The Effect of Dietary Choline Chloride and Inositol on the Depletion Rate of Heptachlor from Birds[1]

by C. R. CREGER and L. F. KYBENA[2]
Department of Poultry Science
Texas A&M University
College Station, Texas 77843

The extent of environmental contamination by pesticides has become increasingly clear during the past few years as a result of the development of analytical methods for the detection of smaller quantities of pesticides. There exists the possibility of contamination of birds reared for human consumption which would necessitate the destruction of these birds, at a great economic loss to the producer. Various methods have been employed in attempts to hasten pesticide depletion from body tissues of poultry. Workers (7,8) observed that the use of low protein diets facilitates the early removal of DDT from the tissue and egg of the laying hen. These workers and Donaldson et al. (2) observed an increase in the DDT residue when high fat diets were fed or when hens were periodically starved. Smith et al. (4) reported an increase in concentration of DDT in adipose tissue and in egg yolk when laying hens were force molted.

For several years choline chloride has been used in conjunction with vitamin B_{12}, vitamin E, and antibiotics (1) for the mobilization of infiltrated hepatic fat in laying hens affected with the Fatty Liver Syndrome. Inositol in combination with choline chloride has been reported to be effective in mobilizing hepatic fat (Reed et al., 1968; Griffith, 1969).

---

[1]This is a contribution of the Texas Agricultural Experiment Station supported in part by the Caesar Kleberg Research Program in Wildlife Ecology.

[2]Present address: South Central Poultry Research Laboratory
State College, Mississippi 39762

Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 3, 1971, published by Springer-Verlag New York Inc.

The experiments reported herein were initiated to determine the effect of choline chloride and inositol on the depletion rate of heptachlor from Japanese quail (Coturnix c. japonica).

Methods and Materials

Two trials were conducted utilizing a total of 160 one-week-old unsexed Japanese quail (Coturnix c. japonica) to determine the effect of choline chloride and inositol supplementation on the depletion rate of heptachlor. The diet fed to all groups in both trials was a practical type grower diet containing 25% protein adequately supplemented with vitamins and minerals.

Trial 1. Eighty one-week-old unsexed Japanese quail were randomly divided into four groups of 20 birds each and placed in conventional tier-type batteries. The experimental design was as indicated in Table 1. Each group received its respective diet and water ad libitum for the diration of the experiment. All diets were calculated to be isonitrogenous. This was accomplished by altering the grain and protein sources. All diets contained 10 p.p.m. commercial grade heptachlor for an initial period of 14 days.

The trial was terminated after a 24-day period, and ten birds from each group were individually weighed and killed. The liver was removed from each bird and then analyzed for heptachlor and metabolites. In both trials conducted, determinations for heptachlor and metabolites were made in accordance with the procedure of Stemp et al. (6), with only slight modifications. All experimental samples, containing heptachlor metabolites, were equated to the heptachlor standard.

In both trials conducted, data were treated to analysis of variance (5) and multiple range and multiple F Tests (3).

Trial 2. The Experimental design was the same as Trial 1 except that all groups received 100 p.p.m. commercial grade heptachlor in their respective diets for an initial period of five days instead of 10 p.p.m. so that detectable residues might be present after a longer period of time (Table 2).

On the final day of exposure to heptachlor, ten from each group were individually weighed and killed. The liver and a sample of muscle tissue were taken from individual birds and analyzed for heptachlor and metabolites. The experiment was terminated after 68 days, and ten birds from each group were individually weighed and killed. The liver, a sample of muscle tissue, and one of adipose tissue were taken from individual birds and analyzed for heptachlor and metabolites.

Results and Discussion

Trial 1. Detectable residues of heptachlor and metabolites were found in quail killed ten days after receiving 10 p.p.m. dietary heptachlor for 14 days (Table 1). The residue levels of heptachlor and metabolites present in the liver were affected by the dietary treatments. The livers of the quail receiving the basal diet contained 2.24 p.p.m.; whereas, the livers of those receivng the basal diet supplemented with 2645 grams of choline chloride and 2000 grams of inositol per 1000 kilograms contained significantly less residue (1.35 p.p.m.). The addition of 10% vegetable oil to the basal diet caused more than a two-fold increase in quantity of residue present in the liver. These data are in agreement with Wesley et al. (8) who observed a 40% increase in residual DDT in the adipose tissue of hens fed a diet containing five percent added fat. Supplementation of the diet containing the additional 10% vegetable oil with 2645 grams of choline chloride and 2000 grams of inositol per 1000 kilograms reduced the heptachlor residue to less than one-half that of the group receiving the diet with 10% added vegetable oil and slightly below those receiving the basal diet.

Trial 2. The residue of heptachlor and metabolites zero days after exposure was approximately the same for the group receiving the basal diet, and the group receiving the basal diet supplemented with choline chloride and inositol (Table 2). This indicates that any possible effect of the lipotropic agents, choline chloride, and inositol has not yet begun. The group receiving the diet with 10% added vegetable oil without choline chloride and

inositol supplementation contained 9.78 p.p.m. heptachlor and metabolites; whereas, the group receiving the same diet with choline chloride and inositol supplementation contained significantly less residue (6.91 p.p.m.).

TABLE 1

Effect of dietary treatment on liver heptachlor residue 10 days after exposure.[1]

Group	Dietary treatment[2]	No. of Birds	Heptachlor & metabolites[3] (p.p.m.)
1	Basal	20	2.24[b]
2	Basal + 2645 g. choline chloride + 2000 g. inositol per 1000 kg.	20	1.35[a]
3	Basal + 10% added vegetable oil	20	5.71[c]
4	Basal + 10% added vegetable oil + 2645 g. choline chloride + 2000 g. inositol per 1000 g.	20	2.06[b]

[1]Pre-experimental levels analyzed less than 0.2 p.p.m. for both feed and liver.

[2]Each diet contained 10 p.p.m. commercial grade heptachlor for first 14 days.

[3]Means not having common letter superscripts were significantly different at the 0.05 level of probability.

Muscle tissue residue of heptachlor and metabolites five days after initiation of the trial was the same for the group receiving the basal diet, and

the group receiving the basal diet supplemented with choline chloride and inositol. This is in agreement with the liver content of heptachlor and metabolites for these two groups. The groups receiving the diets with 10% vegetable oil contained significantly more residue than did the groups receiving the diets without the additional vegetable oil.

TABLE 2

Effect of dietary treatment on liver and muscle tissue heptachlor residue zero days after exposure.[1]

Group	Dietary treatment[2]	No. of Birds	Heptachlor & metabolites[3]	
			Liver p.p.m.	Muscle p.p.m.
1	Basal	20	6.52[a]	3.06[a]
2	Basal + 2645 g. choline chloride + 2000 g. inositol per 1000 kg.	20	6.72[ab]	3.07[a]
3	Basal + 10% added vegetable oil	20	9.78[c]	5.33[c]
4	Basal + 10% added vegetable oil + 2645 g. choline chloride + 2000 g. inositol per 1000 kg.	20	6.91[b]	4.25[b]

[1]Pre-experimental levels analyzed less than 0.2 p.p.m. for feed, liver, and muscle tissue.

[2]Each diet contained 100 p.p.m. commercial grade heptachlor for the first five days.

[3]Means not having common letter superscripts were significantly different at 0.05 level of probability.

The group receiving the diet with 10% added vegetable oil supplemented with choline chloride and inositol contained significantly less residue than

did the group receiving the same diet without supplementation.

Only one chromatographic peak emerged upon analysis of all muscle tissue samples; whereas, three chromatographic peaks emerged upon analysis of all liver samples. This indicates a mobilization of the heptachlor from muscle tissue with subsequent metabolism in the liver. After metabolism occurs, there appears to be elimination of the residue and not deposition in body tissue since only heptachlor and no metabolites were found in muscle tissue.

Table 3 shows the effect of dietary treatment on liver, muscle tissue, and adipose tissue heptachlor residue 63 days after exposure. Supplementation of the basal diet with choline chloride and inositol did not significantly reduce the liver heptachlor residue. However, supplementation to the basal diet containing 10% added vegetable oil did significantly reduce the liver heptachlor residue.

Supplementation of the diets, with or without the 10% vegetable oil, resulted in a significant decrease in muscle tissue heptachlor residue. The muscle tissue of the group receiving the basal diet contained 1.89 p.p.m. heptachlor and metabolites; whereas, the muscle tissue of the group receiving the same diet supplemented with choline chloride and inositol contained only 1.28 p.p.m. Supplementation of the diet containing 10% added vegetable oil with choline chloride and inositol resulted in a significant decrease in muscle heptachlor residue (2.30 p.p.m. vs. 1.55 p.p.m.).

Dietary treatment had a significant effect on heptachlor residue in adipose tissue 63 days after exposure. The group receiving the basal diet supplemented with choline chloride contained 17.80 p.p.m. heptachlor residue; whereas, the group receiving the basal diet contained 19.08 p.p.m. The adipose tissue of the group receiving the diet with 10% vegetable oil supplemented with choline chloride and inositol contained 23.28 p.p.m. heptachlor residue; whereas, the group receiving the same diet without choline chloride and inositol contained

25.77 p.p.m. Donaldson et al. (2) observed that dilution of DDT residues in adipose tissue by the deposition of new tissue exerts a major influence on the residue in the tissue. These data appear to be in agreement with the results obtained by these workers.

TABLE 3

Trial 2: Effect of dietary treatment on liver, muscle tissue, and adipose tissue heptachlor residue 63 days after exposure.[1]

Group	Dietary treatment[2]	No. of Birds	Heptachlor & Metabolites[3] Liver	Muscle tissue	Adipose tissue
			p.p.m.	p.p.m.	p.p.m.
1	Basal	20	4.43[a]	1.89[c]	19.08[b]
2	Basal + 2645 g. choline chloride + 2000 g. inositol per 1000 g.	20	4.53[a]	1.28[a]	17.80[a]
3	Basal + 10% added vegetable oil	20	5.88[c]	2.30[d]	25.77[d]
4	Basal + 10% added vegetable oil + 2645 g. choline chloride + 2000 g. inositol per 1000 kg.	20	4.47[a]	1.55[b]	23.28[c]

[1]Pre-experimental levels analyzed less than 0.2 p.p.m. for feed, liver, muscle tissue, and adipose tissue.

[2]Each diet contained 100 p.p.m. commercial grade heptachlor for the first five days.

[3]Means not having common letter superscripts were significantly different at 0.05 level of probability.

Summary

Two trials were conducted utilizing a total of 160 one-week-old unsexed Japanese quail (Coturnix c. japonica) to determine the effect of choline chloride and inositol supplementation on the depletion rate of heptachlor. Supplementation of the diet with a combination of choline chloride and inositol was effective in reducing the heptachlor residue in the tissue samples studied. The metabolism of heptachlor appears to occur in the liver with subsequent elimination of the residue and not deposition in body tissue. The addition of 10% vegetable oil to the basal diet caused significant increase in the heptachlor residue in the tissue samples analyzed.

References

1. COUCH, J.R., Feedstuffs, 46 (November, 1956).

2. DONALDSON, W. E., T. J. SHEETS and M. D. JACKSON, Starvation effects on DDT residues in chick tissues, Poultry sci. 47, 237-243 (1968).

3. DUNCAN, D. B., Multiple range and multiple F tests, Biometrics 11, 1-42 (1955).

4. SMITH, S. I., C. W. WEBER and B. L. REED, Dietary pesticides and contamination of yolks and abdominal fat of laying hens, Poultry Sci. 49, 233-237 (1970).

5. SNEDECOR, G. W., Statistical methods, The Iowa State College Press, Ames, Iowa (1957).

6. STEMP, A. R., B. J. LISKA, B. E. LANGLOIS, and W. J. STADELMAN, Analysis of egg yolk and poultry tissues for chlorinated insecticide residues, Poultry Sci. 43, 273-275 (1964).

7. WESLEY, R. L., A. R. STEMP, B. J. LISKA, and W. J. STADELMAN, Depletion of DDT from commercial Layers, Poultry Sci. 45, 321-324 (1966).

8. WESLEY, R. L., A. R. STEMP, R. B. HARRINGTON, B. J. LISKA, R. L. ADAMS, and W. J. STADELMAN, Further studies on depletion of DDT residues from laying hens, Poultry Sci. 48, 1269-1275 (1969).