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### THE ANALYST

## PROCEEDINGS OF THE SOCIETY OF PUBLIC ANALYSTS AND OTHER ANALYTICAL CHEMISTS

### Deaths

WITH deep regret we record the deaths of the following members:

Mr. Frank George Edmed, a former Member of Council (January 22nd).

Sir Gilbert Morgan, Honorary Member (February 1st).

Dr. Frank T. Shutt, Formerly Dominion Chemist, Ottawa (January 5th).

# The Phytic Acid Content of some Poultry Feeding Stuffs

By R. H. COMMON, Ph.D., A.I.C.

In view of the probable significance of phytic acid in the calcium-phosphorus metabolism of the fowl (Lowe, Steenbock and Krieger, Common<sup>2</sup>) the phytic acid phosphorus content of a number of feeding stuffs as sold for poultry feeding has been determined in this laboratory. The method adopted was essentially that of McCance and Widdowson, modified with a view to convenience when large numbers of analyses are being made simultaneously.

A quantity of the feeding stuff containing about 4 to 8 mg. of phytic acid phosphorus was dried at 100° C. and extracted for three hours with 100 ml. of N/2 hydrochloric acid in an end-over-end shaker. The extract was filtered, and 25 ml. of the filtrate were pipetted into a 50-ml. graduated flask, neutralised to phenolphthalein with 25 per cent. sodium hydroxide solution, re-acidified with a few drops of N/2 hydrochloric acid, made up to the mark and mixed. portions (usually 20 ml.) for duplicate determinations were pipetted into 50-ml. centrifuge tubes and made up to 20 ml. with water when necessary. Four ml. of a solution of ferric chloride in N hydrochloric acid, containing 1.0 g. of iron per litre. were then introduced into each tube, and the tubes were heated in the water-bath Each tube was then cooled and centrifuged, the supernatant for fifteen minutes. liquid was decanted from the precipitate of ferric phytate, and the tube was drained and dried inside with filter-paper. The precipitate was broken up with 2 to 4 ml. of N/2 hydrochloric acid and re-centrifuged, the wash liquid being poured off and the inside of the tube drained and dried as before.

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The precipitate was broken up by blowing in 2 ml. of water from a fine pipette, and decomposed by heating for fifteen minutes on the water-bath after further addition of 2 ml. of 2 per cent. sodium hydroxide solution. The contents of the tube were filtered through a 7-cm. filter-paper (Whatman No. 40) into a 50-ml. silica dish, hot water being used for washing. Two ml. of 20 per cent. w/v calcium acetate solution were then mixed with the filtrate, the mixture was dried at  $100^{\circ}$  C. overnight in the electric oven, and the residue was gently ignited in an electric muffle to a white ash. This was taken up by heating with about 6 ml. of 2N hydrochloric acid and washed with hot water through a filter into a 100-ml. graduated flask. The contents of the flask were nearly neutralised with 25 per cent. sodium hydroxide solution and cooled, and phosphorus was determined directly by the method\* of Fiske and Subbarrow.

From time to time the silica dishes used in the determination should be cleaned with hydrofluoric acid.

Table I

Comparison of Perchloric Acid Oxidation and Dry Ashing
Techniques in Phytic Acid Phosphorus Determinations

		Phytic acid phosphorus				
		By wet ashing with sulphuric and perchloric acids Per Cent.	By dry ashing with calcium acetate Per Cent.			
Wheat meal		$\begin{array}{c} 0.264 \\ 0.256 \end{array}$	$\begin{array}{c} 0.262 \\ 0.262 \end{array}$			
Maize meal	• •	$0.192 \\ 0.213$	$0.257 \\ 0.259$			
Pollard	• •	$\begin{array}{c} 0.194 \\ 0.209 \end{array}$	$0.250 \\ 0.254$			

The modified method was tested on a sample of pure sodium phytate prepared by Professor D. C. Harrison. The total phosphorus of the sample was found to be 10.8 per cent. and the phytic acid phosphorus to be 10.7 per cent., so that the method is capable of giving a recovery of phytic acid phosphorus of the order of 98 to 99 per cent.

The aliquot part of the phosphate solution under examination, which should contain about 0.4 mg. of phosphorus, and be only slightly acid, is diluted to about 75 ml., treated first with 10 ml. of reagent (a) and then with 4 ml. of reagent (b), shaken and made up to 100 ml. The final acid concentration is about 0.5 N.

The standard for comparison is prepared by mixing 5 ml. of the original standard phosphate solution, 70 ml. of water and 10 ml. of the molybdate reagent (a), adding 4 ml. of reagent (b) and making up to 100 ml. The colorimetric comparison is made after about 30 minutes. If the aliquot portion contains up to  $1\cdot0$  mg. of phosphorus the comparison is made against 10 ml. of standard phosphate solution.

<sup>\*</sup> Fiske and Subbarrow's Colorimetric Method.—The following reagents are required:—(a) Molybdate solutions.—A solution of 25 g. of ammonium molybdate in 400 ml. of water is added to 500 ml. of 10N sulphuric acid and made up to 1 litre with water. (b) 1:2:4-aminonaphthol sulphonic acid reagent.—0.5 g. of the acid, 30 g. of sodium bisulphite and 6 g. of sodium sulphite (crystals) in 250 ml. of water. The standard phosphate solution is prepared by dissolving 0.3509 g. of potassium dihydrogen phosphate in water, adding 10 ml. of 10 N sulphuric acid and making up to 1 litre (5 ml. contain 0.4 mg. of phosphorus).

The modified method was also tested against the original method of oxidation with sulphuric and perchloric acids. In some instances agreement was good, but in others the perchloric acid oxidation seemed to give more erratic results, as may be seen from Table I. This may have been due to the difficulty of removing the last traces of perchloric acid; traces of perchloric acid have been shown to interfere with colour development in the method of Fiske and Subbarrow (Snook).5

Experiments were carried out on the recovery of phytic acid phosphorus The recovery was satisfactory, as may be seen added to extracts of meals. from Table II.

TABLE II Recovery of added Phytic Acid Phosphorus from N/2HYDROCHLORIC ACID EXTRACT OF WHEAT

	Phytic acid
	phosphorus found
Solution	mg.
20 ml. of $N/2$ HCl extract of wheat	1.245
20 ml. of $\dot{N}/2$ HCl extract of wheat	
+ 5 ml. of sodium phytate solution	1.760
5 ml. of sodium phytate solution	0.525
$Recovery = \frac{(1.760 - 1.245)}{0.525} \times$	100 = 98 per cent.

Total phosphorus was determined by a modification of the method of Fiske and Subbarrow, which has been described elsewhere (Common<sup>6</sup>).

Table III gives maximum and minimum figures for the phytic acid phosphorus contents of samples of cereals and other feeding stuffs, and Table IV records the results obtained with typical samples of the different products. The origin of most of the samples was known.

TABLE III

		Phytic acid phosphorus Per cent. on dry matter		Total phosphorus Per cent. on dry matter		Phytic acid phosphorus Per cent. of total phosphorus	
		Max.	Min.	Max.	Min.	Max.	Min.
Wheat (11 samples)		0.305	0.111	0.418	0.204	$77 \cdot 6$	$52 \cdot 9$
Oats (5 samples)		0.312	0.218	0.505	0.365	$74 \cdot 3$	$59 \cdot 1$
Barley (3 samples)		0.217	0.178	0.369	0.328	$59 \cdot 2$	$54 \cdot 3$
Yellow maize meal							
(3 samples)		0.314	0.277	0.407	0.364	$78 \cdot 3$	$76 \cdot 1$
Bran (6 samples)		1.030	0.512	1.571	0.571	77.7	$59 \cdot 3$
Extracted soya bean		0.000	0.941	0.671	0.044	~0.0	<b>50.0</b>
(3 samples)	• •	0.388	0.341	0.671	0.644	53.9	$52 \cdot 9$

Phytic acid phosphorus accounted for about two-thirds to three-quarters of the total phosphorus of wheat, oats, barley and maize. The samples of barley examined tended to have a lower proportion of their phosphorus in the form of phytic acid than did the samples of wheat. About the same ratio of phytic acid phosphorus to total phosphorus was found in bran as in pollard of grades similar to "weatings." Both total phosphorus and phytic acid phosphorus were lower in the Australian wheats than in the British wheats, in agreement with the observations of Snook (1938). Rice bran meal, tapioca root flour and hemp-seed all had about one-third of their phosphorus in the form of phytic acid phosphorus. Earthnut cake, extracted soya bean meal, maple peas, beans, millet and sunflower seed had from one-half to three-quarters of their total phosphorus in the form of phytic acid phosphorus. Dried grass contained only a very small proportion of its total phosphorus as phytic acid, alfalfa meal containing a greater proportion.

proportion.		_				
		$T_A$	BLE IV			
T. V. 4 6		Disconficial	Crude fibre Per cent. on dry	Phytic acid phosphorus Per cent. on dry	Total phosphorus Per cent, on dry	Phytic acid phosphorus Per cent. of total
Feeding stuff		Place of origin	matter	matter	matter	phosphorus
Wheat meal				0.301	0.458	$65 \cdot 7$
Oatmeal		Ireland	11.24	0.242	0.355	68.3
Oats	• •	Ireland (Co. Down)	_	0.272	0.434	$62 \cdot 7$
Sussex ground oats		Ireland		0.218	0.365	59.7
Pinhead oatmeal		Ireland		0.299	0.495	60.5
Barley meal			5.03	0.216	0.355	61.0
Yellow maize		River Plate		0.276	0.368	$75 \cdot 0$
,, ,,		N. America		0.149	0.199	75.0
Bran		Australia	10.92	0.512	0.676	75.8
,,		Canada	12.54	0.974	1.258	77.5
,,		Holland	12.36	1.030	1.571	$65 \cdot 5$
Pollard		-	10.43	0.277	0.350	$79 \cdot 1$
White sharps			7.55	0.312	0.467	66.8
Fine pollard		River Plate	7.19	0.962	1.238	$77 \cdot 7$
Coarse pollard		River Plate	10.53	0.760	1.044	$72 \cdot 8$
Middlings		Holland	6.98	0.481	0.936	$51 \cdot 3$
Fine white sharps			7.03	0.364	0.558	$65 \cdot 3$
Rice bran meal		(E. Indies?)	6.66	0.577	1.558	37.0
Tapioca root flour			1.91	0.028	0.083	33.8
Earthnut cake				0.402	0.668	$60 \cdot 2$
Dried yeast				0.058	1.514	3.8
Alfalfa meal		Canada		0.027	0.131	20.5
Dried grass		Ireland		0.007	0.351	$2 \cdot 0$
Millet		Smyrna		0.206	0.289	71.2
Sunflower seed		Hungaria	*****	0.374	0.453	$82 \cdot 8$
Hempseed		Manchuria		0.285	0.692	41.2
Beans		England		0.501	0.662	75.7
Maple peas		Tasmania		0.194	0.334	$58 \cdot 1$

Small amounts of phytic acid were found in dried yeast. This presumably came from the malt, since phytic acid has not been recorded among the phosphorus compounds present in yeast (Macfarlane<sup>7</sup>).

It is evident that in an ordinary poultry ration about three-quarters of the phosphorus derived from cereals will be in the form of phytic acid. Experiments designed to ascertain the minimum calcium and phosphorus requirements of poultry should take cognizance of this fact.

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AGRICULTURAL CHEMISTRY DEPARTMENT

THE QUEEN'S UNIVERSITY, BELFAST