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An improved method for the determination of vitamin B? and vitamin B? by spectrophotometric means.

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AN IMPROVED METHOD FOR THE DETERMINATION OF VITAMIN B_1 AND VITAMIN B_2 BY SPECTROPHOTOMETRIC MEANS

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TITLE OF THESIS An Improved Method for the Determination of Vitamin B_1 and Vitamin B_2 by Spectrophotometric Means

APPROVED BY READING COMMITTEE COMPOSED OF THE FOLLOWING MEMBERS

C. C. Vernon

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DATE October 17, 1944

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TABLE OF CONTENTS

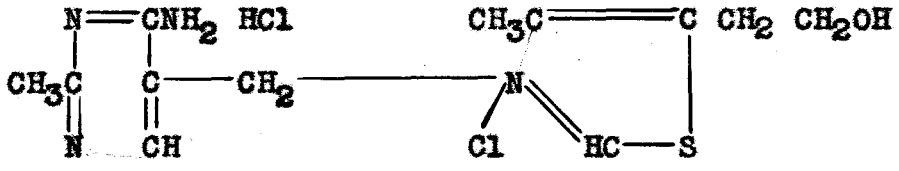
OBJECTIVE

Thiamine or vitamin B_1 has recently been established as one of the nation's most needed vitamins. With the addition of Thiamine to many food products, there has arisen a need for a rapid and accurate method of its determination. In this research the establishment of such a method has been attempted. It should be noted that before this research was completed, the A. O. A. C. had tentatively adopted a short procedure somewhat similar to the one used in this research.

There is also an urgent need for a rapid, accurate method of determining vitamin B_2. The development of a relatively simple procedure for this determination has also been attempted. Although the method gave fairly consistent results, it was not as successful as the B_1 determination.

INTRODUCTION

Vitamin B_1 is known as Thiamine Hydrochloride. Thiamine chloride; Anti-Beriberi or Anti-Neuritic Vitamin. Its chemical formula is $C_{12}H_{18}ON_4SCl_2$ and its molecular weight is 337.26. It contains 16.61% Nitrogen, 9.51% Sulfur and 21.02% Chlorine. This substance occurs in rice husks, cereal grains, yeast, milk, green leaves, roots and tubers. It was first obtained in crystalline form from rice polishings in 1926 by Jansen and Donath, and is now produced synthetically. Its structual formula is shown below.



It exists in the form of crystals or crystalline powder, and has a slight yeast like odor. It melts at 245-248°C. with some decomposition. One gram may be dissolved in about one cc. of water in 100 cc. of 95% alcohol, or in 18 cc. of glycerin. It is insoluble in ether and benzene, and has a pH of about 3.5. On exposure to air of average humidity, it absorbs an amount of water corresponding to nearly one mol., forming a hydrate. The article of commerce contains about 4% water, which is removable by drying at 100°C. or in a vacuum over sulphuric acid.

In the dry form vitamin B_1 is stable, and heating at 100°C. for 24 hours does not diminish its potency. In water solution it can be sterilized at 110°C., but if the pH of the solution is above 5.5, it is destroyed rapidly. One gram of crystalline vitamin B_1 is equivalent to 333,000 International Units.

Vitamin B_1 is used in correcting and preventing Beriberi and Anorexia; in securing optimum growth in infants and children, and in impaired lactation. Vitamin B_1 deficiency in man involves the nervous and circulatory systems. The minimum daily requirement for man has been estimated to be 0.5 to 1 milligram, while the optimum is 1 to 2 milligrams.

Riboflavin is commonly known as Lactoflavin, Vitamin G, or Vitamin B_2. Its chemical formula is $C_{17}H_{20}O_6N_4$ and its molecular weight is 376.19. It exists in the form of fine orange yellow needles, or powder, and melts at about 275°C. with decomposition. In the solid form it is not appreciably affected by diffused light, but in solution, and especially in alkaline solutions, it is rapidly deteriorated on exposure to light.

One hundred ml. of water will dissolve 11 milligrams of this vitamin. It is less soluble in alcohol than in water; it is sparingly soluble in amyl acetate, phenol or cyclohexanol; is very soluble in dilute sodium hydroxide, and is insoluble in acetone, chloroform, ether and benzene. Its aqueous solution is a pale greenish yellow and has an intense green fluoresence, which disappears on the addition of an alkali or acid.

An average dose is thought to be between 3 and 5 milligrams per day, with a minimum daily requirement of 2 milligrams.

Several photometric methods for the determination of vitamin B_1 and vitamin B_2 have been published. The short method for vitamin B_1, herein described, embodies the principles recorded by various authors in the literature, and also principles suggested by Dr. C. G. Harrell of Pillsbury Flour Mills.

Since the short photometric method gave satisfactory results for the determination of vitamin B_1, similar methods, with modifications, were employed in the determination of vitamin B_2.

A referee sample of flour was analyzed by the long extraction method for the purpose of comparing results to those obtained by the short extraction method.

EXPERIMENTAL FOR VITAMIN B_1

CALIBRATION OF THE SPECTROPHOTOMETER FOR THE DETERMINATION OF VITAMIN B_1

Several types of Spectrophotometers are available for photometric determinations. In this research a Coleman Universal Model 11 was used. All accessories were for this type apparatus.

The spectrophotometer was calibrated with standard solutions suggested by Dr. C. G. Harrell. (3)

It was necessary that the filters be in the proper position and that the Ultra Violet Illuminator, used in obtaining light of the desired wave length, be directed correctly into the sample.

An 8 to 10 volt storage battery was connected to the Ultra Violet Illuminator fitted with the filter (UV-1), used in the determination of vitamin B_1. The Illuminator was then placed directly over the sample chamber. The proper filter for screening the photoelectric cell (PC-1) was then placed in position. After the Illuminator had warmed up for about three minutes, the machine was ready for the photometric determinations.

Calibration of the Spectrophotometer

TABLE NO. 1

Calibration No.	Cuvettes	Quinine Setting	Reading for 1 ug. std.
1	U-11-S	100	19.0
			18.0
			20.5
2	U-11-E	100	25.0
			24.5
			23.5
			24.0
			22.5
			25.5
3	U-11-E	100	24.5
			23.5

From the data shown in Table 1, it was concluded that when the U-11-E cuvettes were used, one microgram of vitamin B_1 was equivalent to a reading of 23.0 to 26.0 units on the black scale of the fluorophotometer.

A blank determination was made using the empty cuvettes and no reading was obtained.

In a comparison test, both the isobutanol and distilled water gave a fluorescence reading of 2.0 on the galvonometer.

Calibration of U-11-E Cuvettes

TABLE NO. 2

Cuvette No.	Reading of Empty Cuvette	Reading when containing standard quinine soln.
1	10.8	73.5
		75.5
		74.0
2	10.8	72.0
		76.0
		74.5
3	10.5	72.0
		75.0
		74.0

Analyses of a standard vitamin B_1 solution

TABLE NO. 3

Standard Quinine Setting	Reading for 1 ug. of B_1
69.5	46.5
63.5	45.0
70.0	50.5
70.0	42.0
60.0	34.5
60.0	39.0

From the readings shown in Table 3 it was concluded that the spectrophotometer should be made more sensitive. This was accomplished by changing the angle of the mercury light.

Analyses of a standard B_1 solution after changing the mercury light angle are shown in Table 4.

TABLE NO. 4

Standard Quinine Setting	Reading for 1 ug. of B_1
70.0	50.0
70.0	49.0
70.0	50.0
70.0	49.6

SAMPLES USED FOR THE DETERMINATION OF VITAMIN B_1 BY THE PHOTOMETRIC METHOD

1. A refree sample of enriched patent flour
2. Enriched patent flour
3. Unenriched patent flour
4. Wheat middlings
5. Wheat bran
6. Wheat germ
7. Specially processed flour (Earle process)

DETERMINATION OF VITAMIN B_1 BY THE LONG EXTRACTION METHOD

The following steps were used for the determination of "Total Thiamine" by the long extraction method. (9)

The thiamine was extracted from the sample with 2% acetic acid.

After adjusting the pH and filtering, the thiamine was absorbed by passing the solution through a tube containing zeolite.

The thiamine was then eluated from the zeolite with boiling potassium chloride.

This eluate was treated with an oxidizing agent and isobutanol.

The fluorometric determination was then made on the dried isobutanol.

A blank determination was carried out by eliminating the oxidizing agent from the regular procedure.

Standardization with Pure Vitamin B_1

Since the fluorometric determination is primarily a comparison, an equivalent of one microgram of pure vitamin B_1 was carried through the same procedure as the unknown, and the

fluorometric readings compared.

The results obtained by the long extraction method listed above were compared to results obtained by the short extraction method in Table 5.

SHORT EXTRACTION METHOD FOR THE DETERMINATION OF VITAMIN B_1

The short extraction method, which had been recommended for "added" vitamin B_1 only, was carried out as follows:

Thiochrome Method for "Added" Vitamin B_1 (3)

The sample of enriched material was ground and thoroughly mixed. Three grams of the sample were then added to a 100 ml. volumetric flask containing 15 ml. of potassium chloride (25% potassium chloride in 0.1 normal hydrochloric acid). After the addition of the sample, another 65 ml. of the potassium chloride solution were added and the solution shaken continuously for five minutes. The sample was then diluted to the mark with water, mixed, and filtered through a dry quantitative filter paper. The first 10 ml. of the filtrate were discarded and the remainder collected. A 5 ml. aliquot of the filtrate was then placed in a reaction vessel containing 15 ml. of isobutanol. Three ml. of alkaline ferricyanide (29 ml. of 15% NaOH plus 1 ml. of 1% potassium ferricyanide - made fresh daily) were added and the sample shaken vigorously for sixty seconds. The reaction vessel was then centrifuged for forty-five seconds at 500 revolutions per minute. After centrifuging, the aqueous layer was drawn off and the remaining isobutanol dried with two grams of anhydrous sodium sulfate. The reaction vessel was again centrifuged for ten seconds to remove any sodium sulfate present. The sample was then decanted into a cuvette for a fluorescence reading on

the fluorophotometer.

The standard solutions used in this method are listed below.

A stock solution of vitamin B_1 was prepared by dissolving 0.1000 gram of pure vitamin B_1 (dried over sulphuric acid) in one liter of 20% ethanol. The pH was then adjusted to 3.5 with dilute hydrochloric acid.

A working solution was prepared by diluting 10 ml. of the stock solution to one liter with distilled water. A standard containing one microgram of vitamin B_1 in 5 ml. of solution was prepared by diluting 50 ml. of the working solution to 250 ml. with distilled water.

A stock quinine solution was prepared by dissolving 0.0108 gram of quinine sulfate in one liter of 0.1 normal sulphuric acid.

A working solution of quinine sulfate was then prepared by diluting 25 ml. of the stock solution to one liter with 0.1 normal sulphuric acid.

As in the procedure for the long extraction method, it was necessary to establish a fluorometric reading for one microgram of pure vitamin B_1.

A standard sample containing one microgram of vitamin B_1 was analyzed by the rapid method as follows:

Five ml. of the standard solution containing one microgram of vitamin B_1 were added to 15 ml. of isobutanol. Four ml.

of the KCl extraction solution and three ml. of the alkaline ferricyanide were then added and the procedure completed similiarly to an unknown sample. A blank determination was made by eliminating the addition of the alkaline ferricyanide solution from the above procedure.

The following calculations were used to convert the readings of the fluorophotometer to milligrams of vitamin B_1 per pound.

$$\frac{\text{1 microgram}}{\text{Reading vitamin } B_1 - \text{reading of blank}} = \text{K (constant)}$$

(Reading Sample-reading of blank) X K X 1,001 ÷ 333 = milligrams of vitamin B_1 per pound.

It was apparent upon examination of the procedures listed for the long and short extraction methods that the only difference existed in the preparation of the sample for the fluorometric determination.

After preparation of the sample, the fluorometric determinations were carried out in a similar manner.

The standard quinine solution was set at a certain reading and the unknown and blank values were determined. The values of a standard solution of vitamin B_1 and blank were then determined using the same quinine reading as was used for the unknown.

The amount of vitamin B_1 present was estimated by comparing the unknown reading to the reading obtained for the standard vitamin B_1 solution.

In order to check the reliability of the determinations made in this research, a specially prepared referee sample was sent to several other laboratories for vitamin B_1 analysis. The comparative results are shown in Table 5.

TABLE NO. 5

Laboratory	Sample	Location	Mg. of B_1 per lb. of flour
Mid West	1	Columbus, Ohio	2.04
Merck & Co.	1	Rahway, N. J.	1.59 Av. 1.82
W. E. Long Co.	1	Chicago, Ill.	1.82
This research			
Exp. No. 1	1	Long extraction method	1.81
2	1	" " "	1.52
3	1	" " "	1.11 Av. 1.38
4	1	" " "	1.01
5	1	" " "	1.43
6	1	Short " "	1.87
7	1	" " "	1.84
14	1	" " "	1.83 Av. 1.87
17	1	" " "	1.91
18	1	" " "	1.86
35	1	" " "	1.90

Analysis of flour samples using the long extraction method of Andrews and Nordgren. (9) These analyses were made for comparison to results obtained by the short extraction method. The comparison is made in Table 5, on the preceding page.

TABLE NO. 6

Exp. No.	Sample	Std. Quinine set.	Read. for 1 ug. B_1	Read. of Unknown	Mg. B_1 per lb. of flour
1	Referee (1)	50.0	83.8	Off Scale	
1	" "	30.0	50.4	91.0	1.81
2	" "	50.0	86.3	24.2	1.52
3	" "	50.0	73.5	15.0	1.11
4	" "	50.0	48.45	38.4	1.01
4	" "	80.0	75.7	58.9	1.01
5	" "	70.0	58.9	61.8	1.43

Analyses of flour samples using the short method of extraction for "Added" vitamin B_1. (A standard solution of vitamin B_1 was analyzed with each unknown until satisfactory technique was developed.)

TABLE NO. 7

Exp. No.	Sample	Std. Quinine set.	Read. for 1 ug. B_1	Read. of Unknown	Mg. B_1 per lb. of flour
6	Referee (1)	50.0	44.5	27.5	1.87
7	" (1)	70.0	50.0	30.4	1.84
8	Enriched (2) Pat. flour	50.0	36.6	23.7	2.01
9	" (2)	50.0	35.6	25.0	2.12
10	" (2)	50.0	27.5	20.0	2.16
11	" (2)	78.0	58.8	37.0	1.90
12	" (2)	76.5	53.0	37.3	2.12
13	" (2)	70.0	50.0	34.0	2.10
14	Referee (1)	80.0	48.8	25.7	1.83
15	Enriched (2) Pat. flour	70.0	45.7	31.5	2.09
16	" (2)	50.0	27.5	18.0	1.97
17	Referee (1)	80.0	62.5	37.5	1.91
18	" (1)	80.0	37.0	22.8	1.86
19	Enriched (2) Pat. flour	80.0	60.8	50.5	2.51
20	Dup. of #19	80.0	60.8	48.5	2.42
21	Enriched (2) Pat. flour	80.0	60.8	44.0	2.20
22	Dup. of #21	80.0	60.8	44.2	2.22

From the experimental data shown in Table 7 it was concluded that the natural vitamin B_1 present in flour could be determined by the short method of extraction.

The following table shows results obtained in the analysis of unenriched flour by the short extraction method.

TABLE NO. 8

Exp. No.	Sample	Std. Quinine set.	Read. for 1 ug. B_1	Read. of Unknown	Mg. B_1 per lb. of flour
23	Unenriched (3) Pat. flour	70.0	45.7	1.2	.08
24	" (3)	85.0	59.8	8.5	.43
25	" (3)	85.0	62.5	10.7	.50
26	" (3)	85.0	62.5	7.0	.36
27	Dup. of #26	85.0	62.5	6.9	.35
28	Dup. of #26	85.0	62.5	7.8	.45
29	Dup. of #26	85.0	62.5	5.9	.38
30	Unenriched (3) Pat. flour	95.0	63.5	4.0	.20

Since the short method of analysis had given satisfactory results on flours, it seemed possible that it could be used for the analysis of feeds.

Before the analysis of feeds was started, several determinations of a standard vitamin B_1 solution were carried out. These results are shown in Table 9.

The results of the analyses of feeds are shown in Table 10, with comparative results reported in the literature.

TABLE NO. 9

Standard Quinine Setting	Reading for 1 ug of B_1
80.0	64.0
80.0	65.5
80.0	66.0

Analysis of Feeds for Vitamin B_1 by the Short Extraction Method

TABLE NO. 10

Exp. No.	Sample	Std. Quinine set.	Read. for 1 ug. B_1	Read. of Unknown	I.U. B_1 per gr.	Literature Results
31	Wheat (4) middlings	80.0	65.1	58.0	6.2	6.2 (4)
32	Wheat (5) bran	80.0	61.88	35.0	2.65	2.0 (5)
						1.75 (6)
						3.44 (4)
33	Wheat (6) germ	80.0	61.88	62.0	7.1	6.5 (5)
						9.0 (4)
34	Dup. of #33	80.0	61.88	65.3	7.4	6.5 (5)
						9.0 (4)

During the course of the research, a small 1-1/2 volt battery, connected to the potentiometer, became exhausted. In the search for the trouble, the angle of the mercury light was changed. The data for the readjustment of the mercury light are shown in the following table.

TABLE NO. 11

Reading 1st adj.	Reading 2nd adj.	Reading 3rd adj.	Reading 4th adj.	Reading 5th adj.	Reading final adj.
120.0	115.0	67.0	70.5	76.0	85.0
121.6	112.0	67.5	74.0	76.0	84.8
119.5	110.0	68.0	68.5	78.0	85.0
123.0	108.0	70.5	71.5		86.0
121.3	102.0		67.5		
			68.0		
			66.5		

After replacing the 1-1/2 volt battery and readjusting the mercury light, several more samples of flour were analyzed. These analyses are recorded in the table below.

TABLE NO. 12

Exp. No.	Sample	Std. Quinine set.	Read. for 1 ug. B_1	Read. of Unknown	Mg. B_1 per lb. of flour
35	Referee (1)	80.0	64.7	38.7	1.90
36	Enriched (2) Pat. flour	80.0	64.7	43.0	2.12
37	" (2)	80.0	64.7	41.0	2.02
38	" (2)	80.0	64.7	40.0	1.98
39	" (2)	80.0	64.7	36.0	1.78
40	" (2)	80.0	50.8	30.7	1.90
41	" (2)	80.0	50.8	34.0	2.08
42	Earle Pro- (7) cessed flour	80.0	64.7	31.7	1.56
43	" (7)	80.0	64.7	31.2	1.55

At this time a modified long extraction method for the determination of vitamin B_1, somewhat similar to the method used in this research, was tentatively adopted by the A. O. A. C.

The method tentatively adopted resembled the method used in this research in the following ways:

Yeast samples could be analyzed with omission of the zeolite absorption step; and the production of the thiochrome was carried out in a solution of 25% potassium chloride.

The differences were found in the following steps:

Flours were analyzed by absorbing the vitamin B_1 on zeolite and then eluating.

In the short extraction method used in this research, the zeolite absorption step was completely eliminated. Omission of this step shortened the time required for the analysis considerably.

DISCUSSION AND CONCLUSIONS

Since the short extraction method had been recommended for added vitamin B_1 only, it was doubted that it would completely extract all the vitamin B_1 present. However, results of the analysis of a referee sample by the short extraction method were higher than results obtained by the long extraction method. The analyses of the referee sample by the short extraction method also agreed with the results obtained by several other laboratories.

The feeds analyzed by the short extraction method were well within the limits of error for the values listed in the literature.

Since results obtained by the short extraction method could be duplicated and checked, it was concluded that this method could well be adapted to the analysis of flours and feeds for vitamin B_1.

EXPERIMENTAL FOR VITAMIN B_2

CALIBRATION OF THE SPECTROPHOTOMETER FOR THE DETERMINATION OF VITAMIN B_2

The same type spectrophotometer was used, but different filters were used. The Ultra Violet Illuminator was fitted with filter No. UV-2, and the photoelectric cell was screened by filter No. PC-2.

The standard solutions used are listed under (13) in the experimental data.

Since the quinine sulfate solution used in the determination of vitamin B_1 could not be used for the adjustment of the mercury light, it was necessary to find a material that would fluoresce through the filters used in this determination. It was mentioned in the literature (13) that fluorescin possessed fluorescing properties similar to those of vitamin B_2. A solution of fluorescin was adjusted to such a concentration that the mercury light could be regulated and the fluorophotometer calibrated.

A standard solution containing one microgram of vitamin B_2 per ml. was made up as follows:

0.100 gram of pure vitamin B_2 was dissolved in 500 ml. of the acid-acetone mixture (13). Ten ml. of this solution were then diluted to 200 ml. with the acid-acetone mixture.

Calibration #1

Four ml. of a standard vitamin B_2 solution containing one microgram of B_2 per ml. were added to 46 ml. of the acid-acetone mixture. This sample was then refluxed and carried through the regular procedure.

The fluorophotometer was adjusted to the highest sensitivity obtainable by having both the "Fluor" knob and the "Galv" knob turned all the way clockwise.

Reading for standard	6.2
blank	0.0
	6.2

This reading was for an equivalent of 0.04 micrograms per ml. (Solution was made to 100 ml. instead of 200 ml.) The reading was too small to give satisfactory results.

Calibration #2

50 cc. of the acid-acetone mixture containing 50 micrograms of riboflavin were carried through the regular procedure and made to 100 cc.

Reading for standard containing 0.5 ug. of riboflavin per cc. 50.0

Blank 2.0

48.0

Reading for .25 ug. per cc. 25.0

Blank 2.0

23.0

It was believed that the concentration of the standard was too low, therefore, a new standard containing 4 ug. of riboflavin per cc. was prepared. (50 cc. of stock solution diluted to 250 cc. with the acid-acetone mixture.)

Readings for several ranges of the standard.

Reading for 1 ug.	132.0	
Blank	2.0	
	130.0	
Reading for 0.8 ug.	107.5	
Blank	2.0	
	105.5	
Reading for 0.6 ug.	79.0	
Blank	2.0	
	77.0	

SAMPLES USED FOR THE DETERMINATION OF VITAMIN B_2

1. Dried Skim Milk
2. Chick Growing Mash
3. Starting Mash
4. Growing Mash
5. Broiler Ration
6. Laboratory Prepared Growing Mash
7. Distiller's Solubles
    a. Produlac
    b. Di-Gra-Sol
    c. Samuel's
8. Fish Meal
9. Poultry Mash
10. Specially Processed Flour (Earle Process)

FLUOROMETRIC DETERMINATION OF VITAMIN B_2

Before the fluorometric determination of vitamin B_2 in an unknown could be carried out, it was necessary to separate the vitamin B_2 from any protein present. This was accomplished by the use of an acid-acetone mixture which presumably denatured and precipitated the protein.

"Because of its accuracy (2.2%), rapidity, and specificity, the fluorometric method is perhaps more suitable than either the biological or the colorimetric method."

The procedure used in this research for the determination of vitamin B_2 was similar to the procedure listed under (12), except that the standard B_2 solutions were made up in an acid-acetone mixture. An unknown sample was analyzed as follows:

Five grams of sample were weighed and placed in a condensing flask containing 50 ml. of the acid-acetone mixture.

The lumps were broken and the sample was gently refluxed for one hour.

The sample was then cooled and transferred to a 100 ml. flask. The condenser was then rinsed with 30 ml. of tri sodium phosphate, and the rinse solution added to the original solution.

The pH was then adjusted to 7.5 with additional tri sodium phosphate and the sample made to volume with water.

After thorough mixing, the sample was filtered through a qualitative filter paper and a 50 ml. aliquot pipetted into a 200 ml. volumetric flask.

The volume was made to around 175 ml. with water and 2 ml. of sodium hydrosulfite and 2 ml. of a stannous chloride solution added.

The solution was then mixed, made to volume, and allowed to stand for ten minutes.

The solution was then poured into a one liter Erlenmeyer flask and shaken with access to air for five minutes.

A suitable portion of the sample was then poured into a cuvette for a fluorometric determination.

In all experimental work, a standard solution of vitamin B_2 was carried through the same procedure as an unknown, and the fluorometric readings compared.

Analysis of Vitamin B_2 by the photometric method.

TABLE NO. 13

Exp. No.	Sample	Read. for Std. B_2 sol.	Read. of Unknown	Ug. of B_2 per gram	Calc. or Literature Value
1	Condensed milk (1)	.6 ug-80.0	36.0	36.0	20 to 30 (12)
2	Growing mash (2)	.125 ug-65.0	63.4	9.76	9.76
3	Starting mash (3)	.125 ug-65.0	59.0	5.68	
			60.0		
			58.0		
4	Growing mash (4)	.125 ug-65.0	57.5	8.72	
			56.0		
			56.8		
5	Starting mash (3)	.125 ug-65.0	74.0	7.75	
			73.0		
			74.0		
6	Broiler ration (5)	.125 ug-65.0	65.0	10.0	
			65.0		
			65.0		
7	Dup. of #6	.125 ug-65.0	64.0	9.68	
			63.0		
			61.0		
8	Growing mash (4)	.2 ug-107.0	64.2	9.6	

TABLE NO. 13 (Continued)

Exp. No.	Sample	Read. for Std. B_2 sol.	Read. of Unknown	Ug. of B_2 per gram	Calc. or Literature Value
9	Earle process flour (10)	.2 ug-107.0	21.7	2.0	1.7 to 2
10	Dist. solub. Di-Gra-Sol (7)	.2 ug-39.8	32.0	12.85	20 to 25
11	Dist. solub. Produlac (7)	.2 ug-39.8	25.1	10.1	
12	Dup. of #10	.2 ug-36.4	38.4	14.05	20 to 25
13	Fish meal (8)	.25 ug-43.3	31.5	14.55	
14	Dup. of #10	.25 ug-43.3	36.5	16.8	
15	Fish meal (8)	.2 ug-49.0	40.3	13.1	
16	Dist. solub. Produlac (7)	.2 ug-39.5	28.5	11.55	
17	Dist. solub. Samuel's (7)	.2 ug-49.0	38.75	15.3	
18	Dist. solub. Di-Gra-Sol (7)	.2 ug-46.5	46.4	16.0	
19	Poultry mash (9)	.2 ug-50.0	26.9	8.61	9 to 10 ug

In order to check the reliability of the vitamin B_2 determinations made in this research, two samples were sent to other laboratories for vitamin B_2 analysis. The comparative results are shown in the table below.

TABLE NO. 14

Exp. No.	Sample	Laboratory	Referee Results	Results obtained in this research
19	Poultry (9) mash	Merck & Co.	4.1 ug/gram	8.61 ug/gram
14	Dist. Solub. Di-Ora-Sol (7)	Lucidol Corp.	23.0 ug/gram	16.8
12	" (7)			14.05
10	" (7)			12.85

DISCUSSION AND CONCLUSIONS

The determination of vitamin B_2 by the fluorometric method was more difficult than the determination of vitamin B_1.

The greatest difficulty was experienced in eliminating fluorescing impurities.

As vitamin B_2 is destroyed by light, it is necessary to carry out the determinations with a minimum of light exposure.

Since the vitamin B_2 analyses obtained in this research did not compare favorably with analyses obtained by other laboratories, it was concluded that the procedure for the determination of vitamin B_2 used in this research was not satisfactory. However, further research in this field would be worth while.

SUMMARY

1. The methods for the photometric determination of vitamin B_1 and vitamin B_2 are recorded.

2. The calibration of a Coleman Spectrophotometer for the determination of vitamin B_1 and vitamin B_2 is reported.

3. A short method for the determination of vitamin B_1 is recorded and utilised in this research.

4. The experimental data for the determination of vitamin B_1 and vitamin B_2 are given.

5. The conclusions drawn from the experimental data are reported.

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