Vitamin B_6 Content of Feedstuffs

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The vitamin B_6 content of many animal feedstuffs obtained from representative geographic areas of the United States has been determined by microbiological assay with the yeast, *Saccharomyces carlsbergensis*. Data are presented illustrating the necessity for varying the extraction procedure to obtain maximum values for different feedstuffs. Where comparative data are available, the results agree well in most cases with values reported by others for similar feedstuffs in the United States, Canada, Switzerland, and Japan. Calculations have been made from these data of the possible range of the vitamin B_6 content of various mixed feed formulations. Considering the variability in potency of given feedstuffs and the factors that influence vitamin B_6 availability and animal requirements, supplementation with vitamin B_6 to provide a margin of safety in some types of commercial poultry rations appears justified.

At a recent symposium on vitamin B_6, emphasis was placed on the need for additional reliable data on the vitamin B_6 content of both foods (Sebrell, 1964) and feeds (Fuller, 1964) to aid in the assessment of the adequacy of human and animal diets with respect to this important vitamin. In the animal area the available data cover only a limited number of feedstuffs, and the results by the variety of assay methods used have not always been in agreement. Although it generally has been assumed that the vitamin B_6 content of feeds was adequate, changing conditions such as the introduction of new strains of poultry, increasing feed efficiencies, controlled growth conditions, and widespread use of medicated feeds have prompted renewed concern about adequacy of vitamin B_6 intake of both poultry and swine. The present study was undertaken to provide more comprehensive data on the vitamin B_6 content of feedstuffs currently available in the United States. On the basis of a careful review of the literature and our own experience, the microbiological assay using *Saccharomyces carlsbergensis* was chosen as the most reliable method available.

METHODS

The vitamin B_6 assay procedure used was that of Atkin *et al.* (1943) with the following modifications. Wort molasses agar slants of the following composition were used to grow the test organism, *S. carlsbergensis*, since these yielded much heavier growth than malt agar slants. (Wort agar, 18 grams; Bacto agar, 1 gram; black strap molasses, 0.36 gram; and boiling water, 300 ml.) An equivalent amount of sodium citrate was used in the buffer in place of potassium citrate as a matter of convenience.

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The assay medium was supplemented with niacin so that each tube contained 25 μg. to eliminate possible nonvitamin B_6 stimulation of the test organism (Hopkins and Pennington, 1947).

Atkin *et al.* (1943) found that maximum liberation of vitamin B_6 was usually obtained with 0.055*N* H_2SO_4 but that wheat and wheat products required a stronger concentration of acid—namely, 0.44*N*. Rabinowitz and Snell (1947) showed that HCl was as effective as H_2SO_4 in extracting vitamin B_6. In the present study both acid concentrations were used for the first one or two samples of each particular type of feedstuff to determine which of the two extraction methods yielded higher results. This method was then employed for the remainder of the samples of that type. Where no difference was found, 0.055*N* acid was used. Extractions were carried out by autoclaving at 127° C. with 180 ml. of 0.055*N* H_2SO_4 for 15 to 16 hours or 100 ml. of 0.44*N* HCl for 2 hours. The quantity of sample used was such that when the pH was adjusted to 5 and the extract diluted either to 250 ml. after extraction with 0.055*N* acid, or to 1000 ml. after extraction with 0.44*N* acid, the assay points fell in the range of the standard curve (5 to 40 ng.). All samples were filtered prior to addition to the assay tubes.

RESULTS

Comparative Effectiveness of Extraction with 0.055*N* *vs.* 0.44*N* Acid. In Table I the feedstuffs are categorized according to the relative effectiveness of the two acid extraction procedures in liberating vitamin B_6 from them. In a few cases the differences found are substantial, but in most instances they are relatively small. It was of interest to examine the pyridoxamine, pyridoxal, and pyridoxine contents of the few feedstuffs for which data are available to see whether this might be related to the type of extraction required for maximum liberation of vitamin B_6.

Table I. Comparison of $0.055N$ H_2SO_4 and $0.44N$ HCl Extraction Procedures for Vitamin B_6

	(1) $0.055N$ H_2SO_4, μg./G.	(2) $0.44N$ HCl, μg./G.	$\frac{(1)}{(2)} \times 100$, %
Feedstuffs Yielding Higher Results with $0.055N$ H_2SO_4			
Meat and bone meal, 50% protein	0.94	0.79	119
Fish meal,			
Peruvian	3.5	2.2	159
Menhaden	1.7	1.4	121
Brewers' dried yeast	26.5	22.3	119
Brewers' dried grains	3.1	2.6	119
Safflower meal, 20% protein	14.3	12.1	118
Peanut meal	4.5	4.0	112
Rapeseed	12.8	10.2	125
Feedstuffs Yielding Similar Results with $0.055N$ H_2SO_4 and $0.44N$ HCl			
Corn gluten meal	7.3	7.1	103
Corn, yellow	5.1	5.2	98
Milo	3.4	3.4	100
Cottonseed meal, 50% protein	6.7	6.8	98
Cottonseed meal	6.9	6.6	104
Barley, whole	4.4	4.5	98
Rice bran	5.3	5.4	98
Malt sprouts	11.4	11.8	97
Distillers dried grains	8.2	7.9	104
Oats	1.9	1.8	106
Linseed meal	9.1	8.6	106
Feedstuffs Yielding Lower Results with $0.055N$ H_2SO_4			
Soybean meal	6.7	7.4	91
Wheat mill run	10.4	11.1	94
Wheat bran	13.5	16.2	83
Dehydrated alfalfa meal	7.9	8.5	93
Hominy feed	8.5	9.2	92

A number of studies (Polansky *et al.*, 1964; Rabinowitz and Snell, 1948; Toepfer *et al.*, 1960, 1963) have revealed wide variations in the relative amounts of the three forms of vitamin B_6 from one material to another. There is some indication that $0.055N$ acid extraction is more effective where pyridoxamine is predominant (Brewers' dried yeast and fish meal) or where pyridoxal is the major component (meat and bone meal). In several cases where pyridoxine is the predominant form (wheat and soybean meal) $0.44N$ acid extraction is more effective, whereas in other such samples (barley and rice), similar results were obtained by both extraction methods.

Vitamin B_6 Content of Feedstuffs. The complete tabulation of the vitamin B_6 assays of various feedstuffs with *S. carlsbergensis* in the present study is shown in Table II together with the extensive data of Walter (1965) and other recent data (Midwest Research Institute, 1965; National Research Council, 1964; Polansky *et al.*, 1964; Waggle *et al.*, 1967; Yoshida and Morimoto, 1960). To facilitate the evaluation of these feedstuffs as sources of vitamin B_6, they are listed in Table III according to the following categories based on the vitamin B_6 content in μg. per gram: excellent (10 or greater); good (5.0 to 9.9); fair (2.0 to 4.9); and poor (less than 2). Since the results of Waggle *et al.* (1967) are generally much lower than other values in the literature, the classification of wheat and wheat products is based on the higher values.

DISCUSSION

The results of the present study are generally in good agreement with those reported recently in the literature even though the feedstuffs originated in various geographical areas. Yoshida and Morimoto (1960) used *S. carlsbergensis* for the assay of a number of Japanese feedstuffs. Polansky *et al.* (1964) also used *S. carlsbergensis* to determine the vitamin B_6 content of a number of American grains and cereal products. This group investigated not only the total amount of vitamin B_6 present in unchromatographed extracts but also the amounts of pyridoxine, pyridoxal, and pyridoxamine present after chromatographic separation on Dowex-50 ion exchange columns. The total vitamin B_6 determined from assay of the unchromatographed extracts as well as from the sum of the chromatographed fractions is included in Table II. Walter (1965) used both *S. carlsbergensis* and *Kloekera brevis* to determine the vitamin B_6 content of a variety of feedstuffs available to the Swiss. The U. S. National Research Council (1964) has published tables based on data for American and Canadian feeds. Waggle *et al.* (1967) studied wheat varieties and their milled products using blended samples of wheat from different areas in the United States, which were milled in a university flour mill using standard procedures. Extensive studies of the nutrient content of various types of commercial grades of dehydrated alfalfa conducted by the Midwest Research Institute (1965) include data on vitamin B_6.

An exception to the agreement among recent investigators is found in the report of Friesecke and Kirchgessner (1961) who ran *Neurospora sitophila* assays on 23 of the 27 feedstuffs analyzed by Walter (1965). Of these, 20 showed a much lower vitamin B_6 content and three a considerably higher vitamin B_6 content than found by Walter (1965). These discrepancies are surprising as both the *S. carlsbergensis* and the *N. sitophila* methods are considered to yield excellent results with the former being simpler, faster, and more convenient. The relationships of values by the two assay methods are shown in Table IV which compares recent *S. carlsbergensis* results with *N. sitophila* values from the literature. Since for barley and wheat bran the results of Friesecke and Kirchgessner (1961) are considerably lower than results with *N. sitophila* previously reported and also lower than the yeast assays, it appears likely that there has been a rather consistent negative bias in most of the results reported by these workers.

Assay of Mixed Feeds. As shown in Table I maximum liberation of vitamin B_6 from some feedstuffs may require hydrolysis with either $0.055N$ acid for 16 hours or $0.44N$ acid for 2 hours, while for other feedstuffs both concentrations of acid yield the same results. This variation in

Table II. Vitamin B_6 Contents of Feedstuffs, µg./G.

Comparison of Data Obtained in the Present Study and Reported Recently in the Literature

	Present Study			Literature Values			
	No. of samples	Average	Range	No. of samples	Average	Range	Literature references
Alfalfa meal	5	9.2	6.6–10.8	5	8.2	6.1–10.1	Walter (1965)
				[a]	6.6		Yoshida and Morimoto (1960)
Alfalfa meal, 20%	5	8.4	7.0–9.8	[a]	7.9		Midwest Research Institute (1965)
Alfalfa pellets	2	8.4	8.1–8.7				
Alfalfa meal, 13%	3	6.8	5.8–8.6				
Alfalfa crumbles	1	9.2					
Barley	2	4.5	4.2–4.7	9	2.7	1.1–4.9	Walter (1965)
				18	2.9 (99[b])		NRC (1964)
				2	3.0	2.1–3.9[c]	Polansky *et al.* (1964)
					3.1	2.2–3.9[d]	
Brewers' dried grain	5	2.2	1.5–3.0	[a]	2.0		Yoshida and Morimoto (1960)
Corn	19	5.3	4.1–6.1	5	7.1	5.1–9.5	Walter (1965)
				2	7.2		NRC (1964)
				2	4.9	3.6–6.1[c]	Polansky *et al.* (1964)
					6.2	4.9–7.5[d]	
Corn distillers' dried grains	1	9.9					
Corn distillers' dried solubles	1	13.1					
Corn distillers' dried solubles with grain	1	6.7					
Corn gluten feed	5	13.9	13.3–14.6				
Corn gluten meal	4	7.4	7.0–7.9				
Corn gluten meal, 60%	2	6.4	6.1–6.6				
Cottonseed meal	2	7.0	6.9–7.0	6	6.1	3.7–8.7	Walter (1965)
Distillers' dried grains	3	6.0	3.2–8.4				
Fat, animal and vegetable	7	0.0					
Fish meal							
Peruvian	6	3.6	3.1–4.0				
Menhaden	3	2.1	1.6–2.8				
Herring	3	4.4	3.4–5.9	4	3.7	2.7–5.4	Walter (1965)
				[a]	1.9		Yoshida and Morimoto (1960)
Unspecified	4	2.5	1.6–3.2	9	3.3	1.7–6.5	Walter (1965)
Whitefish	1	4.1					
Fish solubles, condensed	3	2.7	2.6–2.9	[a]	2.8		Yoshida and Morimoto (1960)
Grape pomace, fermented meal	1	1.8					
Hominy feed	1	9.2					
Linseed meal	5	8.8	7.9–9.9	5	16.4	9.7–28.7	Walter (1965)
Malt sprouts, dried	1	12.4					
Meat and bone meal	2	1.0	0.7–1.2	7	2.3	1.2–3.9	Walter (1965)
Milo	2	3.5	2.6–4.3	5	4.1	3.0–5.2	Walter (1965)
				2	4.1		NRC (1964)
Oats	2	2.5	1.9–3.1	4	1.3	0.9–1.9	Walter (1965)
				23	1.2 (42[b])		NRC (1964)
				[a]	0.7		Yoshida and Morimoto (1960)
Peanut meal	1	4.5					
Rapeseed	1	12.8					
Safflower meal, 20%	1	13.8					
Soybean hulls	1	1.7					
Soybean meal	17	6.7	5.4–7.7	5	7.6	5.3–10.6	Walter (1965)
Wheat	1	4.1		[a]	3.9		Yoshida and Morimoto (1960)
				9	2.3	0.9–3.7	Waggle *et al.* (1967)
				7	3.9[c]	3.6–4.3	Polansky *et al.* (1964)
					3.9[d]	3.5–4.1	
Soft				13	4.8(20[b])		NRC (1964)
Hard red				12	4.1 (18[b])		NRC (1964)
Wheat bran	1	16.2		4	10.9	8.9–13.0	Walter (1965)
				9	8.3	7.0–10.7	Waggle *et al.* (1967)
Wheat middlings	1	12.5		4	11.4	9.5–12.8	Walter (1965)
				9	7.0	4.7–9.8	Waggle *et al.* (1967)
Wheat mill run	1	11.1					
Yeast, Brewers' dried	7	29.9	15–40	14	43.3 (46[b])		NRC (1964)
				[a]	25.5		Yoshida and Morimoto (1960)

[a] Not specified.
[b] Coefficient of variability.
[c] Assay of total vitamin B_6 (unchromatographed).
[d] Sum of pyridoxine, pyridoxal, and pyridoxamine fractions (chromatographed).

Table III. **Relative Value of Feedstuffs as Sources of Vitamin B_6**

Excellent (10 or Greater)[a]	Good (5 to 9.9)[a]	Fair (2 to 4.9)[a]	Poor (Less than 2)[a]
Wheat bran[b]	Corn gluten meal	Fish meal	Soybean hulls
Wheat middlings	Corn	Fish solubles, condensed	Fermented grape pomace meal
Wheat mill run	Cottonseed meal	Barley	Meat and bone meal[c]
Brewers' dried yeast	Soybean meal	Milo	Oats
Corn gluten feed	Distillers' dried grains	Brewers' dried grain	Fat, feed grade
Dried malt sprouts	Corn distillers' dried solubles with grain	Wheat	
Safflower meal	Linseed meal	Peanut meal	
Corn distillers' dried solubles	Alfalfa meal		
Rapeseed	Hominy feed		
	Corn distillers' dried grains		

[a] Amounts shown in parentheses represent μg. per gram.
[b] See text.
[c] See Table II.

Table IV. **Comparison of Vitamin B_6 Assays by *S. carlsbergensis* and *N. sitophila* for Various Grain Products, μg./G.**

	S. carlsbergensis			*N. sitophila*				
Product	Polansky *et al.* (1964)	Present report	Walter (1965)	Friesecke and Kirchgessner (1961)	Morris *et al.* (1949)	Price (1946)	Stokes *et al.* (1943)	Wright (1945)
Wheat	3.5–4.1	...	...	...	...	...	3.7	...
Rye	2.9	...	...	...	...	...	3.5	...
Barley	3.9	4.3	2.7	1.9	...	...	4.6	5.6
Wheat bran	...	16.2	10.9	6.0	...	...	...	13.8
Wheat germ	13.1	...	...	...	...	...	14.8	16.0
Whole wheat flour	3.1	...	...	...	...	...	3.8	4.3
Rice	2.0	...	...	...	...	3.4	...	...
Corn meal, yellow	2.4	...	...	...	5.6	...	...	...

optimum hydrolytic procedure for individual feedstuffs indicates that, for a feed made up of a variety of feedstuffs, complete liberation of vitamin B_6 may not be achieved with either 0.055*N* or 0.44*N* acid. However, for feeds of known composition the data in Table I should enable the analyst to select the more efficient extraction method. For feeds of unknown composition, it may be desirable to try both methods, but, in general, one may expect that hydrolysis for 16 hours with 0.055*N* acid will extract at least 95% of the vitamin B_6.

Adequacy of Vitamin B_6 in Commercial Chicken, Turkey, and Swine Rations. Chickens, turkeys, and swine are grown commercially under confined conditions and are fed calculated blended rations. These feeds are composed of individual ingredients which may vary greatly in their vitamin B_6 content from lot to lot (Table II). The knowledge of the possible minimum vitamin B_6 content of a feed and its relationship to requirement is therefore important. The results of the present study have been used to calculate possible minimum vitamin B_6 contents of several chicken, turkey, and swine feeds utilizing current commercial-type formulas as a means of estimating the possibility of deficiency in rations. For these calculations the lowest vitamin B_6 value found for each individual feedstuff was used. Literature values were used for the few feedstuffs not covered in this study. The composition and calculated vitamin B_6 content of chicken and turkey breeder feeds are shown in Tables V and VI. Calculations for the other feeds were done similarly. These calculated minimum contents are compared to the corresponding National Research Council (NRC) recommendations in Table VII. The NRC recommendations are for minimum intakes required for proper animal feeds. Breeder feeds containing ingredients with minimal vitamin B_6 would not meet the NRC recommendation for chickens, and presumably for turkeys whose requirements per kilogram of feedstuff are generally accepted to be the same as those of chickens.

Table V. **Calculated Minimum Vitamin B_6 Content of Typical Chicken Breeder Ration**

	Amount Added, G./Kg.	Vitamin B_6 Minimum Value, μg./G.	Vitamin B_6 Contributed, Mg./Kg.
Dehydrated alfalfa meal, 20%	50	7.0	0.35
Corn, yellow	250	4.1	1.02
Limestone	51.5	...	...
Milo	373	2.6	0.97
Whey, dried	25	2.1	0.05
Salt	5	...	...
Defluorinated phosphate	15	...	...
Fish meal, pilchard	50	1.6	0.08
Stabilized animal fat	20	...	...
Soybean meal, 48%	157.5	6.6	1.05
Vitamin premix	3	...	...
Total vitamin B_6 content			3.5

Table VI. Calculated Minimum Vitamin B_6 Content of Typical Turkey Breeder Ration

	Amount Added, G./Kg.	Vitamin B_6 Minimum Value, μg./G.	Vitamin B_6 Contributed, Mg./Kg.
Dehydrated alfalfa meal, 17%	50	7.0	0.35
Corn, yellow	249	4.1	1.02
Fat	15	...	...
Fish meal, herring	50	3.4	0.17
Limestone	35	...	...
Milo	413	2.6	1.08
Soybean meal, solvent, 44%	137	5.4	0.74
Salt	5	...	...
Trace minerals	0.8	...	...
Defluorinated phosphate	17.5	...	...
Distillers' dried solubles with grains	25	6.7	0.17
Vitamin premix	2.7	...	...
Total vitamin B_6 content			3.5

Table VII. National Research Council Recommendations *vs.* Vitamin B_6 Content of Commercial Rations

		Calculated	
	NRC[a] (1966)	Minimum B_6, mg./kg.	Average B_6, mg./kg.
Chicken			
Broiler, starter	3.0	4.3	...
Laying ration	3.0	4.0	...
Breeder ration	4.5	3.5	4.9
Turkey			
Prestarter	[a]	4.6	...
Breeder	[a]	3.5	4.9
Swine			
Prestarter	1.1	3.4	...
Starter	1.1	3.3	...
Grower and finisher	1.1 (Up to 75 lb.)	4.3	...
Pregestating and gestating			
Hand fed	...	4.3	...
Self fed	...	3.4	...
Lactation, No. 1	...	3.7	...
No. 2	...	3.7	...

[a] See text.

Factors Affecting Utilization and Availability of Vitamin B_6 in Feeds. Although the data summarized in Table VII suggest that only chicken and turkey breeder feeds might be low in vitamin B_6, a number of factors affect vitamin B_6 requirements and could increase the need for vitamin B_6.

Supplementation of feeds with antibiotics and other drugs can increase the vitamin B_6 requirement of poultry—e.g., nitrofurazone, furazolidine, and arsanilic acid (Dillon, 1962; Fuller and Donahoo, 1959; Harwood and Stunz, 1950; Newberne and McEven, 1957).

The level of protein intake has been reported to affect the vitamin B_6 requirement of a number of animals. An increased vitamin B_6 requirement with increased protein intake has been reported for rats (Cerecedo and Foy, 1944; Sheppard and McHenry, 1946; Terroine, 1951), mice (Miller and Baumann, 1945), and dogs (Morgan *et al.*, 1946). Although no data are available, birds raised on a relatively high protein diet may require a greater vitamin B_6 intake than birds raised on a relatively low protein diet.

In the presence of a limiting amount of an essential amino acid, improved growth and efficiency of gain was obtained by further addition of vitamin B_6, the amino acid, or both (Sauberlich, 1961). This may account for reports that supplemental vitamin B_6 has improved growth on practical feeds which, either by assay or by calculation, contained more than minimal requirements of vitamin B_6 (Bird and Rubin, 1946; Dillon, 1962; Fuller and Kifer, 1959).

The three natural forms of vitamin B_6 (pyridoxine, pyridoxamine, and pyridoxal) show varying activity for poultry. Thus, pyridoxine is usually more active than pyridoxal and pyridoxamine in promoting growth (Davies *et al.*, 1959; Luckey *et al.*, 1945; Sarma *et al.*, 1946). Waibel *et al.* (1952) have reported that pyridoxal and pyridoxamine are as active as pyridoxine when autoclaved starch is the dietary carbohydrate, but less active than pyridoxine when sucrose or glucose was the carbohydrate.

Biological availability of the various forms of the vitamin may be incomplete in commercial feeds, which are complex mixtures of basic feedstuffs and additives. Although the very limited biological assay data reported for individual feedstuffs usually agree with the microbiological assay data obtained with *S. carlsbergensis*, there do not appear to be similar comparisons between microbiological and biological assays of commercial feeds. Possibly with certain combinations of ingredients, the vitamin B_6 available from the feed may be significantly less than indicated by microbiological assay.

The presence in linseed meal of a factor antagonistic to vitamin B_6 was reported by Kratzer and Williams (1948) and Kratzer *et al.* (1954). Recently, the toxic principle has been isolated and is probably 1-[(*N*-γ-L-glutamyl)-amino]-D-proline (Klosterman *et al.*, 1967). Although linseed meal is not used in commercial poultry feeds, the existence in other feedstuffs of this or other vitamin B_6 antagonists cannot be excluded. The only manifestation of such antagonism would be a response to vitamin B_6 added to the feed.

Finally, genetic differences between breeds can affect the vitamin B_6 requirement. Lucas *et al.* (1946) reported that Red Rock chicks have a higher requirement for vitamin B_6 and that this difference in requirement could be due to genetic factors. Yoshida and Morimoto (1960) have reported differences in the vitamin B_6 requirement of two strains of chicks.

On the basis of the above evidence, the addition of vitamin B_6 to certain critical feeds—i.e., poultry breeder feeds—would appear justified to assure adequate margins of safety in line with commercial practice on other vitamins.

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