Rapid Determination of Calcium in Feedstuffs

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The back titration method with EDTA for the determination of calcium in feedstuffs has been compared to the classical oxalate method. The back titration method gave significantly lower results than the oxalate method for feed samples but not for bone samples. Standard curves obtained by both methods of analysis did not differ significantly and demonstrated that the difference between methods could be corrected by the establishment of a standard curve with the back titration method. The back titration method is simple and much faster than the oxalate method in this laboratory, especially when a small number of samples are analyzed.

THE CLASSICAL METHOD of oxalate precipitation and subsequent permanganate titration for the determination of calcium in feedstuffs is tedious and time consuming. Attempts to simplify this determination in other materials have included the use of flame photometry and chelatometric titration. Magnesium interference is a problem in the latter method but can be overcome by the use of high pH and indicators specific to calcium at this pH. Phosphate interference, a problem in both methods, has been eliminated by ion exchange (*3*, *5*), precipitation (*4*), masking (*6*), and back titration (*2*, *8*).

This paper describes a method for the determination of calcium in feedstuffs based on the EDTA back titration method. It is an adaptation of many methods previously described for other materials.

Experimental

Reagents. Calcein indicator solution. Dissolve 4.0 mg. of dye in 25 ml. of 0.25*N* KOH and store in a brown bottle under refrigeration. Solution is stable for 1 month.

Standard EDTA solution. Dissolve 5.579 grams of dried disodium ethylenediaminetetraacetic acid in 1 liter of distilled water. Standardize against 0.04*M* and 0.005*M* calcium chloride solutions and store in polyethylene bottles.

Recovery experiments were performed with added calcium and phosphate. Calcium was added as calcium chloride solution while the phosphate was dissolved monobasic sodium phosphate. Standard curves were obtained with solutions of tricalcium phosphate. All chemicals were of reagent grade.

Procedure. Samples of mixed feeds were taken into solution according to the A.O.A.C. procedure (*1*). Aliquots (1 ml.) were transferred to 21 × 70 mm., flat-bottomed vials, and an excess of EDTA (3 ml.) was added. The pH of the solution was adjusted to approximately 13 by the addition of 3*N* KOH (5 ml.). Indicator (2 drops) was added and the excess EDTA titrated with 0.005*M* Ca solution from a microburet. The titration was carried out with magnetic stirring and illumination from a long wave ultraviolet lamp (3660 A.) so that the operator could see only the fluorescence from the solution. A dull background and darkened room increased the sensitivity of the end point which was the persistence of fluorescence.

Six samples of feed were analyzed by the oxalate method (*1*) and the back titration method. Methods of analysis were compared by recovery experiments and by determining the standard curves of both methods using solutions of calcium phosphate up to concentrations of calcium which were equivalent to 6% calcium feeds. The effect of excess phosphate was tested by raising the concentration of phosphate to 15 times that of the calcium present and redetermining the calcium present. All analyses were replicated five times.

Since bone analysis had been reported (*8*) to be satisfactory by the back titration method, six fat-extracted, left tibias from mature hens were dry ashed, dissolved, and included in some of the tests.

Results and Discussion

Table I shows that the values for the feed samples obtained by the back titration method were consistently lower than by the oxalate method. Analysis of variance (*7*) of these data (Table II) showed that the methods gave significantly different ($P = 0.01$) results for the calcium content of the feed samples but not of the bone samples. A correction for this difference could be made by establishing the relationship (standard

Table I. Calcium Content of Feed and Bone Samples Determined by the Oxalate and Back Titration Methods

Feed Sample	Oxalate Method, % Ca ± Std. Error	Back Titration Method, % Ca ± Std. Error	Difference, % Ca
1	2.282 ± 0.0040	2.260 ± 0.0008	−0.022
2	2.931 ± 0.0029	2.902 ± 0.0008	−0.029
3	4.069 ± 0.0039	4.065 ± 0.0013	−0.004
4	2.348 ± 0.0016	2.334 ± 0.0019	−0.014
5	3.131 ± 0.0048	3.101 ± 0.0020	−0.030
6	4.183 ± 0.0051	4.152 ± 0.0007	−0.031
Mean	3.157	3.136	−0.021
Bone Sample			
1	25.493 ± 0.0266	25.504 ± 0.0149	+0.011
2	22.118 ± 0.0101	21.937 ± 0.0114	−0.181
3	25.226 ± 0.0396	25.359 ± 0.0185	+0.133
4	23.533 ± 0.0479	23.365 ± 0.0050	−0.168
5	24.309 ± 0.0107	24.196 ± 0.0145	−0.113
6	23.547 ± 0.0215	23.313 ± 0.0047	−0.234
Mean	24.038	23.946	−0.092

Table II. Analysis of Variance of Results in Table I

Source of Variance	Degrees of Freedom	Mean Squares: Feed samples	Mean Squares: Bone samples
Total	59	...	...
Subgroup	11	3.0535	7.73190
Sample	5	6.71606[a]	16.93349[a]
Method	1	0.00670[b]	0.11625
Sample × method	5	0.00029	0.05343
Within sample	48	0.00004	0.00265

[a] Significant at $P = 0.001$.
[b] Significant at $P = 0.01$.

Table III. Recovery of Calcium Added to Feed and Bone Solutions

Sample	% Recovery ± Std. Error: Oxalate method	Back titration method
Feed		
1	99.63 ± 0.16	99.44 ± 0.04
3	100.04 ± 0.04	99.33 ± 0.03
5	99.80 ± 0.06	98.90 ± 0.03
Bone		
1	100.22 ± 0.08	100.11 ± 0.07
2	100.07 ± 0.10	100.08 ± 0.04
3	99.63 ± 0.08	99.90 ± 0.05

Table IV. Effect of Added Phosphate on the Determination of Calcium in Feed by the Back Titration Method

Feed Sample	Calcium Present, Mmoles	Phosphate Added, Mmoles	% Recovery ± Std. Error
1	0.02116	0.080	99.99 ± 0.09
1	0.02116	0.160	99.95 ± 0.09
1	0.02116	0.320	100.12 ± 0.04
3	0.04036	0.080	99.92 ± 0.03
5	0.03100	0.080	100.07 ± 0.05

curve) of volume of back titrant to known concentrations of calcium phosphate solution for each set of samples. This was checked by obtaining standard curves with both the oxalate and back titration methods. The respective equations of the standard curves for the oxalate and back titration methods were $\hat{Y} = 0.9780x - 0.0023$, and $\hat{Y} = 0.9902x - 0.0011$, while the respective standard deviations from regression (7) were 0.947×10^{-4} and 0.155×10^{-3}.

Statistical tests showed no significant differences between the two regression equations.

Recovery of known amounts of calcium added to the feed and bone solutions are presented in Table III. As in the feed analysis data of Table I, the standard error was lower with the back titration than with the oxalate method for both the feed and bone samples, which is contrary to the standard curve results. Recoveries with both methods were good and within the working range of our laboratory.

The effect of phosphate on the back titration method is presented in Table IV. The presence of a 15-fold concentration of phosphate did not influence the results provided the EDTA was added before the solution was made basic. Reversing this order of adding reagents resulted in varying results.

Literature Cited

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 9th ed., p. 292, 1960.
(2) Coolidge, T. B., *Anal. Biochem.* **1,** 93 (1960).
(3) Gehrke, C. W., Affspung, H. E., Lee, Y. C., *Missouri Univ. Agr. Expt. Sta. Res. Bull.* **570** (1954).
(4) Malmstadt, H. V., Hadjiioannou, T. P., J. AGR. FOOD CHEM. **7,** 418 (1959).
(5) Mason, A. S., *Analyst* **77,** 529 (1952).
(6) Middleton, K. R., *Ibid.*, **86,** 111 (1961).
(7) Snedecor, G. W., "Statistical Methods," 5th ed., p. 291, Iowa State College Press, Ames, 1957.
(8) Yalman, R. G., Bruegemann, W., Baker, P. T., Garn, S. M., *Anal. Chem.* **31,** 1230 (1959).

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TOMATO BY-PRODUCTS AS FEEDSTUFFS

Nutritive Value of Dried Tomato Pulp for Ruminants

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DRIED TOMATO PULP, unlike dried tomato pomace and certain other tomato feed by-products, consists of the whole cull fruit. The fruit has been cut and pressed, with the resulting juice concentrated by evaporation and remixed with the press cake for further dehydration. Tomato pomace has been shown useful in feeding fur-bearing animals (*10*), and both tomato pomace and pulp have been found useful for chickens (*7, 8*), but only limited data are available to indicate the nutritive value of tomato by-products for ruminants. Chapman *et al.* (*4*) reported satisfactory gains of yearling steers grazing pasture and consuming 16 to 19 pounds daily of a concentrate containing 10 to 30% tomato pulp. Tomato pulp replaced citrus pulp at 10 to 30% of the concentrate, and the steers fed tomato pulp yielded carcasses equal in grade and acceptability to those fed citrus pulp.

The present study was conducted to determine the nutrient composition and nutrient utilization of dried tomato pulp by steers and lambs.

Experimental Procedure

The first experiment was a conventional digestibility trial conducted with three 2-year-old steers. A 21-day preliminary feeding period was followed by a 7-day total fecal collection period. Nutrient digestibility of the tomato pulp was determined by difference by feeding both Alyce clover hay alone and a mixture of 30% Alyce clover hay and 70% tomato pulp. In the second experiment conducted with the same animals, the tomato pulp was gradually increased in the ration until 100% pulp was fed for 17 days. Total fecal collections were taken during the last 7 days of this period for obtaining digestibility coefficients. Twelve to 14 pounds of the rations were fed daily in two equal feedings which maintained animal body weight. In addition, 25 grams of defluorinated phosphate (4.25 grams P, 8.75 grams Ca) and 25 grams of trace mineralized salt (Table I) were fed daily. Water was provided *ad libitum* during the preliminary period but was supplied twice daily when the steers were in the metabolism stalls.

Ten native lambs averaging 72 pounds in body weight were used to obtain

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