cause ATA. Similarities between ATA and T-2 toxicosis include dermal toxicity (1, 19), inflammation about the nose and mouth (17), oral lesions (30), intradermal hemorrhages (17), and neural disturbances (9, 18, 28). The main piece of clinical evidence missing from the correlation of T-2 toxicosis with ATA is the devastation of the bone marrow and its hematological sequelae characteristic of ATA. The present finding of leucopenia during T-2 toxicosis of laying hens supplies part of the evidence needed to link the toxicosis to the damaging of bone marrow. Furthermore, Ueno et al. (25) reported cellular degeneration and karyorrhexis of the actively dividing cells of the bone marrow of mice receiving fatal dosages of culture filtrates of *Fusarium* strains known to produce T-2 toxin (26). It must be mentioned, however, that Kosuri et al. (14) observed leucocytosis in rats given rapidly lethal doses of T-2 toxin, so there

may be a time factor involved in the effect on bone marrow.

ACKNOWLEDGMENTS

LITERATURE CITED

1. Bamburg, J. R., N. V. Riggs, and F. M. Strong. 1968. The structures of toxins from two strains of *Fusarium tricinctum*. Tetrahedron **24**:3329-3336.

2. Bamburg, J. R., and F. M. Strong. 1971. 12,13-Epoxy-trichothecenes. *In* S. Kadis, A. Ciegler, and S. J. Ajl (ed.), Microbial toxins, vol. 7. Academic Press Inc., New York.

3. Bamburg, J. R., F. M. Strong, and E. B. Smalley. 1969. Toxins from moldy cereals. J. Agric. Food Chem. **17**:443-450.

4. Bitman, J., H. C. Cecil, and G. F. Fries. 1970. DDT-induced inhibition of avian shell gland carbonic anhydrase: a mechanism for thin eggshells. Science **168**:594-596.

5. Bruning, J. L., and B. L. Kintz. 1968. Computational handbook of statistics. Scott Foresman Co., Glenview, Ill.

6. Burmeister, H. R. 1971. T-2 toxin production by *Fusarium tricinctum* on solid substrate. Appl. Microbiol. **21**:739-742.

7. Christensen, C. M., R. A. Meronuck, G. H. Nelson, and J. C. Behrens. 1972. Effects on turkey poults of rations containing corn invaded by *Fusarium tricinctum* (Cda.) Sny. & Hans. Appl. Microbiol. **23**:177-179.

8. Dubowski, K. M. 1962. An o-toluidine method for body-fluid glucose determination. Clin. Chem. **8**:215-235.

9. Forgacs, J., and W. L. Carll. 1962. Mycotoxicoses. Adv. Vet. Sci. **7**:273-382.

10. Friedman, H. S. 1968. Quantitative determination of total lipids in serum. Clin. Chim. Acta **19**:291-245.

11. Gilgan, M. W., E. B. Smalley, and F. M. Strong. 1966. Isolation and partial characterization of a toxin from *Fusarium tricinctum* on moldy corn. Arch. Biochem. Biophys. **114**:1-3.

12. Haugh, R. R. 1933. New method for determining the quality of an egg. U. S. Egg Poultry Mag. **39**:27-49.

13. Hsu, I. C., E. B. Smalley, F. M. Strong, and W. E. Ribelin. 1972. Identification of T-2 toxin in moldy corn associated with a lethal toxicosis in dairy cattle. Appl. Microbiol. **24**:684-690.

14. Kosuri, N. R., E. B. Smalley, and R. E. Nichols. 1971. Toxicologic studies of *Fusarium tricinctum* (Corda) Snyder et Hansen from moldy corn. Am. J. Vet. Res. **32**:1843-1850.

15. Liehner, P. N., and A. Egbert. 1969. Dieldrin and eggshell thickness in ducks. Nature (London) **224**:1218-1219.

16. Longcore, R. R., F. B. Samson, and T. W. Whittendale. 1971. DDE thins eggshells and lowers reproductive success of captive black ducks. Bull. Environ. Contamin. Toxicol. **6**:485-490.

17. Marasas, W. F. O., J. R. Bamburg, E. B. Smalley, F. M. Strong, W. L. Ragland, and P. E. Degurse. 1969. Toxic effects on trout, rats, and mice of T-2 toxin produced by the fungus *Fusarium tricinctum* (Cd.) Synd. Hans. Toxicol. Appl. Pharmacol. **15**:471-482.

18. Mayer, C. F. 1953. Endemic panmyelotoxicosis in the Russian grain belt. Part one: The clinical aspects of alimentary toxic aleukia (ATA); a comprehensive review. Mil. Surg. **113**:173-189.

19. Mayer, C. F. 1953. Endemic panmyelotoxicosis in the Russian grain belt. Part two: The botany, phytopathology, and toxicology of Russian cereal food. Mil. Surg. **113**:295-315.

20. Mirocha, C. J., and S. Pathre. 1973. Identification of the toxic principle in a sample of poaefusarin. Appl. Microbiol. **26**:719-724.

21. Natt, M. P., and C. A. Herrick. 1952. A new blood diluent for counting erythrocytes and leucocytes in the chicken. Poultry Sci. **31**:735-738.

22. Smalley, E. B., W. F. O. Marasas, F. M. Strong, J. R. Bamburg, R. E. Nichols, and N. R. Kosuri. 1970. Mycotoxicoses associated with moldy corn, p. 163-173. *In* M. Herzberg (ed.), Proc. First U.S.-Japan Conf. Toxic Microorganisms. U. S. Dept. of Interior and UJNR Panels on Toxic Microorganisms, Washington, D.C.

23. Smith, J. W., and P. B. Hamilton. 1970. Aflatoxicosis in the broiler chicken. Poultry Sci. **49**:207-215.

24. Sunderman, F. W., R. P. MacFate, D. A. McFayden, G. G. Stevenson, and B. E. Copeland. 1953. Symposium on clinical hemoglobinometry. Am. J. Clin. Pathol. **23**:519-598.

25. Ueno, Y., K. Ishii, K. Sakai, S. Kanadea, H. Tsunoda, T. Tanaka, and M. Enomoto. 1972. Toxicological approaches to the metabolites of Fusaria. IV. Microbial survey on "Bean-hulls poisoning of horses" with the isolation of toxic tricothecenes, neosolaniol and T-2 toxin of Fusarium solani M-1-1. Jpn. J. Exp. Med. **42**:187-203.

26. Ueno, Y., N. Sato, K. Ishii, K. Sakai, H. Tsunoda, and M. Enomoto. 1973. Biological and chemical detection of tricothecene mycotoxins of *Fusarium* species. Appl. Microbiol. **25**:699-704.

27. Wooton, I. D. P. 1964. Micro-analysis in medical biochemistry. Grune and Stratton, Inc., New York.

28. Wyatt, R. D., W. M. Colwell, P. B. Hamilton, and H. R. Burmeister. 1973. Neural disturbances in chickens caused by dietary F-2 toxin. Appl. Microbiol. **26**:757-761.

29. Wyatt, R. D., J. R. Harris, P. B. Hamilton, and H. R. Burmeister. 1972. Possible field outbreaks of fusariotoxicosis in avians. Avian. Dis. **16**:1123-1129.

30. Wyatt, R. D., B. A. Weeks, P. B. Hamilton, and H. R. Burmeister. 1972. Severe oral lesions in chickens caused by ingestion of dietary fusariotoxin T-2. Appl. Microbiol. **24**:251-257.