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Dieldrin-^{14}C Elimination From Turkeys

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Abstract. A series of experiments were conducted to find ways of removing dieldrin[2] residues from tissues of broiler-type turkeys *(Meleagris gallopavo)*. The turkeys were contaminated with dieldrin and dieldrin-^{14}C by oral dosing. Elimination was measured by assaying for ^{14}C in the droppings. Carcass retention was measured by assaying the tissue for ^{14}C, and in one experiment, dieldrin residues were measured by electron capture gas-liquid chromatography. Charcoal, Colestipol, and cholestyramine at dosages approximately equal to an intake of 5% of the diet were ineffective gastrointestinal adsorbants for removing dieldrin residues from the turkeys. Starvation accelerated the elimination of dieldrin from the turkeys, but only if the body lipids were reduced to approximately 10% or less of the carcass dry matter.

Dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-*endo-exo*-5,8-dimethanonaphthalene, HEOD) residues above tolerances established by the United States Food and Drug Administration as acceptable for human consumption were discovered in body fat of turkeys in two flocks in North Dakota in 1974. Some turkeys from one of these flocks were purchased by North Dakota State University (NDSU) and used to investigate the effectiveness of phenobarbital, a hepatic microsomal enzyme inducer, for causing the birds to eliminate the dieldrin. Phenobarbital effectively increases the rate of elimination of dieldrin from body stores of rats (Engebretson and Davison 1971), cattle (Wilson and Cook 1970) and pigs (Dobson and Baugh 1976), but it did not affect the rate of elimination of dieldrin from these contaminated turkeys nor did sodium barbital affect the rate of elimination of dieldrin from turkeys in a subsequent experiment conducted at NDSU (Sell *et al.* 1977).

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[2] Reference to company name or product does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

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We report herein the results of balance experiments conducted to test other ways of accelerating the elimination of dieldrin residues from body stores of turkeys given dieldrin and dieldrin-^{14}C.

Methods

Four balance experiments were conducted with broiler-type male turkeys. The turkeys were housed in individual 65- × 70- × 50- to 75-cm high wire cages provided with 44- × 44-mm mesh floors made of 5-mm diameter stainless steel wire. Droppings collected on trays suspended beneath the floors were removed twice weekly. Water was always available. The diet was composed of corn and a 40% protein commercial supplement.

At completion of the experiments, the turkeys were exsanguinated and plucked, and the blood and feathers were discarded. In specified experiments, the gall bladders and gastrointestinal (GI) tracts were removed. The carcass remains and GI tracts were separately ground, mixed, and sampled, and the samples were lyophilized. Gall bladders were lyophilized intact. Lyophilized samples were combusted in triplicate with a Packard Model 306 Oxidizer[2] and assayed for radioactivity by conventional liquid scintillation techniques. Lipids in lyophilized carcasses were determined by Soxhlet extraction, with petroleum ether, 30 to 60°C B.P., as the solvent.

The data were analyzed by analysis of variance techniques. When differences were significant, differences between individual means were further examined by least significant difference. Differences were considered significant at $P < 0.10$ for all statistical techniques.

Test Chemicals

Technical dieldrin was used. This material contained 87% of the active ingredient, HEOD. All dosages were corrected to 100% of the active ingredient; hereafter, when the word dieldrin is used, it refers to the amount of HEOD. Dieldrin-^{14}C, specific activity 64 mCi/mmol, contained no impurities detectable by thin-layer and gas-liquid chromatography.

Colestipol (diethylenetriamine-epichlorohydrin copolymer HCl), an anion exchange resin used medically for binding bile acids, was used as supplied by the Upjohn Company, Kalamazoo, MI.

Cholestyramine (polystyrene trimethylbenzylammonium Cl^-) was used as purchased from Mead-Johnson Research Center, Evansville, IN.

Charcoal, Darco S-51, was obtained from a local sugar refinery.

Experiment 1

Twelve 16-week-old turkeys, purchased from a commercial turkey grower, were given 2.08 μCi of dieldrin-^{14}C and 67 μg of dieldrin in gelatin capsules daily for 7 days. This dieldrin dosage was approximately equal to 0.3 ppm of the feed. The turkeys were randomly assigned, with the aid of a table of random digits, to three groups of four birds each on the 8th day:

Group 1 turkeys (control group 1) were killed immediately to determine the amount of radioactivity accumulated in their bodies.

Group 2 turkeys (control group 2) were fed *ad libitum* for 10 days of depletion.

Group 3 turkeys were starved for 10 days.

At the end of depletion, the turkeys were killed. The carcasses of all turkeys, with no parts removed, were ground and assayed for carbon-14.

Experiment 2

Twelve 14-week-old turkeys, raised at NDSU, were given 2.28 μCi of dieldrin-^{14}C and 60 μg of dieldrin in gelatin capsules daily for 7 days. They were randomly assigned to four groups of three birds each on the 8th day:

Group 1 turkeys (control) were fed *ad libitum* for 10 days of depletion.

Group 2 turkeys were starved for 10 days.

Group 3 turkeys were fed *ad libitum* and given 15 g of Colestipol in gelatin capsules daily for 10 days.

Group 4 turkeys were starved and given 15 g of Colestipol in gelatin capsules daily for 10 days.

At the end of depletion, the turkeys were killed. The gall bladders and contents and GI tracts were removed from the carcasses and assayed for carbon-14 separately.

Experiment 3

Twelve 18-week-old turkeys were given 2.24 μCi of dieldrin-^{14}C and 60 μg of dieldrin daily for 7 days. They were randomly assigned to four groups of three birds each on the 8th day:

Group 1 turkeys (control) were fed *ad libitum* for 10 days of depletion.

Group 2 turkeys (control) were fed *ad libitum* for 21 days of depletion.

Group 3 turkeys were given cholestyramine at 5% of the diet fed *ad libitum* for 10 days.

Group 4 turkeys were given cholestyramine at 5% of the diet fed *ad libitum* for 21 days.

Procedures at the end of depletion were the same as those of Experiment 2.

Experiment 4

Twelve 26-week-old turkeys were given 2.38 μCi of dieldrin-^{14}C in capsules daily for 7 days and were fed dieldrin at 0.3 ppm of their diet during the same 7 days. Feed consumption of individual birds ranged from 185 to 330 g/day and averaged 215 g/day for the 12 birds. They were randomly assigned to four groups of three turkeys each on the 8th day:

Group 1 turkeys (control) were fed *ad libitum* for 14 days of depletion.

Group 2 turkeys were starved for 14 days.

Group 3 turkeys were fed charcoal at 5% of the diet fed *ad libitum* for 14 days.

Group 4 turkeys were fed cholestyramine at 5% of the diet fed *ad libitum* for 14 days.

The turkeys were killed after 14 days of depletion. Bile was withdrawn from the gall bladders with a syringe, and the GI tracts were removed before the carcasses were ground. Other procedures at the end of depletion were the same as those of Experiment 2.

Results

Experiment 1

Data from Experiment 1 are tabulated in Table 1. About 49% of the radioactivity remained in the control turkey carcasses at the end of the 7-day dosing period and 35% of the radioactivity was eliminated in the droppings. The mean value for the amount of radioactivity eliminated in droppings during the 8th to 17th days for starved birds was not significantly higher than that for control group 2 birds. Carcasses of starved birds contained significantly less radioactivity and significantly less lipids than carcasses of control group 2 birds.

One control group 2 turkey quit eating during the depletion period and lost 1.7 kg in weight. Gross lesions were not observed when this bird was killed. The loss

Table 1. Influence of starvation on elimination of carbon-14 by turkeys given dieldrin-^{14}C experiment 1[a]

Treatment	Initial weight (kg)	Final weight (kg)	Recovery of carbon-14, % of dose in				Carcass lipids (%)[b]	Carcass dry weight (kg)
			0–7 day droppings	8–17 day droppings	Carcass	Total		
Control group 1 (accumulation)	5.4 ± 0.4	5.4 ± 0.6	35.0 ± 1.3	—	48.8 ± 2.4	83.8 ± 3.1	15.0 ± 1.4	1.48 ± 0.20
Control group 2 (depletion)	5.4 ± 0.2	5.1 ± 0.6	34.6 ± 1.6	12.6 ± 2.5	37.4 ± 3.2[c]	83.1 ± 2.4	16.3 ± 3.4	1.51 ± 0.21
Starved 10 days	5.6 ± 0.2	4.7 ± 0.5	33.5 ± 1.4	14.6 ± 1.3	29.9 ± 4.7[d]	81.1 ± 2.9	5.1 ± 1.1[e]	1.12 ± 0.13

[a] Data are means ± standard error of the means for four turkeys per group
[b] Percentage of the carcass dry matter
[c] Differed from mean of control group 1, $p < 0.05$
[d] Differed from mean of control group 2, $P < 0.10$
[e] Differed from means of either control group, $P < 0.01$

of appetite was attributed to nervousness and the handling necessary in doing the experiment. This bird eliminated 20% of the radioactivity in droppings during this period, and its carcass contained only 30% of the radioactivity administered and 3.4% lipid. All turkeys used in this experiment remained nervous during the experiment, so it was decided that turkeys for subsequent experiments would be raised in confinement at NDSU. The total recovery of radioactivity, averaging 83%, was consistently low; however, we know of no experimental deficiencies that would explain this low recovery.

Experiment 2

Results from Experiment 2 are shown in Table 2. The control turkeys excreted about 21% of the administered radioactivity during the 7-day dosing period and about 13% during the 10-day depletion period. When control birds were killed, about 6% of the radioactivity remained in the GI tracts and about 50% remained in the carcasses. Turkeys that were starved excreted more radioactivity in their 8- to 17-day droppings and contained more radioactivity in their gall bladders than any other group of turkeys. Turkeys that were starved or that were given Colestipol and starved contained less radioactivity in their GI tracts and less carcass lipids than the control turkeys or the turkeys given Colestipol but not starved. Results for the group given Colestipol but not starved did not differ significantly from those for the controls in any parameter measured.

The turkeys of Experiment 2 contained more carcass lipids and retained more radioactivity than the turkeys of Experiment 1.

Experiment 3

Data from Experiment 3 are shown in Table 3. One turkey in the 21-day control group was removed from the experiment because of a broken leg.

The 10-day control turkeys excreted about 21% of the radioactivity in their droppings during the 7-day dosing period and about 9% during the 10-day depletion period. They retained about 5% of the radioactivity in their GI tracts and 62% in their carcasses at slaughter. The 21-day controls eliminated an additional 5.4% of the radioactivity in their droppings during the added 11-day depletion period.

The addition of cholestyramine to the diet did not affect the amount of radioactivity eliminated in the droppings during the 8- to 17-day period, but it did increase ($P < 0.05$) the amount eliminated during the 18- to 28-day period; however, the increased amount of radioactivity eliminated in the droppings was not large enough to reduce significantly the amount of radioactivity remaining in the carcasses of these turkeys when compared with that in the 21-day controls.

The amount of radioactivity remaining in carcasses of the 21-day control turkeys and the turkeys given cholestyramine for 21 days was lower than the amount remaining in the carcasses of the other two groups of turkeys; but the differences among treatment groups were not great enough to be significant.

Table 2. Influence of starvation and Colestipol on elimination of carbon-14 by turkeys given dieldrin-^{14}C—Experiment 2[a]

Treatment	Initial weight (kg)	Final weight (kg)	Recovery of carbon-14, % of dose in						Carcass lipids (%)[c]	Carcass dry weight (kg)
			0–7 day droppings	8–17 day droppings	Gall bladder	GI tract[b]	Carcass	Total		
Control	6.4 ± 0.5	7.0 ± 0.5	20.8 ± 0.9	13.2 ± 1.3	0.03 ± 0.003	5.7 ± 0.2	50.4 ± 2.1	90.1 ± 0.4	23.8 ± 0.9	2.27 ± 0.20
Starved 10 days	6.3 ± 0.1	5.0 ± 0.1	21.0 ± 0.6	18.3 ± 1.6[d]	0.30 ± 0.05[e]	2.6 ± 0.1[f]	51.3 ± 2.3	93.5 ± 0.2	11.7 ± 1.3[e]	1.29 ± 0.03
15 g Colestipol, 10 days	6.0 ± 0.1	6.4 ± 0.1	20.6 ± 0.8	15.3 ± 1.2	0.07 ± 0.01	5.1 ± 1.1	52.8 ± 1.9	93.9 ± 1.5	22.2 ± 1.2	1.74 ± 0.08
15 g Colestipol and Starved 10 days	6.1 ± 0.4	4.9 ± 0.3	18.5 ± 0.7	13.9 ± 0.7	0.07 ± 0.02	3.1 ± 0.5[f]	56.1 ± 2.7	91.6 ± 1.8	14.1 ± 1.9[e]	1.25 ± 0.07

[a] Data are means ± standard error of the means for three turkeys per group

[b] GI = gastrointestinal tract; includes tissue and ingesta

[c] Percentage of carcass dry matter

[d] Different from the control group or the group that was given Colestipol and starved, $P < 0.05$, but not from the group given Colestipol alone

[e,f] Differed from unmarked means in the column; e = $P < 0.001$; f = $P < 0.05$

Table 3. Influence of cholestyramine on elimination of carbon-14 by turkeys given dieldrin-^{14}C—Experiment 3[a]

Treatment	N	Initial weight (kg)	Final weight (kg)	Recovery of carbon-14, % of dose in						Carcass lipids (%)[c]	Carcass dry weight (kg)
				0–7 day droppings	8–17 day droppings	18–28 day droppings	GI tract[b]	Carcass	Total		
Control, 10 days	3	7.6 ± 0.3	8.3 ± 0.3	20.7 ± 0.8	9.1 ± 1.0	—	5.0 ± 1.0	62.3 ± 2.7	97.0 ± 1.0	27.3 ± 4.9	2.49 ± 0.15
Control, 21 days	2	7.6 ± 0.4	9.5 ± 0.3	18.6 ± 1.5	8.6 ± 1.8	5.4 ± 1.0	6.6 ± 0.6	56.9 ± 3.3	97.4 ± 1.0	30.7 ± 3.4	2.62 ± 0.15
5% Cholestyramine, 10 days	3	7.8 ± 0.4	8.5 ± 0.5	20.1 ± 0.8	10.2 ± 1.6	—	6.4 ± 0.9	61.2 ± 2.0	97.8 ± 2.0	27.0 ± 1.0	2.58 ± 0.15
5% Cholestyramine, 21 days	3	8.3 ± 0.1	9.6 ± 0.03	17.7 ± 0.4	9.4 ± 0.5	8.1 ± 0.3[d]	5.2 ± 0.6	54.9 ± 0.9	95.2 ± 0.6	33.9 ± 4.3	2.80 ± 0.10

[a] Data are means ± standard error of the means
[b] GI = gastrointestinal tract; includes tissue and ingesta
[c] Percentage of the carcass dry matter
[d] Differs from the 21-day control mean, $p < 0.05$

Experiment 4

Results of Experiment 4 are shown in Table 4. One turkey in the starved group was removed from the experiment because of a swollen hock and inability to walk.

The control birds eliminated about 12% of the administered carbon-14 in their droppings during the 7-day dosing period and 10% in their droppings during the 14-day depletion period. They retained about 4% of the radioactivity in their GI tracts and 66% of the radioactivity in their carcasses. Within the sensitivity of this experiment, 14 days of starvation did not significantly reduce the amount of carcass lipids in these mature turkeys, nor did it enhance elimination of carbon-14 when compared to any other treatment. However, the bile from starved turkeys contained more radioactivity than the bile from other turkeys.

Charcoal or cholestyramine fed at 5% of the diet for 14 days did not significantly alter the rate of elimination of carbon-14 from these turkeys. Feed consumption during the 8- to 21-day period averaged 325 ± 10, 273 ± 27, and 200 ± 19 g (means and standard error of the means) for the control, charcoal-fed, and cholestyramine-fed turkeys, respectively. Obviously, both charcoal and cholestyramine reduced feed consumption.

Discussion

Use of charcoal, Colestipol, or cholestyramine as gastrointestinal adsorbants to remove dieldrin from the bodies of turkeys shows little potential. Cholestyramine did increase the amount of radioactivity eliminated in the droppings during the 10- to 21-day depletion period of Experiment 3, but it did not increase elimination *via* droppings measurably during the first 10-day depletion period of that experiment or during the 14-day depletion period of Experiment 4. Charcoal and Colestipol never increased the amount of radioactivity eliminated *via* droppings measurably, and charcoal, Colestipol, or cholestyramine never decreased the amount of carbon-14 remaining in the turkey carcasses.

Starvation increased the rate of elimination of radioactivity and lowered body residues in some instances, but the benefits of starvation were inconsistent. It appeared that the success of starvation for reducing the amount of radioactivity in the body was related to the amount of lipids remaining in the turkey carcasses. Therefore, the relationships of percentage of radioactivity eliminated in the droppings and percentage of radioactive dosage remaining in the carcasses to percentage of carcass lipids were examined by regression analysis (Figure 1).

Radioactivity elimination in droppings for Experiments 1 and 2 decreased significantly as carcass lipids increased. Radioactivity remaining in carcasses for Experiment 1 increased significantly with carcass lipids. In fact, asymptotic curves best fit the data obtained from Experiment 1. According to the curves, when carcass lipids were reduced to about 7% and below, as in Experiment 1, the amount of radioactivity eliminated in droppings increased markedly and the amount of radioactivity remaining in the carcasses decreased markedly.

In Experiment 2, carcass lipids ranged from approximately 10 to 25%. Although the amount of radioactivity eliminated in droppings increased, accord-

Table 4. Influence of starvation, cholestyramine, and charcoal on elimination of carbon-14 by turkeys given dieldrin-^{14}C—Experiment 4[a]

Treatment	N	Initial weight (kg)	Final weight (kg)	Recovery of carbon-14, % of dose in						Carcass lipids (%)[c]	Carcass dry weight (kg)
				0–7 day droppings	8–21 day droppings	Bile	G.I. tract[b]	Carcass	Total		
Control	3	10.1 ± 0.7	11.6 ± 0.7	12.5 ± 0.8	10.1 ± 2.1	0.02 ± 0.003	4.4 ± 0.3	66.1 ± 1.6	93.2 ± 2.2	29.6 ± 2.8	3.53 ± 0.26
Starved, 14 days	2	10.5 ± 0.6	8.8 ± 0.4	11.2 ± 0.4	9.8 ± 2.3	0.29 ± 0[d]	4.3 ± 1.7	65.6 ± 1.9	88.9 ± 0.5	23.2 ± 5.4	2.67 ± 0.10
5% Charcoal, 14 days	3	10.9 ± 0.6	10.6 ± 0.6	11.5 ± 1.5	14.2 ± 0.6	0.01 ± 0.003	3.1 ± 0.6	64.7 ± 3.0	93.5 ± 2.1	23.8 ± 1.5	3.18 ± 0.16
5% Cholestyramine, 14 days	3	10.6 ± 0.8	10.7 ± 0.9	10.6 ± 0.7	12.1 ± 1.4	0.01 ± 0.001	3.6 ± 0.2	66.9 ± 2.6	93.6 ± 1.4	25.8 ± 4.9	3.24 ± 0.32

[a] Data are means ± standard error of the means
[b] GI = gastrointestinal tract; includes tissue and ingesta
[c] Percentage of the carcass dry matter
[d] Differs from all other means in this column, $P < 0.001$

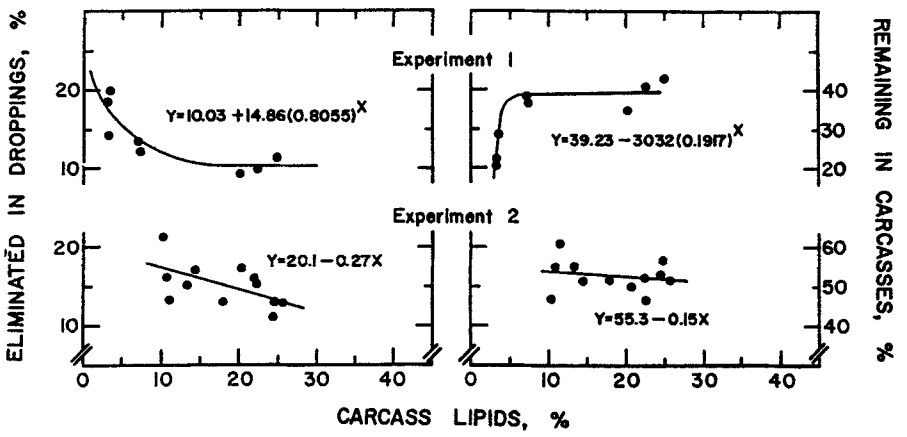

Fig. 1. Relationship of carcass lipids to elimination of carbon-14 in droppings and to the amount of carbon-14 remaining in the carcasses of dieldrin-^{14}C-contaminated turkeys. Regressions were significant ($P < 0.05$) for radioactivity eliminated in droppings *vs.* carcass lipids for Experiments 1 and 2 and for the radioactivity remaining in the carcass *vs.* carcass lipids for Experiment 1. Regression for the radioactivity remaining in the carcasses *vs.* carcass lipids for Experiment 2 was not significant

ing to the regression, with decreasing carcass lipids, the amount of radioactivity eliminated by these turkeys was not sufficient to cause a detectable decrease in the amount of radioactivity remaining in their carcasses. The turkeys in Experiment 3 were never starved, and their carcass lipids were all greater than 20%. Even the starved turkeys of Experiment 4 contained carcass lipids above 17% at the end of the trial. In neither Experiment 3 nor Experiment 4 were regressions significant for radioactivity eliminated in droppings vs. body lipids or for radioactivity retained in carcasses vs. body lipids.

The amount of dieldrin that remained in the carcasses of turkeys in Experiment 3 was measured by electron capture gas-liquid chromatography (Davison 1970) to verify the assumption that most of the carbon-14 residues remaining in the carcasses were present as dieldrin. The recovery of dieldrin in the carcasses of these 12 turkeys by gas-liquid chromatography was 68 ± 3.2% (mean and standard error of the mean). The recovery of dieldrin-^{14}C in these same carcasses, based on combustion and carbon-14 assay, was 60 ± 1.7%. Thus, the two methods yielded similar results, and it seemed reasonable to assume that most of the radioactivity remaining in the carcasses was present in dieldrin rather than in dieldrin metabolites. Based on previous experiences with dieldrin metabolism in sheep (Davison 1970, Hedde *et al.* 1970, Feil *et al.* 1970), we expected that radioactive measurements would be a good indicator of the amount of dieldrin residue in the turkey carcasses. In our laboratory, the radioactive measurements are more rapid and precise than the gas-chromatographic measurements.

We concluded from these experiments that starvation might be an effective means for removing dieldrin residues from contaminated turkeys; but the extent of starvation must be severe enough to reduce body lipids below 10% of the carcass dry matter. With this information, a practical-type experiment was conducted at NDSU with larger numbers of turkeys contaminated with dieldrin.

These turkeys were decontaminated successfully by intermittent periods of starvation and feeding (Sell *et al.* 1977).

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