Table III. Comparative Residue Values for Paraquat and Diquat in Soils

Soil	Org matter, %	ppm: Gc method	ppm: Ion-exchange method
	Paraquat		
Clay loam	3.8	0.92	1.25
Organic	82.4	12.35	10.56
	Diquat		
Fine sandy loam	3.0	0.30	0.22

ther increase in the weight of the catalyst did not increase the recovery. The amount of the catalyst required for maximum recovery of residues depends on the soil type; organic soil required more catalyst, indicating an increasing demand for catalyst with an increase in organic matter contents of the soil. However, 25 mg of the catalyst per g of soil was adequate for the maximum recovery of residues from the three different types of soil. Under these conditions the hydrogenation of soil extracts was completed in 1 hr as further increase in time of hydrogenation did not improve the recovery (Figure 7). The maximum recovery of residues was not affected by varying the pH, over the range 1–9, of the acid extracts of soil prior to hydrogenation.

The method described in this paper is rapid and simple. No cleanup is necessary for routine soil samples. The lower limit of sensitivity for this method is approximately 0.01 ppm for diquat and paraquat.

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Estimation of Methionine and Cystine in Compounded Poultry Rations

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Reliable chemical methods for the estimation of sulfur-containing amino acids have been developed. With these procedures, the content of these two amino acids which are important for good egg production can be assessed in chick feeds. The determination of methionine was carried out by ascending thin-layer chromatographic separation. The separated spots were sprayed with ninhydrin and the color read by the Beckman DB-G spectrophotometer. The interfering spots were separated with the help of column chromatography over Dowex-1. Cystine was estimated according to the method of Schram *et al.* (Schram, E., Moore, S., Bigwood, E. J., *Biochem. J.* **57**, 33 (1954)). The method was based on the hydrolysis of oxidizing product, produced by performic acid treatment of the rations. The prepared hydrolysate was passed through a column of Dowex-2 and estimated according to the photometric ninhydrin method of S. Moore and W. H. Stein (*J. Biol. Chem.* **176**, 307 (1948)).

It is known that the nutritive value of a food protein depends on the amino acid content. Thus the evaluation of the nutritive quality of compounded poultry rations may be obtained by analyzing their amino acids.

The minimum requirements of methionine and cystine in a balanced diet for chickens were reported to be 0.45 and 0.35%, respectively (Bose, 1972). As these two sulfur-containing amino acids were among the more deficient nutrients in many poultry rations, the need for the estimation of these two substances was badly realized.

Some existing methods (Lavin, 1943; Toennis and Callan, 1939; Albanese *et al.*, 1944) were tried for the analysis of methionine in the compounded poultry rations and found unsuitable. The paper describes a simple method of methionine estimation by ascending thin-layer chromatography after necessary purification. Cystine was determined as cysteic acid using the column chromatographic method of Schram *et al.* (1954) and gave good results. This method was based on the performic acid oxidation of cystine followed by ion-exchange chromatography.

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ESTIMATION OF METHIONINE

Reagents and Apparatus. Thin-layer plates consisted of thin layers of 0.4 mm thickness on 20 × 10 cm frosted glass plates using silica gel (Gouri Chemical, 28/3D Haray Kristo Sett Lane, Calcutta 50, India), and dried overnight at room temperature. The solvent system consisted of a mixture of *n*-butyl alcohol, glacial acetic acid, and water (1:4:1, v/v). The developing reagent was a 0.1% solution of ninhydrin in *n*-butyl alcohol. Solutions of L-methionine hydrochloride in the range of 0.030–0.185 mg/ml in water were used as standards.

Methods. *Preparation of the Sample.* Finely ground fat-free material was hydrolyzed with 6 *N* HCl for 18–24 hr at 110°. The contents were then cooled to room temperature

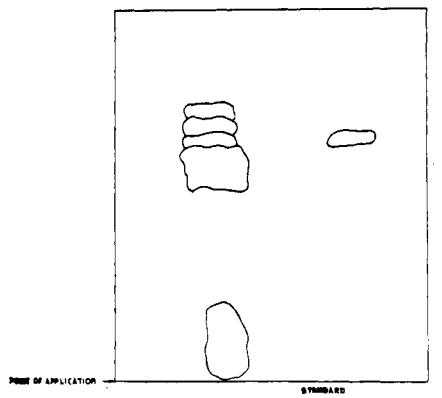

Figure 1. Thin-layer chromatogram of the feed hydrolysate (after extraction with ether) for the estimation of methionine with the solvent system butanol–acetic acid–water (1:4:1).

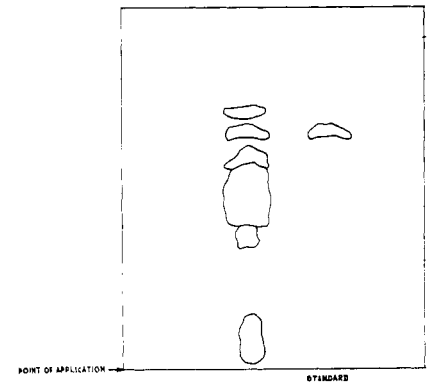

Figure 2. Thin-layer chromatogram of the feed hydrolysate (after extraction with ether and then passing through a Dowex column) for the estimation of methionine with the solvent system butanol–acetic acid–water (1:4:1).

and filtered. After washing the residue with a minimum amount of distilled water several times, the filtrate plus washings were evaporated to dryness. The residue was again taken up with a minimum of water and reevaporated. This process was repeated three–four times to remove most of the HCl. Finally the dried residue was transferred to a volumetric flask and made up to a definite volume with distilled water.

Purification of the Sample. In a separatory funnel, 2 ml of the feed hydrolysate was extracted with ether three–four times to remove ether-soluble amino acids. The aqueous phase was evaporated to dryness and the residue was dissolved in 2.5 *N* HCl. The prepared solution was then introduced in a 10 × 1 cm Dowex-1 column (300–400 mesh), previously equilibrated with 2.5 *N* HCl. Fractions (2 ml) of the effluent were collected manually at a flow rate of 20 ml/hr using 2.5 *N* HCl as eluent. Discarding two–three tubes, all the fractions of the next 12 tubes were collected and mixed together. The number of tubes collected for the methionine portion from the Dowex-1 column was characterized by passing a standard solution of L-methionine hydrochloride in 2.5 *N* HCl at the same flow rate stated above and observing the color reaction noted by McCarthy and Sullivan (1944). The mixed fraction was then evaporated to dryness and the residue was taken up in 2 ml of distilled water. The solution was then subjected to thin-layer chromatography.

Thin-Layer Chromatography. Samples and standards (0.01 ml) were applied to the silica gel plates using a chromaplot. The chamber was covered and, before introducing the tlc plates, it was allowed to equilibrate with the freshly prepared solvent mixture for at least 1 hr. The spots were air dried and the plates were introduced into the chamber. After 2.5 hr the plates were removed from the chamber and allowed to air-dry. When the plates were perfectly dried, they were carefully sprayed with ninhydrin reagent for developing the spots and kept in an air-oven at 110° for 5–10 min. Methionine spots were separated as a purple color and characterized with the help of standards.

Quantitative Estimation. For quantitative estimation, the methionine spots were scrapped out carefully in centrifuge tubes and 4 ml of 1% Na_2CO_3 solution was added in each tube. The tubes were heated to 50–60° in a water bath for 15–20 min and cooled at room temperature. The color of the centrifugate was read at 560 mμ on a Beckman DB-G spectrophotometer. A calibration curve was prepared with the help of a standard solution of L-methionine hydrochloride and the amount of methionine present in the feed hydrolysate was calculated.

Table I. Methionine Content of Compounded Poultry Rations by Thin-Layer Chromatography and the Horn *et al.* Method

Ration	% protein[a]	% methionine[a] Thin-layer method	% methionine[a] Horn *et al.* method
H	19.7	0.42	0.43
G	19.1	0.41	0.415
P	18.2	0.33	0.35
D	18.8	0.38	0.395

[a] Values given are on a moisture-free basis.

Table II. Recovery Experiments by Adding Known Amount of Methionine in the Feed Hydrolysates

Ration	Methionine Added, mg	Added amount found, mg	Recovery, %	Mean recovery, %
H	0.4	0.386	96.5	
	0.7	0.672	96.0	
G	0.6	0.582	97.0	
	0.9	0.875	96.7	
				96.41
P	0.5	0.480	96.0	
	0.8	0.768	96.3	
D	1.0	0.963	96.3	
	2.0	1.91	96.5	

Results and Discussion. When the aqueous phase of the feed hydrolysate was subjected to thin-layer chromatography after separating with ether, the separation of methionine from the other free amino acids was not good (Figure 1). A better separation was obtained when the ether extracted feed hydrolysate was subjected to thin-layer chromatography after passing through a Dowex-1 column (Figure 2).

Table I shows the percentage of methionine content of the compounded poultry rations, procured from the local market. These results were compared to the estimated values obtained by the method of Horn *et al.* (1946). It was observed that the values obtained by the two methods showed no significant differences. The advantage of the present method over the method of Horn *et al.* was also noted. It was observed that interference due to histidine and tryptophan (Csonka and Denton, 1946) could not be fully avoided in some experiments although phosphotungstic acid was used to remove these two amino acids. In the present method no such interference was observed.

Recovery experiments were also carried out by adding known amounts of pure methionine hydrochloride to the feed hydrolysates. Table II shows that the mean recovery percentage of methionine was found to be 96.41. The

Table III. Cystine Content of Compounded Poultry Rations by the Methods of Schram *et al.* and Goa

Ration	% cystine[a]	
	Schram *et al.*	Goa
H	0.32	0.28
G	0.34	0.30
P	0.29	0.26
D	0.36	0.33

[a] Values given are on a moisture-free basis.

Table IV. Recovery Experiments by Adding a Known Amount of Cysteic Acid in the Feed Hydrolysates

Ration	Cysteic acid			
	Added, mg	Added amount found, mg	Recovery, %	Mean recovery, %
H	0.2	0.197	99.5	
	0.4	0.397	99.2	
G	0.6	0.594	99.0	
	0.8	0.796	99.5	99.28
P	0.3	0.298	99.4	
	0.5	0.496	99.2	
D	0.7	0.695	99.3	
	0.9	0.892	99.1	

method is simple and probably will give satisfactory results with other types of material.

ESTIMATION OF CYSTINE

Reagents and Apparatus. Performic acid was prepared by the addition of 1 vol of 30% (w/w) H_2O_2 to 9 vol of 88% (w/w) formic acid. Standard solutions of 0.1–0.3 μg of cysteic acid/ml in 0.1 *N* chloroacetic acid were prepared.

Methods. Performic acid (30 ml), previously cooled to 0°, was added to the finely ground feed. The flask with the contents was kept immersed in an ice–water bath which was placed in a refrigerator for 18 hr. At the end of the reaction, 3–4 ml of 48% hydrobromic acid was added and the vessel swirled in an ice bath to remove excess performic acid (Moore, 1963). The solution was concentrated at 30–40° using a flash evaporator. The syrupy residue was then hydrolyzed with 6 *N* HCl for 20 hr. After filtering the hydrolysate, the filtrate was evaporated to dryness and taken up with 0.1 *N* chloroacetic acid. The pH was adjusted to 5 by using 0.05 *N* NaOH. The prepared hydrolysate (2 ml) was then chromatographed in a 0.9 × 15 cm Dowex-2 (200–400 mesh) column of the chloroacetate form. The effluent was collected in 1-ml fractions at 5 ml/hr using 0.1 *N* chloroacetic acid as eluent and analyzed by the photometric ninhydrin method of Moore and Stein (1948).

Results and Discussion. Table III shows that the cystine contents of the four samples designated as H, G, P, and D were found to be 0.32, 0.34, 0.29, and 0.36%, respectively, when the method of Schram *et al.* (1954) was applied. The protein contents of these rations were already described in Table I. Recovery experiments with cysteic acid were carried out using the same ion-exchange resin. The results (Table IV) revealed that an average recovery of 99.28% was possible. All the rations were again estimated for cystine plus cysteine according to the method of Goa (1961). These determinations were performed to see how these results agreed with those of Schram *et al.* (1954). The data furnished in Table III indicated that the method of Schram *et al.* gave higher results than that of Goa. It is already reported that Goa (1961) obtained 100% recovery of cystine by his method, but 99.28% recovery of cystine was also obtained according to the method of Schram *et al.* in the present investigation. Comparing these two values it can be stated clearly that the method of Schram *et al.* with slight modification was superior to that of Goa in the determination of cystine content of poultry rations, as the former method gave better results.

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