# Influence of 2,4-D and 2,4,5-T on In Vitro Digestion of Forage Samples

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**Highlight:** *The influence of 2,4-D [(2,4-dichlorophenoxy) acetic acid] and 2,4,5-T [(2,4,5-trichlorophenoxy) acetic acid] on in vitro digestion of dried ground corn silage (*Zea mays *L.) and bermudagrass (*Cynodon dactylon *L.) foliage was determined using a modification of the Tilley and Terry method for determining in vitro dry matter digestibility of forage plants. Neither herbicide influenced the digestion of plant samples when treated with a herbicide concentration range of $10^{-8}$ to $10^{-4}$ M. Solutions containing $10^{-4}$ M of either herbicide did not influence the growth of microbial populations in incubated rumen liquor. The influence of rumen microorganisms on degradation of 2,4-D and 2,4,5-T was also investigated. Samples containing sucrose or plant material, buffered rumen liquor, and $10^{-4}$ M concentrations of either herbicide were incubated for 10 day periods. Data from periodic quantification of herbicide remaining in the samples indicated that neither herbicide used in these experiments was degraded by the rumen microorganisms. Results indicate that: (1) 2,4-D and 2,4,5-T do not alter the rumen microbial functions or development and (2) these herbicides are not readily degraded in the rumen by the rumen microorganisms.*

For almost three decades 2,4-D [(2,4-dichlorophenoxy) acetic acid] has provided the American farmer with an effective and economical means of controlling broadleaf weeds in crops and pasture lands. The related compound, 2,4,5-T, [(2,4,5-trichlorophenoxy) acetic acid] was introduced to help in the control of brush and more resistant weeds in pastures and rangeland. Recently, use of these herbicides has met with much adverse publicity from certain ecologists and environmentalists. They have been concerned that ingestion of these herbicides from treated pastures and ranges could result in contamination of the meat, making it unfit for human consumption.

Many scientists have undertaken research to determine the magnitude of herbicide contamination in by-products from animals fed varying amounts of these herbicides (Clark et al., 1964; Clark and Palmer, 1971; Leng, 1972). In general, they have concluded that: (1) residues of phenoxy herbicides are not likely to exceed 300 ppm in or on forage immediately after treatment with these herbicides at recommended rates for control of weeds and brush in pastures and rangelands; (2) generally, such residues decline rapidly with a half-life of 1 to 2 weeks depending on geographic location; and (3) residues of phenoxy herbicides and their phenolic moities are not likely to occur in milk, meat, or fat.

Previous studies have indicated that sheep and cattle can tolerate rather large quantities of 2,4-D salts and esters for extended periods of time (Radeleff and Bushland, 1960). However, whether 2,4-D is metabolized, stored, or excreted unchanged from sheep or cattle has received little attention. Clark et al. (1964) found that following the oral administration of 2,4-D-$^{14}$C, the majority of the activity had passed through the blood within a 24-hour period and by the end of 28 hours post treatment 90% of the 2,4-D-$^{14}$C was recovered in the sheep urine. Similar results have been reported for 2,4,5-T (Clark and Palmer, 1971; St. John et al., 1964). Little research has been accomplished on the influence of these herbicides on the digestive functions and development of the rumen microorganism or on the influence of these microorganisms on the degradation of 2,4-D and 2,4,5-T over prolonged periods of time.

This study was undertaken to determine: (1) the influence of these herbicides on the in vitro dry matter digestibility (IVDMD) of corn (*Zea mays* L.) and bermudagrass (*Cynodon dactylon* L.) forage samples, (2) the influence of 2,4-D and 2,4,5-T on rumen microbiota growth, and (3) the in vitro degradation of 2,4-D and 2,4,5-T by rumen microbiota.

## Methods and Materials

### Experiment 1.

The influence of 2,4-D and 2,4,5-T on the IVDMD of ground corn and bermudagrass plant samples was determined by using a modification of the Tilley and Terry two-stage method for determining the IVDMD of forage crops (Tilley and Terry, 1963). Separate half gram samples of dried bermudagrass foliage and samples of equal portions of ear, leaf, and stem material from ensilage corn were ground to pass through a #40 mesh screen. The plant foliage samples were incubated with 45 ml of buffered rumen liquor in 50-ml centrifuge tubes. The rumen liquor used in all the experiments was removed, through a permanent fistula, from a mature steer maintained on a grass diet for the duration of these experiments. In treatments containing 2,4-D and 2,4,5-T, quantities of a buffered (pH 6.9) solution of each technical grade herbicide were added to give final herbicide concentrations of 0, $10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$, or $10^{-4}$ M in the buffered rumen liquor. A sample consisted of a single concentration for each herbicide. Herbicide solutions were added preceding the addition of the buffered rumen liquor. This experiment was repeated twice and the data analyzed by the appropriate analysis of variance test (Steele and Torrie, 1960).

### Experiment 2.

In an attempt to determine the influence of 2,4-D and

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2,4,5-T on the growth of microbiota in rumen liquor, an experiment was conducted using 0.2 g sucrose to replace the plant material and only the first stage of the two-stage technique for the IVDMD of forage crops. Treatments consisted of $10^{-4}$ M 2,4-D or 2,4,5-T and no herbicide. The herbicide treatments were conducted the same as in experiment 1. At 0, 2, 4, 7, 9, and 11 days after adding the herbicides, quadruplicate samples were removed from the incubator and centrifuged for 15 min at 1800x*g*. After discarding the supernatant, the insoluble residue was washed once with 40 ml distilled water, centrifuged, and the supernatant discarded. The insoluble residue was dried at 88°C for 48 hours and the dry weights of the residue calculated. This experiment was repeated twice and all data were statistically analyzed by the appropriate analysis of variance tests.

**Experiment 3.**

The purpose of this experiment was to determine the influence of the rumen microorganisms on the degradation of 2,4-D and 2,4,5-T. Forty-five ml of buffered rumen liquor were placed into 50 ml centrifuge tubes containing 0.5 g dried ground bermudagrass foliage or in tubes without the plant material. The latter series of treatments were included to determine the influence of forage material on the microbial degradation of these herbicides. Treatments consisted of final concentrations of 0 or $10^{-4}$ M 2,4-D or 2,4,5-T. The sample tubes were incubated at 38° C throughout the experiment. At 0, 2, 4, 6, 8, and 10 days after beginning the experiment, quadruplicate samples from each treatment were removed from the incubator. The samples were centrifuged at 1800x*g* for 15 min and 10 ml aliquots of supernatant were removed from each sample tube for herbicide quantification. Herbicide remaining in the 10 ml aliquots was extracted, methylated, and quantified by a method similar to that used by Smith (1972). The 10 ml aliquots were acidified with 1 N HCl. The herbicide was extracted from the acidified solution with two 20 ml aliquots of diethyl ether and the ether evaporated in an 80° C water bath. The herbicide was esterified with 6 ml of $BF_3$/methanol (125 g/L) (Merkle and Davis, 1966). Methyl esters of the herbicides were taken up, quantitatively, in hexanes and quantified using an F&M 700 gas chromatograph equipped with a nickel-65 electron capture detector. A 1.8 M spiral glass column packed with 80-100 mesh chromosorb W coated with 4% SE30 was used. Flow rate of the argon/methane (95/5 percent) carrier gas was 40 ml/min. Column, injection port, and detector temperatures were 200, 250, and 230° C, respectively. The experiment was repeated twice, and the data were statistically evaluated by the appropriate analysis of variance test (Steele and Torrie, 1960).

## Results and Discussion

Residues in or on forage following field applications of herbicides in pasture or rangeland depend upon the amount of the spray intercepted by the overstory, the rate or mode of application, and the time interval following treatment. Research has shown that initial residues in or on grass from such treatments are not likely to exceed 100 ppm for each pound of herbicide applied (Getzendaner et al., 1969; Morton et al., 1967). Herbicide concentrations in excess of $10^{-4}$ M in rumen solution would be unlikely following recommended application rates and procedures for 2,4-D and 2,4,5-T. Results of my experiments, conducted to determine the influence of these herbicides on the IVDMD of forage samples (Table 1), indicate $10^{-8}$ - $10^{-4}$ M solutions of either herbicide does not significantly alter the digestion activities of the rumen microorganisms when compared with the untreated check. In an attempt to verify the point that the development of the microbial populations was not being altered by these herbicides, dry weights of the treated and untreated rumen liquor, developed in sucrose solutions, were obtained periodically over an 11-day treatment period. Data from this experiment (Table 2) show that the weight increase due to the growth of microorganisms over the 11-day treatment period was not altered by $10^{-4}$ M solutions of either herbicide. These data indicate that herbicide residues remaining on or in forage samples following spray treatments with 2,4-D or 2,4,5-T apparently will not influence the herbage digestion by ruminants.

**Table 1. Influence of 2,4-D and 2,4,5-T concentration (M) on corn and bermudagrass in vitro dry matter digestibility (%).**

| Herbicide concentration | In vitro dry matter digestibility | | | |
|---|---|---|---|---|
| | 2,4-D treated | | 2,4,5-T treated | |
| | Corn | Bermudagrass | Corn | Bermudagrass |
| 0 | 48.3[1] | 43.0 | 53.0 | 42.5 |
| $10^{-8}$ | 49.6 | 42.9 | 50.4 | 43.3 |
| $10^{-7}$ | 49.8 | 42.5 | 50.1 | 42.6 |
| $10^{-6}$ | 50.4 | 42.7 | 51.0 | 43.0 |
| $10^{-5}$ | 51.0 | 43.8 | 52.3 | 42.9 |
| $10^{-4}$ | 48.8 | 43.8 | 53.5 | 42.9 |

[1] All means within a column are not significantly different at $P = 0.05$ as tested by analysis of variance test.

**Table 2. Influence of 2,4-D and 2,4,5-T on the growth of rumen liquor microbial populations.**

| Days after treatment | Weight (mg) of dried rumen liquor | | |
|---|---|---|---|
| | Herbicide treated | | |
| | 2,4-D | 2,4,5-T | Check |
| 0 | 30[1] | 31 | 30 |
| 2 | 33 | 32 | 33 |
| 4 | 34 | 35 | 34 |
| 7 | 37 | 38 | 36 |
| 9 | 38 | 37 | 38 |
| 11 | 40 | 37 | 38 |

[1] All means within a horizontal line are not significantly different at $P = 0.05$ as tested by analysis of variance test.

The role of microorganisms in the degradation of the phenoxyacetic acid herbicides is well established (Audus, 1969; Audus, 1951; Kearney and Kaufman, 1969). The majority of this research has been accomplished with soil solutions or microorganisms isolated from soils. Very little research is reported on the degradation of 2,4-D and 2,4,5-T by organisms from sources other than soil. Data from my research (Table 3) indicate that microorganisms in rumen liquor do not degrade 2,4-D or 2,4,5-T over a 10-day incubation period. This was true for samples with and without bermudagrass foliage as an energy source. In vivo methods have indicated that 24 hours following the oral administration of 2,4-D-$^{14}$C, the majority of the activity had passed through the blood; and by the end of 28 hours post treatment, 90% of the 2,4-D-$^{14}$C was recovered from sheep urine (Clark et al. 1964). However, previous investigations with microorganisms common in soils have shown that degradation of 2,4-D by microorganisms is preceded by a lag phase lasting up to 6 days during which the herbicide is not appreciably degraded (Smith, 1972; Audus, 1964). This lag phase was followed by a period of rapid substrate disappearance. The lag phase might result from the time required either for development of an effective population of herbicide degrading organisms or time required

Table 3. Influence of rumen microorganisms on the degradation of 2,4-D and 2,4,5-T with and without bermudagrass foliage present.

| Days after treatment | Herbicide recovered μg/ml) | | | |
|---|---|---|---|---|
| | With bermudagrass | | Without bermudagrass | |
| | 2,4-D | 2,4,5-T | 2,4-D | 2,4,5-T |
| 0 | 22.0[1] | 24.2 | 22.1 | 24.2 |
| 2 | 22.5 | 22.9 | 22.4 | 23.2 |
| 4 | 21.2 | 23.9 | 22.3 | 23.5 |
| 6 | 19.7 | 23.3 | 21.3 | 23.2 |
| 8 | 19.8 | 23.8 | 22.9 | 23.9 |
| 10 | 20.1 | 23.6 | 20.6 | 23.1 |

[1] All means within a column are not significantly different at $P = 0.05$ as tested by an analysis of variance test.

for appropriate enzyme induction in a population already present (Kearney and Kaufman, 1969; Akamine, 1951). Results from the in vivo studies did not allow for determining the degradation of 2,4-D or 2,4,5-T following a lag phase of up to 6 days. Data from my experiments indicate that these herbicides are not degraded by microorganisms common to the rumen when incubated for treatment periods as long as 10 days. Therefore, it appears that irrespective of degradation scheme these herbicides are not degraded by the microorganisms common to ruminants under grazing conditions.

Results of the study indicate that: (1) 2,4-D and 2,4,5-T do not alter the rumen microbial functions or development and (2) these herbicides are not readily degraded by rumen microorganisms.

## Literature Cited


**Akamine, E. K. 1951.** Persistence of 2,4-D toxicity in Hawaiian Soils. Bot. Gaz. 112:312-318.

**Audus, L. J. 1949.** The biological detoxication of 2,4-dichlorophenoxy-acetic acid in soil. Plant Soil 2:31-34.

**Audus, L. J. 1951.** The biological detoxication of hormone herbicides in soil. Plant Soil 3:170-175.

**Audus, L. J. 1964.** Herbicide behavior in the soil, p. 163-206. *In* L. J. Audus, ed. The Physiology and Biochemistry of Herbicides. Academic Press, New York.

**Clark, Donald E., and Jack S. Palmer. 1971.** Residual aspects of 2,4,5-T and an ester in sheep and cattle with observations on concomitant toxicological effects. J. Agr. Food Chem. 19:761-764.

**Clark, Donald E., Joel E. Young, R. L. Younger, L. M. Hunt, and J. K. McLaren. 1964.** The fate of 2,4-dichlorophenoxyacetic acid in sheep. J. Agr. Food Chem. 12:43-45.

**Getzendaner, M. E., J. L. Herman, and B. Van Glessen. 1969.** Residues of 4-Amino-3,5,6-trichloropicolinic acid in grass from application of tordon herbicides. J. Agr. Food Chem. 17:1251-1256.

**Kearney, P. C., and D. D. Kaufman. 1969.** Degradation of herbicides. Marcel Dekker, Inc., New York. 394 p.

**Leng, Marquerite L. 1972.** Residues in milk and meat and safety to livestock from the use of phenoxy herbicides in pasture and rangeland. Down to Earth. 28:12-20.

**Merkle, M. G., and F. S. Davis. 1966.** The use of gas chromatography of picloram and 2,4,5-T. Proc. SWC 19:557-562.

**Morton, H. L., E. D. Robinson, and R. E. Meyer. 1967.** Persistence of 2,4-D and 2,4,5-T and dicamba in range forage grasses. Weeds 15:268-271.

**Radeleff, R. D., and R. C. Bushland. 1960.** The nature and fate of chemicals applied to soils, plants, and animals. Symposium, Agr. Res. Serv. 20-9, Sept. 1960, U. S. Dep. of Agr., p. 146.

**Smith, A. E. 1972.** Influence of calcium hydroxide and sulfur on 2,4-D degradation in soil. Soil Sci. 113:36-41.

**St. John, L. E., D. C. Wagner, and D. J. Lisk. 1964.** Fate of atrazine, kuron, silvex, and 2,4,5-T in the dairy cow. J. Dairy Sci. 47:1267-1270.

**Steele, R. G. D., and J. H. Torrie. 1960.** Principles and procedures of statistics. McGraw-Hill Book Co., Inc. 481 p.

**Tilley, J. M. A., and R. A. Terry. 1963.** A two-stage technique for the *in vitro* digestion of forage crops. J. Brit. Grassl. Soc. 18:104-111.