FEEDING DDT AND ALFALFA SPRAYED WITH DDT TO CALVES[1]

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Extensive use has been made of DDT in spraying forage crops, but no data have been available heretofore on the effects of feeding the sprayed crops to growing calves. Also, no information has been available on the amount of DDT stored in the body tissues of calves when they are fed DDT either as residue or the pure compound. Other investigators (2, 4, 9) have published limited data on the storage of DDT in the body tissues of sheep, cows and pigs that were fed this compound. This experiment was undertaken in order to obtain similar information with young dairy calves.

METHODS

On July 29, 1947, a field of alfalfa was sprayed with 0.6 lb. of technical DDT per acre. Representative portions of the field were cut on August 6, August 18 and September 3. Immediately after the hay was cut, it was mow-dried in the barn. The difference in cutting interval provided hays having varying concentrations of DDT residues and gave information on the amount of weathering of the residue on the standing crop. The alfalfa was baled after mow-drying, then stored and, as the calves were placed on the experiment, it was fed to them until they were 8 mo. of age.

All calves used were Jersey males. When 10 days of age, they were fed skimmilk, a carotene concentrate, a grain mixture and the assigned roughage. The amount of alfalfa consumed varied with each calf's appetite up to 3 or 4 mo. of age, after which time alfalfa consumption was limited to 1.5 lb. per 100 lb. of body weight. Skimmilk gradually was withdrawn from the diet beginning at 3 mo. of age, and after 4 mo. no milk was fed. After the calves were 3 mo. old, the total digestible nutrient intake was limited to Morrison's requirement.

The hay that was selected for use as a control was obtained on the market and fed to the calves in group 4 (see table 1). A preliminary sample did not contain DDT. However, on 12 out of 15 monthly composite samples, the analysis of this hay showed 1.4 to 21.3 ppm. of DDT present. Consequently, the calves in group 6, which were fed dried beet pulp and grain and no DDT, were used for control animals.

All calves were slaughtered at 8 mo. of age, except no. 2, which was slaughtered at 6 mo. of age. Various internal organs and endocrine glands were weighed at slaughter. Samples of tissue from liver, kidney, heart, intestine, brain, spinal cord, muscle, lung, lymph nodes, spleen, abomasum and pituitary

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gland were taken for histological study. Samples of kidney fat, body and mesenteric fat, liver and rib and loin meat were taken for DDT analysis. The kidney- and body-fat samples were analysed separately except for nos. 14 and 15, which were combined into one sample. The samples of rib and loin meat were taken to approximate the edible portion in the region of the 12th and 13th rib and first and second lumbar vertebrae.

At intervals during the experiment, hemoglobin, vitamin A, carotene, calcium and phosphorus concentrations were determined on blood samples of each calf. Body weight gains and general appearance also were noted.

After this experiment was completed, two more calves were fed larger amounts of DDT in oil solution. These calves were raised to 80 days of age on a normal ration. From the 80th to the 256th day (when slaughtered) they were fed timothy hay and a grain mixture composed of 97 per cent corn, 2 per cent bonemeal, 1 per cent salt and 10 ml. of cod-liver oil daily. In addition, they were given DDT dissolved in corn oil at the rate of 100 mg. DDT per kg. of dry matter consumed. This was given once daily in a capsule. The ration fed these two calves was very low in protein. This type of diet has been shown to enhance the toxic effects of DDT in mice (7).

A sample of each of the hays fed was taken five times each week and the samples were combined by monthly periods. DDT analyses were made on these monthly composite samples by the total organic chlorine method (1). Occasionally, the Schechter-Haller colorimetric method was used as a specific check for DDT (8).

The preliminary treatment accorded the various calf meat and fat samples analysed for DDT deposits was as follows: The liver samples were ground and thoroughly mixed and the rib and loin cuts were boned out and thoroughly ground. In each case, a 50-g. sample was taken and mixed well to dryness with anhydrous Na_2SO_4. The dried samples then were placed in a Waring Blendor and thoroughly extracted four times with redistilled chloroform, each time decanting off the chloroform. The combined extract then was filtered through a 24-cm. folded filter paper before analysis.

Preliminary studies on body- and kidney-fat samples showed that when solid fat was used, the amount of liquid fat that could be rendered from the fat sample varied considerably, due to the varying amounts of connective tissue and water present. To put all the samples of fat on a comparable basis, they were rendered out and dried first and then 5-g. samples of pure fat were weighed out on an analytical balance.

All samples of meat and fat were analysed as outlined by Schechter *et al.* (8).

RESULTS

The results are given in table 1. As indicated by the monthly composite samples, the alfalfa cut 8 days after spraying averaged 39 ppm. of DDT when fed to the calves. The alfalfa cut 20 days after spraying averaged 11 ppm. and that cut 36 days after spraying averaged 9 ppm. The average DDT content of samples of the alfalfa bought on the market averaged 5.7 ppm.

TABLE 1

Showing DDT intake of dairy calves and the concentration of DDT in the body tissues

Calf no.	DDT intake			Average daily gain in weight	Concentration of DDT in ppm			
	Total intake	Ppm. of dry matter consumed	Daily intake as mg./kg. body wt.		Body fat	Kidney fat	Rib and loin meat	Liver
	(*g.*)			(*lb.*)				
	Group 1. Fed alfalfa cut 8 d. after spraying							
1	10.2	22.1	0.64	1.15	100.0	104.0	1.7	0.2
2	6.5	21.7	0.58	1.17	80.0	83.2	1.2	0.2
3	8.9	16.8	0.48	1.06	84.8	87.3	1.7	1.1
4	5.3	10.8	0.30	1.19	71.8	75.0	0.6	0.2
	7.7	17.8	0.50	1.14	84.2	87.4	1.3	0.4
	± 2.2	± 5.3	± 0.15	± 0.06	± 11.8	± 12.2	± 0.5	± 0.4
	Group 2. Fed alfalfa cut 20 d. after spraying							
5	3.7	6.8	0.20	1.26	8.1	10.0	0.0	0.0
6	2.6	4.8	0.10	1.24	23.0	23.5	0.2	0.0
	3.2	5.8	0.15	1.25	15.6	16.8	0.1	0.0
	± 0.84	± 1.4	± 0.07	± 0.01	± 10.5	± 9.5	± 0.1	
	Group 3. Fed alfalfa cut 36 d. after spraying							
7	2.7	5.3	0.16	1.14	9.3	10.2	0.0	0.0
8	1.5	3.2	0.08	1.03	4.4	6.1	0.0	0.0
9	2.2	4.3	0.12	1.29	3.4	4.2	0.0	0.0
10	2.1	4.1	0.12	1.25	4.2	7.0	0.6	0.0
	2.1	4.2	0.12	1.18	5.3	6.9	0.2	0.0
	± 0.5	± 0.9	± 0.03	± 0.12	± 2.7	± 2.5	± 0.3	
	Group 4. Fed alfalfa bought on the market (Commercial alfalfa)							
11	1.2	2.2	0.07	1.14	2.0	3.0	0.0	0.0
12	1.2	2.6	0.09	1.00	22.0	22.9	0.4	0.0
13	1.5	2.8	0.08	1.38	5.3	7.9	0.0	0.0
	1.3	2.5	0.08	1.17	9.8	11.3	0.1	0.0
	± 0.2	± 0.3	± 0.01	± 0.19	± 10.7	± 10.3	± 0.2	
	Group 5. Fed timothy hay and corn and DDT by capsule							
14	34.5	106.1	2.9	0.64		340.0	12.7	1.9
15	37.6	103.0	2.7	0.68		345.0	13.1	3.9
	36.1	104.6	2.8	0.66		342.5	12.9	2.9
	± 2.2	± 2.2	± 0.04	± 0.03		± 3.5	± 0.3	± 1.1
	Group 6. Fed dried beet pulp and grain.							
16	0.0	0.0	0.0	1.37	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	1.60	0.0	0.0	0.0	0.0
				1.49				
				± 0.16				

± values indicate standard deviation.

None of the calves showed any gross abnormal symptoms when consuming the alfalfa or the DDT dissolved in oil. The average daily gains of individual calves fed the various alfalfa diets ranged from 1.0 to 1.6 lb. The average daily body weight gains of the groups that were fed alfalfa hay were practically identical but slightly less than the two calves receiving beet pulp. However, by the "t" test at the 5 per cent level, group 1 (fed early cut alfalfa) gained less than group 6 (fed beet pulp). The body weight gains of the two calves in group 5 were low. This was to be expected when calves were fed a ration low in protein,

and cannot be attributed to the DDT intake. No indications of DDT toxicity were noted in these two calves.

Hemoglobin values for the calves as individuals or as groups were within the normal range. After 90 days of age, individual calves varied from 9.0 to 11.0 g. of hemoglobin per 100 ml. of blood, which is within the normal range found for calves in this herd. Plasma vitamin A and carotene values were normal and showed a normal increase as the calves increased in age. Plasma calcium and phosphorus values also were normal. All groups averaged between 9.3 and 11.1 mg. calcium per 100 ml. at the various ages, while plasma inorganic phosphorus averaged between 6.2 and 9.2 mg. per cent.

At slaughter time, group averages for ratios of body weight to liver, kidney, and thyroid weights showed no consistent or statistical differences. Sarett and Jandorf (6) stated that 0.07 per cent DDT in the diet of rats produced an increase in the relative size of the liver. The two calves in group 5 received only 0.01 per cent DDT in this ration, and their livers were the smallest of all individuals. They averaged 1.50 per cent of body weight, while those fed no DDT (group 6) averaged 1.78 per cent. The individual values for the calves fed the alfalfa diets varied from 1.60 to 1.99 per cent.

Liver tissues from all calves except nos. 4, 8 and 13 were examined histologically. At least two routine paraffin sections stained with hematoxylin and eosin and three frozen sections stained for fat were examined. None of the livers examined showed any degree of fatty change or any other histologic alterations that could definitely be attributed to DDT ingestion.[4] Liver cell alteration of the type reported to occur in rodents on low dosage levels of DDT was not seen in any of these animals. However, the characteristic liver cell damage caused by DDT in rodents (rats, rabbits, mice and guinea pigs) has not been found to occur in animals of a variety of other species that have been treated with this compound (5).

The intake of DDT and the storage of this compound in the fat, meat and liver are presented in table 1. The general tendency for the concentration of DDT in a specific body tissue to increase as DDT intake increased is evident.

The DDT intake, as ppm. or mg. per kg., of group 5 (fed DDT by capsule) was 5.7 times larger than that of group 1 (fed alfalfa cut 8 days after spraying), but the concentration in the fat was 4-fold greater. The concentrations found in liver and meat were seven and ten times greater, respectively. The DDT intake of group 1 was about three to seven times greater than those of groups 2, 3 and 4, while the concentrations found in the fat were about 5- to 15-fold greater.

Only an occasional trace of DDT was found in the meat samples of calves consuming 2.2 to 6.8 ppm. of DDT and none was detected in the liver of these animals, while the calves receiving 10 ppm. or more of DDT showed definite storage of DDT in meat and liver.

In all cases, the kidney fat contained a higher concentration of DDT than did fat taken from the mesentery or other portions of the body.

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DISCUSSION

The increased concentration of DDT found in the body fat of calves when intake was increased is in agreement with the results of Harris *et al.* (4) on sheep. However, at comparable intakes, the calves in this experiment had higher concentrations of DDT than Harris *et al.* found in sheep fed for 4 mo. The longer period during which the calves received DDT may be one explanation of this difference. Laug *et al.* (5) showed that the concentration of DDT in perirenal fat continued to increase for 23 wk. when rats were fed a diet containing 5 ppm. of DDT.

Because of the limited number of calves, this experiment does not give any definite information on the differences in DDT storage as affected by capsule or residue feeding, as noted by other investigators (3, 4).

In this investigation, only the liver tissue was examined histologically. From observations in studies with rats on low levels of DDT intake, this is the organ that is most likely to show changes due to DDT ingestion (5). However, in these calves fed DDT at 2.5 to 105 ppm., there were no detectable hepatic cell alterations similar to those that have been noted in rats when fed DDT at 5 ppm. or more (5).

The occurrence of DDT either as residue or as contamination in hay purchased on the market is worthy of note. Its presence was confirmed by the colorimetric method of analysis. Although the amount was small and variable, it was sufficient to allow definite storage of DDT in the fat of all calves fed the alfalfa purchased on the market. In contrast, the calves simultaneously fed only dried beet pulp and grain had no DDT in their body tissues. Other investigators have found DDT in the fat of control animals used in their experiments (5).

All meat and fat samples, except those of the two animals fed DDT by capsule, showed some off color when analysed by the colorimetric method, which is indicative of decomposition of the technical DDT. This probably was due to exposure to the elements in the field and to some further decomposition upon being metabolized in the plant or in the animal's body. The two primary decomposition products are 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene and bis-(*p*-chlorophenyl)acetic acid, both compounds giving a reddish cast to the characteristic blue color developed by the colorimetric procedure used.

Animals 14 and 15 were given capsules containing technical and some *p,p'*-DDT along with their feed. No off colors were evident in the analysis of the tissues of these animals due to the fact that the DDT was taken into the animal's body in capsule form, and, therefore, not subjected to partial decomposition while on the plants in the field.

SUMMARY

DDT in oil solution and alfalfa containing various amounts of DDT residue were fed to 15 dairy calves. The growth of these calves was not adversely affected by the amounts of DDT ingested. Hemoglobin, vitamin A, carotene, calcium and phosphorus concentrations in the blood also were entirely normal. No

histologic changes ascribed to DDT ingestion were found in the livers of these calves. DDT intake varied from 0.07 to 2.9 mg. per kg. of body weight or 2.2 to 106 ppm. of the dry matter consumed. The DDT feeding period ranged from 160 to 230 days in length. The storage of DDT in the body and kidney fat varied from 2.0 to 345 ppm. and the concentration in the fat was proportional to the DDT intake. When DDT intake exceeded 10 ppm. of the dry matter intake, DDT appeared in the meat of the calves.

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