# Feeding Studies with Ethephon Growth Regulator in the Dairy Cow

A feeding experiment was conducted to study the metabolism and excretion of the plant growth regulator ethephon (2-chloroethylphosphonic acid) in a lactating cow. At a concentration of 5 ppm in the dairy ration, no residues of the compound were found in milk or feces. About 9.8% of intact ethephon was eliminated in the urine. The compound degraded in the presence of fresh rumen fluid.

Ethephon (2-chloroethylphosphonic acid) is a plant growth regulating compound produced by Amchem Products, Inc., Ambler, Pa. It induces flowering, ripening and abscission of fruit and certain other crops. Drift contamination of forage adjacent to treated orchards is well known. There is also a current desire to possibly again include apple pomace in dairy cattle rations, since the use of DDT has been curtailed. Data obtained in this laboratory show that ethephon as a residue in apples is about equally distributed in pomace and juice when the fruit is pressed. It was of interest, therefore, to investigate the stability and routes of excretion of this compound in the bovine.

## EXPERIMENTAL SECTION

A Holstein cow weighing 720 kg and with an average daily milk production of about 12.7 kg (4.1% butterfat) was catheterized and fed ethephon at the 5-ppm level (based on a daily ration of approximately 22.7 kg) for 4 days. The pure compound in water was thoroughly mixed with the evening grain, which the animal consistently consumed in total. Morning and evening subsamples of the total mixed milk were taken 1 day prior to feeding (control sample), daily throughout the feeding period, and for 6 days thereafter. The total daily urine and feces samples were similarly collected, weighed, mixed, and subsampled during the same test period. The manure samples were collected in specially constructed trays. All samples were immediately frozen prior to analysis.

### *In Vitro* STUDY

The stability of ethephon in the presence of fresh rumen fluid was studied. One milliliter of a solution of ethephon in methanol (500 $\mu$g/ml) was thoroughly mixed with 100 ml of fresh filtered rumen fluid and held at 38° in an open system. At measured intervals 5 ml of fluid was removed, 1.25 ml of 8% hydrochloric acid was added, and the solution was made to a volume of 25 ml with methanol. One milliliter of this solution was evaporated (rotary evaporator) to dryness, 0.4 ml of methanol and 5 ml of diethyl ether were added, and the solution was methylated by the procedure of Schlenk and Gellerman (1960), followed by gas chromatographic analysis with an alkali-thermionic detector using essentially the method of Bache (1970). The retention time of ethephon was about 2.8 min.

### EXTRACTION AND METHYLATION OF ETHEPHON IN OTHER BODY FLUIDS

**Milk.** The sample was thoroughly mixed by blending and a 25-g subsample of whole milk was freeze-dried. Twenty grams of the dried sample was shaken (reciprocating shaker) for 1 hr with 75 ml of methanol containing 0.3 ml of 8% hydrochloric acid. The mixture was filtered and evaporated to a total volume of 50 ml. Ten milliliters of the solution was evaporated to about 0.5 ml with nitrogen, and 5 ml of ether was added. The resulting precipitated material was centrifuged and the supernatant liquid was methylated and chromatographed as in the procedure for rumen fluid except that the gas chromatographic column temperature was 120°.

**Urine.** Five grams of urine, 0.5 ml of 8% hydrochloric acid, and 15 ml of methanol were mixed and centrifuged. The supernatant liquid was decanted and diluted to 50 ml with methanol. Five milliliters of the solution was evaporated to dryness, 0.3 ml of 8% hydrochloric acid and 0.2 ml of methanol were added, and the mixture was methylated and chromatographed.

**Feces.** Five grams of feces was shaken for 1 hr with 50 ml of methanol and 1 ml of 8% hydrochloric acid. The mixture was filtered and the filtrate made to a volume of 50 ml; 2 ml of the filtrate was evaporated to dryness, 0.2 ml of 8% hydrochloric acid and 0.3 ml of ethanol were added, and the mixture was methylated and chromatographed.

## RESULTS AND DISCUSSION

Ethephon was not detected in milk or feces. About 9.8% of the intact compound was eliminated in the urine. This total excretion occurred during the first 5 days of the feeding experiment. The recovery of ethephon from body fluids and the estimated analytical sensitivities are listed in Table I. The peak height corresponding to 0.1 ppm of ethephon was about 10 cm. The recovery of the compound from feces was admittedly poor but about 0.5 ppm would have been detectable. Ethephon decomposed when incubated with rumen fluid. Its disappearance is illustrated in Figure 1.

**Table I. Recovery of Ethephon from Body Fluids**

| Sample | Added, ppm | Recovery, % | Estimated sensitivity, ppm |
|---|---|---|---|
| Milk | 0.1 | 70 | 0.01 |
| | 0.2 | 90 | |
| | 0.3 | 93 | |
| Urine | 0.1 | 91, 84, 83 | 0.01 |
| Feces | 0.5 | 16 | |
| | 1.0 | 10 | 0.5 |
| Rumen fluid | 5.0 | 92 | 0.1 |

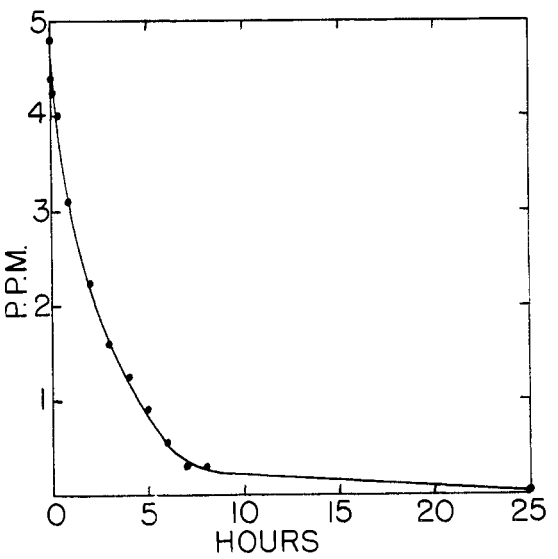


**Figure 1.** Disappearance of ethephon in rumen fluid.

The fate of ethephon in the bovine is speculative. It is possible that the compound may decompose in the presence of rumen fluid, with the release of ethylene gas, dihydrogen phosphite, and chloride ions. This reaction is thought to occur in plant tissues (Technical Service Data Sheet, 1969). The rapid degradation of ethephon in rumen fluid may explain the presence of only 9.8% of the intact compound in urine. Since ethephon is not fat-soluble, its absence in milk at low feeding levels is not surprising. Based on past analysis of field-treated fruit and pomace in this laboratory, concentrations of ethephon in harvested apple fruit and pomace would expectedly be well below 5 ppm, the dose level in this investigation. Residues of ethephon in pomace therefore would not be expected to cause milk contamination if the pomace was included in the dairy ration.

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LITERATURE CITED

Bache, C. A., *J. Ass. Offic. Anal. Chem.* **53,** 730 (1970).
Schlenk, H., Gellerman, J. L., *Anal. Chem.* **32,** 1412 (1960).
Technical Service Data Sheet H-96, "Ethrel," Amchem Products, Inc., Ambler, Pa., April 1969, p 3.

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# Free Amino Acids of Pakistani Wheat Varieties

Free amino acids content of eight Pakistani wheat varieties has been determined by paper chromatography. In all, 13 amino acids have been found in all the varieties. These varieties differ both qualitatively and quantitatively in free amino acids. Glutamic acid, aspartic acid, phenylalanine, cystine, tyrosine, and valine are common to all varieties. Nortino variety contains the greatest number and amount of free amino acids, while C-271 and C-273 contain the least.

Amino acids, one of the most important constituents of our daily diets from a nutritional viewpoint, exist almost invariably in all the food products, both in the free as well as the bound form. Proteolysis and the partial or complete inability of any amino acid to take part in protein synthesis may be the reasons for the presence of free amino acids in foods. Free amino acids have been used as an index of maturity in certain fruits (Rockland and Underwood, 1954). In some foods, these have been taken as an index of decomposition (Fuks and Wierzchowski, 1967), while in bovine muscle their greater quantities have been associated with tenderness (Field and Chang, 1969).

Free amino acids of various food products have been reported in the literature (Coussin and Samish, 1968; Elahi and Khan, 1971; Ghadimi and Pecora, 1963; Petropavlovskii and Troyan, 1970; Tinsley and Bockian, 1959; Wolfgang *et al.*, 1970). Data concerning the free amino acid content of Russian (Ekimovsky and Somin, 1970) and American (Hoseney and Finney, 1967) wheat varieties are present in literature.

In this paper, the free amino acids of eight ripe Pakistani wheat varieties have been reported.

## EXPERIMENTAL SECTION

**Apparatus and Reagents.** Paper chromatography was carried out on Whatman No. 1 paper, using glass chamber (21 × 8 in.). A Beckman DU spectrophotometer was used for optical density measurements.

Ninhydrin reagent was prepared by dissolving 0.5 g of commercial ninhydrin in 100 ml of acetone. Solvents used were of analytical grade.

**Preparation of Samples.** Pure samples of eight wheat varieties (*i.e.*, C-271, C-273, C-591, Penjamo 62, Maxi-Pak red, Maxi-Pak white, Nortino, and Dirk) were collected on June 26, 1970, of the 1970 crop, from Ayub Agriculture Research Institute, Lyallpur. Each variety was ground into whole meal flour in the laboratory test mill after being freed of dirt and foreign seeds.

**Extraction of Amino Acids.** The extraction procedure is essentially that of Pant and Tulsiani (1968). Flour (5 g) of each variety was defatted in a Soxhlet apparatus and was well stirred in 50 ml of 70% ethanol for 30 min and centrifuged. The supernatant layer was decanted and the residue was again stirred with the solvent and centrifuged. The process was repeated until the supernatant layer gave no color with ninhydrin solution. All the ethanol extracts were combined and evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of distilled water. The solution was used for both qualitative and quantitative purposes.

**Qualitative and Quantitative Analysis.** Free amino acids were determined both qualitatively and quantitatively by spotting 0.25–0.50 ml of the extract on Whatman No. 1 paper using one-dimensional ascending paper chromatographic techniques as described earlier (Elahi and Khan, 1971).

The following solvent systems were used: (1) propanol–water (70:30 v/v); (2) phenol–*n*-propanol–water (100:20:20 v/v); and (3) ethylmethyl ketone–propionic acid–water–*tert*-butanol (75:25:30:20 v/v).

Serine and glycine, valine and methionine were not separable in solvent 1 and were separated in solvent 2. Lysine and histidine, alanine and threonine were not separable in solvents 1 and 2 and were separated by first passing solvent 1 and then after drying solvent 3 in the same direction.