Estimation of Methionine Hydroxy Analog in Mixed Feeds

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An analytical procedure for the hydroxy analog of methionine, DL-α-hydroxy-γ-methylmercaptobutyric acid, is required for the control of this supplement in commercial poultry feeds. In the method described, the analog is extracted from the feed with dilute HCl, and the clarified extract is assayed microbiologically with *Lactobacillus arabinosis*. The small amounts of the analog present in commercial feeds (0.05 to 0.10%) have been successfully determined.

THE WIDESPREAD supplementation of poultry feeds with the hydroxy analog of methionine, DL-α-hydroxy-γ-methylmercaptobutyric acid, calcium salt, has necessitated a method to estimate this compound.

In a previous procedure (*2*) for the determination of DL-methionine in mixed feeds, the free amino acid was extracted from the feed with water, and the amount present in the extract assayed microbiologically with *Streptococcus faecalis*. This method was inapplicable to the hydroxy analog because the calcium salt is difficult to extract, and the assay organisms for determining methionine do not respond to the analog. Holden, Wildman, and Snell (*1*) found that most of the hydroxy and keto acids corresponding to the essential amino acids supported growth of *Lactobacillus arabinosis*. Shiota and Clark (*3*) also showed that the hydroxy analog of methionine produced growth essentially equal to that of DL-methionine with this organism. The suitability of *L. arabinosis* for assay of the analog was confirmed, and excellent response curves (Figure 1) were obtained with an assay range from 0 to 40 or 50 μg. The extraction difficulty was overcome by using 0.01*N* HCl. This method has proved satisfactory for checking supplemented commercial poultry feeds.

Assay Procedure

Organism and Preparation of Assay Inoculum. *Lactobacillus arabinosis*, Strain 17-5, ATCC 8014 is used. Stock cultures are carried monthly on agar stabs of Difco microinoculum agar. About 10 ml. of Difco microinoculum broth are inoculated with a stab culture of the organism and incubated at 37° C. for 18 hours. After collection by centrifuging and washing, the cells are suspended in 10 ml. of sterile saline producing a turbidity with an absorbance between 0.13 and 0.14 at 550 mμ. A drop of this suspension is added to each assay tube for inoculation.

Assay Medium. The medium devised by Williams (*4*) for amino acid assays with *L. arabinosis* is also satisfactory for the analog. The composition of this medium is given in Table I.

Preparation of Assay Tubes. The medium is prepared at twice its final concentration and adjusted to pH 6.8. To matched 18 × 150 mm. culture tubes, 5 ml. of the double strength medium are added, diluted with the assay solution and water to 10 ml. They are capped and autoclaved for 5 minutes at 15 p.s.i. The tubes are then cooled,

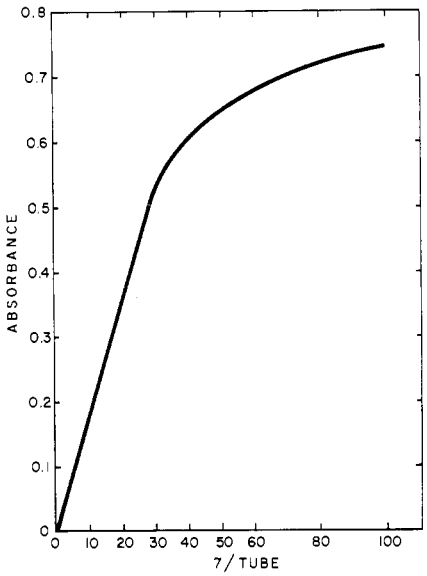

Figure 1. Response of *Lactobacillus arabinosis* to the methionine analog DL-α-hydroxy-γ-methylmercaptobutyric acid, calcium salt

Table I. Basal Medium

Amino Acids and Purines	*Weight of Ingredients for 250 Ml.*
	Mg.
L-Alanine	50
L-Arginine.HCl	100
L-Asparagine	50
L-Aspartic acid	50
L-Cysteine.HCl	25
L-Cystine	25
DL-Glutamic acid	250
Glycine	100
L-Histidine.HCl.H_2O	100
L-Isoleucine	50
L-Leucine	50
L-Lysine.HCl	50
DL-Norleucine	50
L-Phenylalanine	50
L-Proline	50
L-Serine	50
L-Threonine	50
L-Tryptophan	50
L-Tyrosine	50
L-Valine	50
Adenine	5
Guanine	5
Uracil	5
Vitamins	*μg.*
Folic acid	2.50
Biotin	1.25
p-Aminobenzoic acid	50
Thiamine.HCl	250
Riboflavin	250
Pyridoxal	500
Ca-pantothenate	250
Niacin	500
Pyridoxamine.HCl	500
Salts and Glucose	*Mg.*
Salts A	
K_2HPO_4	250
KH_2PO_4	250
Salts B	
$MgSO_4.7H_2O$	100
NaCl	5
$FeSO_4.7H_2O$	5
$MnSO_4.4H_2O$	5
	Grams
Glucose	10
Sodium acetate.$3H_2O$	10

Table II. Standard Curve Responses

μg./Tube	DL-Hydroxy Analog, Absorbance at 42 Hours	L-Methionine, Absorbance at 20 Hours
0	0.015	0.016
5	0.105	0.126
10	0.207	0.233
20	0.372	0.418
30	0.520	0.484
40	0.600	0.533
50	0.640	0.577
60	0.672	0.599
80	0.719	0.647
100	0.740	0.702

Estimation of Analog in Diets

	Diet 9				Diet 24	
	Basal + 0.114% Analog				Basal + 0.05% Analog	
	Extracted, 0.5 Gram in 50 Ml.				Extracted, 1 Gram in 50 Ml.	
	Extract 1		Extract 2			
Ml.	Absorbance	μg./ml.	Absorbance	μg./ml.	Absorbance	μg./ml.
0.5	0.129	12.6	0.119	11.0	0.109	10.0
1.0	0.248	13.0	0.238	12.3	0.202	0.3
1.5	0.347	12.2	0.324	11.5	0.277	9.9
2.0	0.421	11.3	0.420	11.3	0.351	9.3
2.5	0.502	11.4	0.482	10.8	0.419	9.0
3.0	0.534	10.5	0.548	11.0	0.459	8.4
4.0	0.614	10.7	0.593	9.9	0.555	8.5
	Av.	11.6	Av.	11.1	Av.	9.34
		0.116%		0.111%		0.046%

Table III. Comparison of Growth Responses of Isomers and Assay of Diet 29

	Standard Curve Responses			Assay of Diet 29 Basal + 0.05% Analog Extracted, 1 Gram in 50 Ml.		
	Absorbance at 23 Hours		Absorbance at 42 hours,			
μg./tube	L-Methionine	DL-Methionine	DL-analog	Ml.	Absorbance	μg./ml.
0	0.017	0.011	0.015	0.5	0.113	9.4
5	0.147	0.095	0.116	1.0	0.209	9.5
10	0.276	0.214	0.221	1.5	0.274	9.2
20	0.452	0.436	0.390	2.0	0.356	9.0
30	0.545	0.536	0.544	2.5	0.435	9.0
40	0.614	0.621	0.632	3.0	0.508	9.0
50	0.690	0.676	0.687	4.0	0.610	9.1
60	0.699	0.694	0.701		Av.	9.17
80	0.710	0.732	0.745			0.046%
100	0.729	0.734	0.774			

inoculated, and incubated at 37° C. Growth is determined turbidimetrically after 40 to 44 hours of incubation with a Coleman Junior spectrophotometer or similar instrument at 550 mμ.

Preparation of Sample Extracts. The feed mixture to be analyzed is ground to obtain a uniform sample, and the methionine analog extracted with 0.01*N* HCl. For this purpose, 1 gram or less of the powdered feed is weighed into a glass-stoppered flask, 50 ml. of 0.01*N* HCl at room temperature (20° to 25° C.) are added, and the mixture is shaken at intervals for 10 minutes. The extraction flask is next heated at 90° C. for 10 minutes in a water bath to facilitate extraction and to coagulate the extracted proteins. After centrifuging the solution at 1500 r.p.m. for 15 minutes, the clear supernatant extract is poured off and neutralized to pH 7 with 1*N* NaOH. Neutralization causes cloudiness again, but a clear solution suitable for assay is obtained with 5 minutes additional centrifuging.

Standard Used. The assay standard for these tests is the compound used for feed supplementation, the DL-α-hydroxymethionine analog, calcium salt (Hydan, Du Pont DL-α-hydroxy-γ-methylmercaptobutyric acid, calcium salt, minimum purity 90%). An aqueous solution containing 20 μg. per ml. will supply a range of the standard between 5 and 100 μg. per tube. The tubes are prepared in duplicate. The clarified extracts of the diets are similarly assayed with increasing amounts between 0.5 and 5.0 ml.

Assay Results

Typical assays of several poultry diets are reported in the tables. In Table II, Diet 9 contained 0.114% analog; 0.116 and 0.111% were found. Diet 24 contained 0.05%, and 0.046% was found. Extracts of the basal diets containing no added methionine or analog produced slight but insignificant growth above that of the inoculated blanks. In the second assay (Table III), the growth response of the organism to L- and DL-methionine at 23 hours is compared with the response to the analog at 42 hours. At the shorter time interval, little growth occurs with the analog, and therefore, the type of methionine compound used for supplementation may be determined. *L. arabinosis* responds almost equally to the L- and DL-isomers.

Literature Cited

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