Effects of Feeding Systox-Treated Alfalfa Hay to Dairy Cows

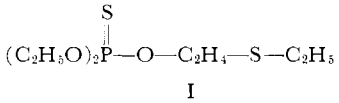
PAUL A. DAHM and NORMAN L. JACOBSON
Iowa State College, Ames, Iowa

Three experiments were conducted to determine whether demeton or demeton residue toxins fed to cows would appear in the milk or affect the health of the animals. When technical demeton was fed in capsules for 3 consecutive days at the rate of 0.1, 0.5, and 2.5 mg. per kg. of body weight, the cow exhibited severe symptoms of organic phosphate poisoning, milk production dropped sharply, and fat percentage increased. When Systox-treated hay containing an average residue of 51 p.p.m. of demeton toxins was fed to the same cow in increasing amounts for 49 days, mildly adverse effects on weight gains and red blood cell cholinesterase activity were noted. When an average of 41 p.p.m. of Systox-treated hay was fed to three cows for 56 days, no adverse effects on weight changes and milk production were observed, but red blood cell cholinesterase activity decreased gradually during the last 6 weeks of feeding.

THE INCREASING USE OF SYNTHETIC ORGANIC CHEMICALS for the control of insects on forage crops has led to considerable concern about the persistency of residues of these materials on and in the crops and the possible appearance of these residues or their metabolites in edible tissue and products, such as milk, of animals consuming these crops. The appearance of DDT and related chlorinated hydrocarbon insecticides in the milk of cows whose diets included small amounts of these materials has been established. This contamination of milk has been the basis for certain precautions in the use of DDT and related insecticides on forage crops that might be fed to lactating cows.

A more recently introduced group of insecticidal chemicals has been referred to as organic phosphates or organic thiophosphates. Hazleton (*3*) has reviewed the pharmacodynamics, toxicity, and hazards of these insecticides. Although varying widely in their chemical and physical properties, they all appear to inhibit cholinesterase. Some, such as demeton, act systemically, penetrate rapidly into many plants, and are translocated upward within these plants following application either as a spray to foliage or as a soil treatment to the root system. An in vivo conversion in plants results in the formation of metabolites with anticholinesterase activity several times greater than that of the technical demeton originally applied (*2, 5, 6*).

The systemic insecticide formulation known as Systox (registered trade-mark of Chemagro Corp., New York 17, N. Y.) is a mixture of an emulsifying agent and demeton, formulated to contain 21.2% by weight of a mixture of the thiono- and thiol-isomers of demeton. The insecticidally active isomers in demeton are *O,O*-diethyl, *O*-(2-ethylmercaptoethyl)-thionophosphate (I) and *O,O*-diethyl, *S*-(2-ethylmercaptoethyl)-thiolphosphate (II); they occur in a ratio of approximately 2(I) to 1(II).



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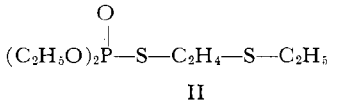


II

Although many data have been gathered on the dissipation of residues of organic phosphate insecticides from crops, less is known about the chemical nature and fate of these residues when fed to animals. In two related studies (*1, 8*) parathion was fed to dairy cows at levels greatly in excess of normal forage residues. No parathion was found in the milk, blood, or urine of these cows.

Because of the potential use of demeton as an insecticide on crops which are fed to dairy cows and other animals, it was considered important to determine whether demeton residue toxins fed to cows would appear in the milk, or would affect the health of the animals. To accomplish this, three experiments were conducted. In Experiment A, technical demeton was fed in capsules to a cow in increasing amounts for 3 consecutive days. In Experiment B, Systox-treated alfalfa hay containing an excessively high residue of demeton toxins was fed in increasing amounts over a period of 49 days to the same cow used in Experiment A. In Experiment C, the Systox-treated alfalfa hay was fed to three cows of different breeds at a uniform rate for 56 days. Data gathered during these experiments included demeton residue toxins in the hay, cow weights, daily milk production, milk fat, cholinesterase inhibition by the milk, and cholinesterase activity of the red blood cells.

Materials and Methods

Preparation of Hay A 2.58-acre field of first-growth alfalfa (var. Kansas Common) on the Iowa State College Agronomy farm was sprayed using a Systox formulation (containing 2 pounds of demeton per gallon) in 35 gallons of water in a Hagie, high clearance sprayer. Approximately 6.2 ounces of actual demeton were applied per acre. The alfalfa was mowed about 27 hours and baled about 75 hours after application of the insecticide. Only 0.09 inch of precipitation fell during the interval from spraying to baling. The total weight of the baled hay at the time of storage was 10,350 pounds (a yield of 2.01 tons of hay per acre).

The hay was prepared by the above procedure to provide an excessively heavy residue of demeton toxins in the hay for the feeding experiments. Good insect-control practices with Systox would normally require a longer interval between application of the insecticide and harvesting of the hay, with resulting lower amounts of residue toxins in the hay.

The percentage of moisture in the alfalfa was determined before and after insecticide application, after mowing, while the hay was drying in the field, and prior to baling. In each case a com-

posite alfalfa sample was obtained by gathering about 50 subsamples at random in the field. These subsamples were brought to the laboratory and mixed thoroughly; triplicate portions were selected for both moisture determinations and residue analyses. The average moisture content of the alfalfa in the field prior to application of the insecticide was about 72% and after mowing decreased to about 12% at the time of baling. Concurrent residue analyses for demeton toxins were made. The baled hay was stored in a barn near the stalls in which the experimental cows were located.

Experimental Animals and Feeding Procedures Six lactating dairy cows were employed in the three experiments. In Experiment A, Holstein cow 3607 received increasing amounts of technical demeton (Chemagro Corp., sample 1-54-42) by capsule at 9 A.M. for three consecutive days as follows: first day, 0.10; second day, 0.51; and third day, 2.5 mg. of demeton per kg. of cow body weight. The cow was weighed and milk and blood samples were taken on the 4 days prior to and several times a day during capsulate feeding. This cow was on a mixed grass and legume pasture of good quality, and, in addition, was fed 12 pounds of a concentrate mixture (15% crude protein) daily.

About a month after Experiment A was concluded and the effects of the demeton had diminished, cow 3607 was employed in Experiment B. Twelve pounds of the concentrate mixture and increasing quantities of Systox-treated hay (average residue 51 p.p.m. of demeton toxins) were fed for 49 days to yield the following average daily intake of demeton residue toxins: the first 15 days, 0.3; next 13 days, 0.6; and final 21 days, 1.0 mg. per kg. of cow body weight per day. A sampling period of 2 days preceded the feeding of Systox-treated hay. Pasture was allowed only when, and if, the daily allotment of hay had been consumed. At the end of this experiment, observations on cow 3607 were continued and milk and blood samples were taken at the same intervals as for animals in Experiment C.

In Experiment C, a Holstein (3718), a Guernsey (3480), and a Milking Shorthorn (3518) were fed Systox-treated hay (average residue 41 p.p.m. of demeton toxins) at a rate of about 1 pound per 100 pounds of body weight daily for 56 days. A sampling period of 11 days, during which normal values were established for all the cows, preceded the feeding of treated hay. Feeding treated hay at the above rate resulted in the following average daily intakes of demeton residue toxins during the 56-day period: cow 3718, 0.38; cow 3480, 0.29; and cow 3518, 0.34 mg. of demeton toxins per kg. of cow body weight per day. Two cows, a Holstein (3747) and a Guernsey (3674), were employed as controls and were fed untreated alfalfa hay free-choice. Each of the five cows received from 8 to 12 pounds of concentrate mixture, the amount depending upon the level of milk production. All cows except 3518 received approximately 36 pounds of corn silage daily. The silage allowance of 3518 was reduced to encourage hay consumption. A record was kept of daily milk production, and twice weekly the cows were weighed, milk samples were taken for determination of percentage fat and cholinesterase inhibition, and jugular blood samples were obtained for determination of cholinesterase activity in the red blood cells.

Cows in all experiments were milked routinely twice daily (at about 4:30 A.M. and P.M.). In Experiment A several milk samples were taken in addition to those obtained from the regular milkings. No cow was placed on experiment earlier than 2.5 months after parturition.

Sampling and Analytical Hay and milk samples were analyzed by slight modifications of the enzymatic inhibition method of Hensel and others (*4*).

Alfalfa. Field samples of sprayed alfalfa were prepared for analysis according to the procedure of Hensel and coworkers. The baled alfalfa required special processing. Each morning, as the sprayed hay was weighed for feeding, cross-sectional portions for analysis were taken from the bales. These portions were stored and at approximately weekly intervals were run through a hammer mill. From 5 to 10 pounds of the ground hay were obtained at the end of each sampling interval. Each sample was mixed well and aliquots were selected for analysis at Iowa State College and at Chemagro Corp. Moisture determinations also were made on all samples.

The following modifications of the procedure of Hensel and coworkers were used: A randomly selected 40-gram sample of the ground alfalfa was placed in a Waring Blendor and reduced to a powder. A Cenco Pinto blade assembly (No. 17248 L54) was most satisfactory for this dry alfalfa. After addition of 300 ml. of distilled water, the mixture was further macerated for about 10 minutes. The contents of the blendor were transferred to the center of a three-thickness fold of cheesecloth and the aqueous extract was squeezed into a beaker. Approximately 60% of the water added was recovered. The extract was adjusted to pH 7.0, using a Beckman Model G pH meter with external electrodes and a magnetic stirrer. The alfalfa extract was slightly acid; 2 drops of concentrated sodium hydroxide (50 grams of sodium hydroxide in 50 ml. of water) and 3 or 4 drops of a more dilute (2*N*) sodium hydroxide solution usually were sufficient to adjust to pH 7.0. Because of the small amounts of caustic solutions added, no correction was considered necessary in the final calculation of parts per million.

Three-milliliter aliquots of the neutral alfalfa extracts were added to 50-ml. volumetric flasks as described by Hensel and coworkers. These aliquots were taken from the beakers immediately after adjusting to pH 7.0, while the beaker was on the magnetic stirrer. Five milliliters of pooled human blood plasma (obtained from Chemagro Corp.) were added to each volumetric flask. Distilled water was added to the 50-ml. mark. The flask was thoroughly shaken, placed in a water bath at 37.5° C., and incubated for 70 minutes. From this point on, the procedure was the same as described by Hensel and coworkers.

A standard curve was prepared as follows: A quantity of unsprayed, baled alfalfa also was processed through a hammer mill. An aliquot (40 grams) of this alfalfa was used to prepare an aqueous extract similar to the sprayed alfalfa extract. Again, 3-ml. portions of the neutral extract were added to volumetric flasks. Four flasks, each containing a 3-ml. portion of the control extract, were prepared. One flask served as a control and provided the delta pH value for calculating per cent inhibition values. To each of the other three flasks were added 1, 2, and 3 ml. of a diluted Systox standard containing, respectively, 4.5, 9.0, and 13.5 γ of demeton. A fresh standard solution was prepared each day analyses were run; 0.212 gram of a Systox standard formulation (containing 21.2% demeton, obtained from Chemagro Corp.) was weighed onto an aluminum foil boat; the boat was transferred to a 1-liter volumetric flask, distilled water added to the mark, and the Systox formulation put into solution using a magnetic stirrer. This standard solution contained 45 γ of demeton per ml. A diluted standard was prepared to contain 4.5 γ of demeton per ml.

Generally three or four sprayed alfalfa samples were run at a time. All samples, including those for the standard curve and control, were run in duplicate. The average delta pH values were used to calculate the per cent inhibition for each sample. Under the conditions of analysis, the inhibition usually ranged between 15 and 75%. Values falling outside these limits were not used, and the samples were rerun using a different volume of the aqueous alfalfa extract. A standard curve was drawn by closeness of fit to the three points obtained by plotting on semilogarithmic paper the per cent inhibition values on the linear scale and the concentration of demeton in parts per million on the logarithmic scale. A close approximation to a straight line usually was obtained.

Although the standard curves for these analyses appeared to be linear over the range of 15 to 75% inhibition, when these curves were projected to 0% inhibition, the line did not intersect the abscissa at zero. It appears that the authors are working actually with a curvilinear function and have merely restricted analyses to a small portion of the curve in which an approximately linear relationship between per cent inhibition and parts per million of residue toxins prevails. As further evidence, the greatest vertical deviation between successive standard curves occurs at the middle residue toxins values. A summary of demeton toxins in the baled, Systox-treated alfalfa is shown in Table I.

Table I. Demeton Toxins in Baled, Systox-Treated Alfalfa Fed to Dairy Cows

(Samples analyzed by modified enzymatic procedure of Hensel)

Composite Sample No.[a]	*Average Demeton Toxins*[b]*, P.P.M.*
Expt. B. Alfalfa fed to cow 3607	
1	29
2	60
3	64
4	51
Av.	51
Expt. C. Alfalfa fed to cows 3718, 3480, and 3518	
5	44
6	41
7	30
8	30
9	48
10	43
11	53
12	42
Av.	41

[a] Each composite sample analyzed was gathered on an approximately weekly basis and consisted of daily aliquots of hay taken from bales as fed.

[b] Based upon average values obtained at Iowa State College and Chemagro Corp. on aliquots of composite samples.

Milk. Whole, unpasteurized milk samples were collected several times a day in Experiment A, from one of the regular milkings on a daily basis in Experiment B, and twice weekly in Experiment C. These samples were used to determine the per cent fat and the cholinesterase inhibition by the milk. During Experiments A and B, milk samples were obtained from Holsteins in the college dairy herd and were used for the preparation of standard curves (% inhibition *vs.* p.p.m.). Again, the enzymatic inhibition method of Hensel and coworkers, with modifications, was employed. Each milk sample was homogenized three times and an approximately 100-ml. aliquot was adjusted to pH 7.0 using a Beckman pH meter and magnetic stirrer as for the hay analyses. A 35-ml. aliquot of the neutralized milk was transferred to a 50-ml. volumetric flask and 5 ml. of pooled human blood plasma were added to each flask. One control flask and three flasks containing 4.5, 9.0, and 13.5 γ, respectively, of the Systox diluted standard described above also were prepared. From here on, the procedure was the same as described by Hensel and coworkers. The per cent inhibition ranged between 15 and 75%. Duplicate samples of milk, randomly selected, from the experimental cows in Experiment C were sent to Chemagro Corp. for determination of cholinesterase inhibition.

Blood. The electrometric method of Michel (7) was used to determine the cholinesterase activity of red blood cells in all three experiments. A 10% potassium oxalate solution was used instead of heparin to prevent clotting of the blood. The cholinesterase activities of the plasma and the red blood cells were determined separately. As the cholinesterase activity in red blood cells (about 0.5 delta pH per hour) is approximately 5 times greater than in plasma, the red blood cells only were used in these experiments. Also, the small nonenzymatic hydrolysis correction factors described by Michel were not determined for the red blood cells, because the authors were concerned chiefly with more obvious changes in pH.

In all three experiments, pretreatment blood samples were used to establish normal cholinesterase activity for the red blood cells. In Experiment C, blood samples were taken concurrently from the two control cows and used as a further basis of comparison. In addition, several dozen blood samples taken at random from cows in the college dairy herd were analyzed.

Results and Discussion

Hay Analyses Samples of sprayed hay were collected in the field 2.5, 27, 51, and 72 hours after spraying. Average demeton residue toxins on and in these samples, as gathered, were 18, 31, 29, and 31 p.p.m., respectively. The demeton residue toxins in Table I represent an average of values obtained at Iowa State College and at Chemagro Corp. Considerable variation occurred in these residue analyses; the Chemagro Corp. values generally were higher than those obtained at Iowa State College. A residue of 46 p.p.m. was reported for an aliquot of sample 7 sent to a third laboratory (R. L. Metcalf, Citrus Experiment Station, University of California, Riverside, Calif.) for analysis. All the ground samples listed in Table I were reanalyzed about a year after the hay had been sprayed. The average residue was 12 p.p.m., an indication of a loss of residue during storage. At about the same time, alfalfa from several bales left over from the feeding experiment was ground and analyzed at both Iowa State College and Chemagro Corp. An average residue of 28 p.p.m. was found in this hay. Thus, a significant quantity of demeton residue toxins was present in the baled hay stored in a barn over a period of approximately 1 year.

To determine the effect of heat upon the demeton residues, the samples used for moisture determination (oven-dried at 105° C. to constant weight) were also analyzed. No residue could be detected in any of the samples. Under these conditions the toxins were apparently eliminated by this heating procedure.

Experiment A. Technical demeton was fed in capsules to cow 3607 in increasing amounts for three consecutive days as follows: first day, 0.1; second day, 0.51; and third day, 2.5 mg. of demeton per kg. of cow body weight. The capsules were administered about 9:00 A.M. each day. By afternoon of the third day the cow appeared listless, showed symptoms of intoxication, had diarrhea, and displayed labored breathing, apparent pain, and anorexia. By evening the diarrhea had subsided but the cow refused to eat and continued to be listless and intoxicated. The cow's physical condition improved during the next 2 days and by the third day after demeton feeding had been discontinued the cow appeared near normal. Concurrent with the physical symptoms there occurred a loss of weight due to the diarrhea and failure to eat and drink.

Milk yield at the morning milking (4:30 A.M.) on the day when the highest level of demeton was fed (at 9 A.M.) was normal. However, in the afternoon no milk could be obtained, even though there appeared to be a considerable amount of milk in the udder. Apparently the milk let down reflex was nonfunctional at this point. The accumulated milk was obtained at the next milking and subsequently milk production gradually increased to near-normal over a period of 3 days. As normally occurs when milk yield declines, the milk fat percentage increased as milk yield decreased.

Milk samples taken 1 and 6 hours after the third feeding contained substances which inhibited the cholinesterase present in the plasma used for analysis. The red blood cell cholinesterase activity declined from a prefeeding value of 0.4 delta pH unit per hour to a low of 0.08 delta pH unit per hour at mid-afternoon of the third day of feeding. The greatest cholinesterase-inhibiting property of the milk and lowest red blood cell cholinesterase activity coincided with the period of greatest physical deterioration.

By the morning of the first day after

Table II. Summary of Feeding Systox-Treated Hay to Holstein Cow 3607

(Experiment B. Values in parentheses indicate number of determinations or samples upon which data are based)

		Sampling Data							
		Systox-Treated Alfalfa Hay				Milk			
			Av. Demeton Toxins						
Consecutive Feeding Periods, Days	Av. Cow Wt., Kg.	Av. consumption, kg./day	In hay; p.p.m.	Consumed mg./day	Consumed mg./kg. cow body wt./day	Av. prod. lb./day	Av. fat, %	Av. CHE[a] inhibition, %	Blood CHE[a] activity, ΔpH/hr.
2	482(2)	0	0	0	0	33.0(2)	3.88[b](2)	...	0.37(2)
no treated hay)									
15	483(15)	2.86(15)	51[c]	146	0.3	29.6(15)	3.75(15)	2.31(14)	0.31(15)
13	503(13)	5.91(13)	51[c]	301	0.6	23.2(13)	3.65(13)	1.37(13)	0.30(13)
21	505(21)	9.75(21)	51[c]	497	1.0	17.4(21)	3.98(21)	4.11(21)	0.10(21)

[a] CHE = cholinesterase.

[b] Average fat content of 11 Holstein herd samples, obtained at intervals during this experiment for preparation of cholinesterase inhibition standard curves, was 3.44.

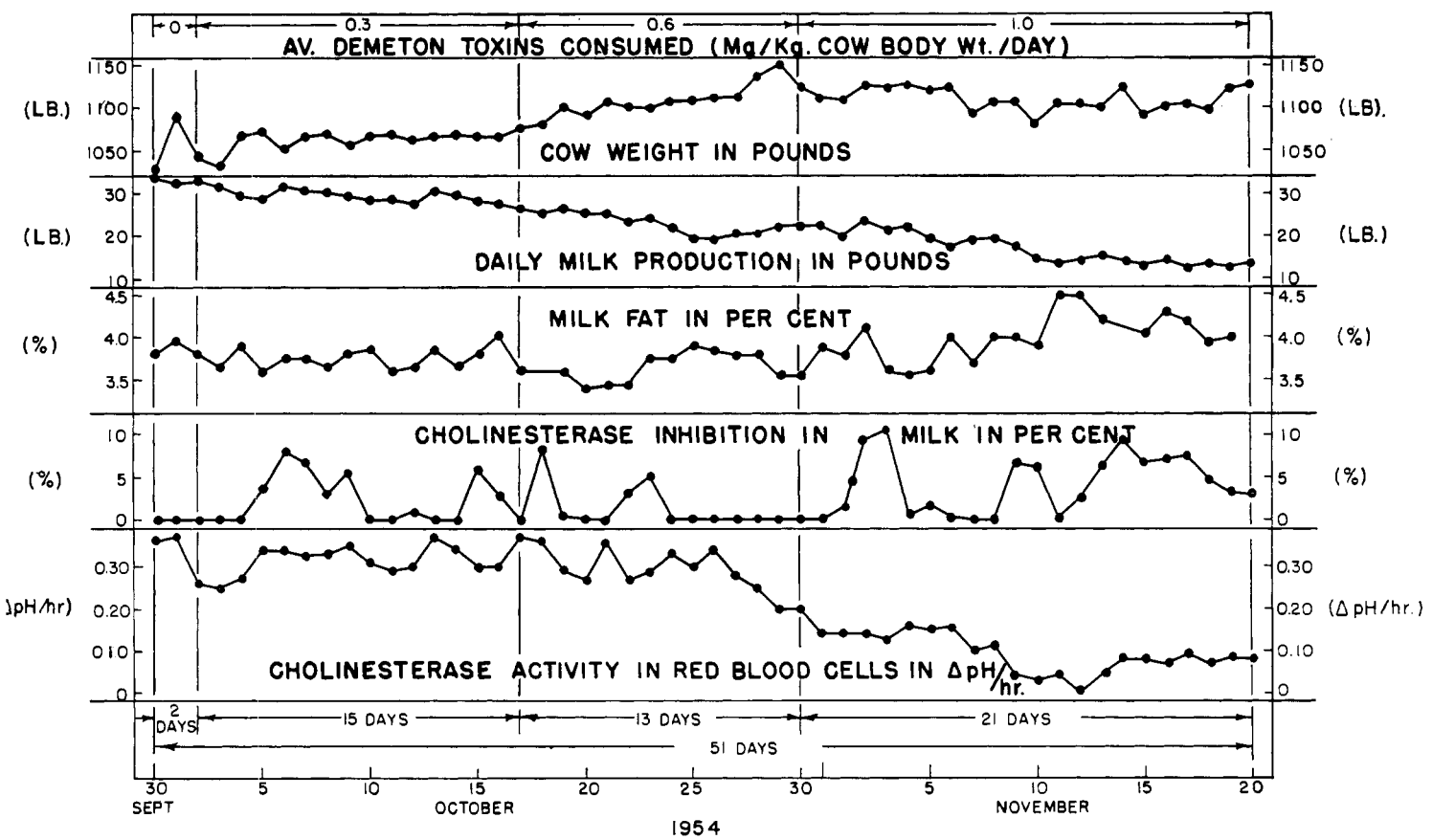
[c] Based upon residue analyses of four composite samples of daily aliquots of hay taken from bales as fed. Analysis values were: 29, 60, 64, and 51 p.p.m. demeton toxins in hay (see also Table I).

demeton feeding was discontinued no substances which inhibited plasma cholinesterase could be detected in the milk, and in the next 2 days the red blood cell cholinesterase activity reached 0.35 delta pH unit per hour. At the two lower feeding levels no significant changes in either the milk or blood could be detected. Thus, it appears that the passage of cholinesterase-inhibiting substances into the milk following the highest level of feeding was transitory. Inactivation of the red blood cell cholinesterase persisted longer.

A sample of the technical demeton fed to cow 3607 was administered (by William B. Deichmann, University of Miami, Coral Gables 34, Fla.) to male rats as a 1% solution (vol./vol.) in corn oil. The approximate lethal dose was 0.005 ml. per kg.

Experiment B. The effects of feeding increasing quantities of treated hay to cow 3607 for 49 days are summarized in Figure 1 and Table II. This experiment was initiated approximately 8 months after parturition and 4 months after conception of 3607. Therefore, a gradual increase in body weight normally would be expected during the experiment. This was observed when 0.3 and 0.6 mg. of demeton toxins per kg. of body weight per day were fed.

Figure 1. Effects of feeding increasing amounts of alfalfa hay containing an excessively high residue of demeton toxins. for 49 days to Holstein cow 3607



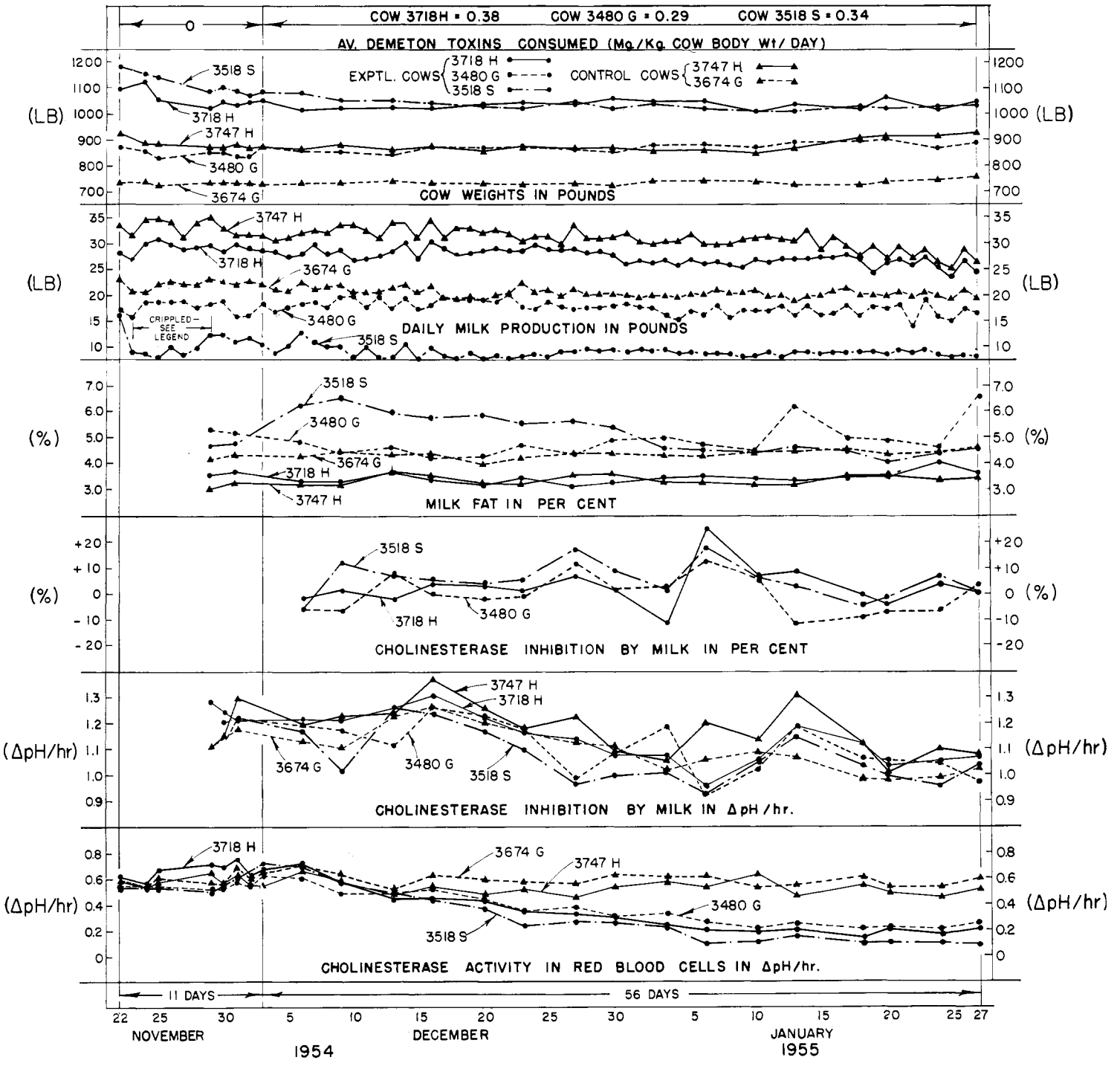


Figure 2. Effects of feeding alfalfa hay containing an excessively high residue of demeton toxins at a uniform rate for 56 days to dairy cows

Cow 3518S was badly crippled for 6 days during pre-experimental feeding period, causing low milk production

However, when the level was increased to 1.0 mg. per kg., weight gains ceased and a slight downward trend occurred, suggesting an adverse effect of this higher feeding level on weight gains, possibly through slight depression of appetite. A downward trend in milk production is normal at this stage of lactation. During the 8- and 12-day periods following increase to the 0.6- and 1.0-mg. levels, respectively, however, the decline seemed unusually rapid. No abnormal trends in percentage of milk fat were apparent. As only one cow was involved, these observations on body weight and milk production changes can be considered suggestive only.

No significant cholinesterase inhibition by the milk occurred while the treated hay was fed. The variations in cholinesterase inhibition shown in Figure 1 are never greater than the 10% level, which is the limit of sensitivity of the analytical method. Furthermore, in the case of many of the 0% inhibition values shown in Figure 1, the delta pH of the milk from cow 3607 was actually greater—i.e., showed less cholinesterase inhibition—than the control milk. Similar data are shown in Figure 2 (Experiment C), where cholinesterase inhibition by milk both in per cent (using positive and negative values) and

in delta pH per hour is plotted. A decline in cholinesterase activity of the red blood cells began about half-way through the 0.6 mg. per kg. feeding period and continued to the end of this period. The cholinesterase activity did not return to normal until about 2 months later. Concurrent milk analyses failed to show any cholinesterase inhibition by the milk over this same period. In this experiment two questions remain unanswered: To what extent did feeding demeton in Experiment A affect the response of the cow to ingestion of treated hay in Experiment B? Does the absence of significant cholinesterase inhibition by the milk and concurrent decrease in cholinesterase activity in the red blood cells suggest a pathway of residue detoxication that does not involve the milk secretory process?

Experiment C. The effects of feeding uniform quantities of treated hay to three cows for 56 days are shown in Figure 2 and Table III. The weight changes for both experimental and control cows appeared to conform to the normal pattern during the preliminary and experimental feeding periods, except for cow 3518. This cow received a leg injury during the preliminary period (see Figure 2) which interfered with mobility and resulted in reduced feed consumption, weight loss, and a sharp decline in milk production. No unusual trends in milk production and milk fat percentage curves (except for 3518) were observed and trends were similar for the two groups.

No significant cholinesterase inhibition by milk occurred; the variations in analyses are shown in Figure 2, where both the percentage cholinesterase inhibition and delta pH per hour values for milk have been plotted. The deviations about zero cholinesterase inhibition are shown for the three cows fed treated hay and the similarity in cholinesterase inhibition by milk from all five cows is shown. The milk samples analyzed by Chemagro Corp. gave changes in delta pH units per hour as great as or greater than the control samples.

The cholinesterase activity in the red blood cells of the three experimental cows began to drop about 2 weeks after feeding of the treated hay was begun. A gradual decline continued throughout the experimental feeding period for these cows. The cholinesterase activity in the blood from the control cows remained fairly constant throughout the experiment. This experiment shows more conclusively than Experiment B that feeding demeton residue toxins in alfalfa hay at levels of 0.29 to 0.38 mg. of residue toxins per kg. of cow body weight per day produces no significant anticholinesterase substance in the milk. Thus it appears that the pathway of residue detoxication does not involve the milk secretory process.

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Table III. Summary of Feeding Systox-Treated Hay to Cows for 56 Consecutive Days

(Experiment C. Values in parentheses indicate number of determinations or samples upon which data are based)

Cow No. and Breed	Consecutive Feeding Periods, Days	Av. Cow Wt., Kg.	Sampling Data: Systox-Treated Alfalfa Hay: Av. consumption, kg./day	Av. Demeton Toxins: In hay, p.p.m.	Av. Demeton Toxins: Consumed, mg./day	Av. Demeton Toxins: Consumed, mg./kg. cow body wt./day	Milk: Av. prod., lb./day	Milk: Av. fat, %	Milk: Av. ΔpH/hr.	Milk: Av. CHE[a] inhibition, %	Blood CHE[a] activity, Δph/hr.
Experimental cows											
3718	11[b]	482(8)	0	0	0	0	29.1(11)	3.6(2)	1.25(2)	...	0.66(8)
Holstein	56	473(16)	4.41(56)	41[c]	180	0.38	27.5(56)	3.4(16)	1.14(16)	3.4(16)	0.33(16)
3480	11[b]	386(8)	0	0	0	0	17.6(11)	5.2(2)	1.18(2)	...	0.55(8)
Guernsey	56	398(16)	2.79(56)	41[c]	114	0.29	17.8(56)	4.9(16)	1.11(16)	0.3(16)	0.35(16)
3518	11[b]	503(6)	0	0	0	0	10.4[d](11)	4.8(2)	1.17(2)	...	0.57(8)
Milking Shorthorn	56	473(16)	3.88(56)	41[c]	158	0.34	9.0(56)	5.2(16)	1.07(16)	6.2(16)	0.27(16)
Control cows											
3747	11	402(8)	0	0	0	0	33.4(11)	3.1(2)	1.19(3)	...	0.58(8)
Holstein	56	396(16)	0	0	0	0	31.2(56)	3.4(16)	1.18(16)	0[e](16)	0.53(16)
3674	11	333(8)	0	0	0	0	22.3(11)	4.2(2)	1.14(2)	...	0.60(8)
Guernsey	56	337(16)	0	0	0	0	20.7(56)	4.4(16)	1.10(16)	0[e](16)	0.60(16)

[a] CHE = cholinesterase.
[b] No treated hay fed during this 11-day period.
[c] Based upon residue analyses of eight composite samples of daily aliquots of hay taken from bales as fed (see Table I for analytical data).
[d] Animal badly crippled for 6 days, causing low milk production.
[e] Milk from control cows was used to prepare standard curves and determine per cent inhibition (for comparison of ΔpH/hr. values for milk from all five cows see Figure 2).