hoped that this paper will encourage food scientists to make their data more accurate and meaningful to others by reporting procedures used for calculating retentions as part of the description of analytical methods. True retentions, rather than apparent retentions, should be reported whenever possible.

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Biotin Content of Feedstuffs

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The biotin contents of a variety of feedstuffs are reported. Preliminary experiments using hydrolysis for 2 hr at 121° with 2 *N* and 6 *N* H_2SO_4 indicated that higher results were obtained with 2 *N* acid for feedstuffs of plant origin and with 6 *N* for feedstuffs of animal origin. On the basis of these results, all subsequent extractions were made with 2 *N* acid for plant materials and with 6 *N* acid for samples of animal origin. Peanut meal, safflower seed meal, streptomyces meal and solubles, brewers' yeast, dried liver, and a whey–yeast product had relatively high biotin contents. Other samples have been grouped in order of decreasing biotin contents.

A number of investigators (Patrick et al., 1942; McGinnis and Carver, 1947; Roblee and Clandinin, 1953; Slinger and Pepper, 1954) have reported biotin deficiency in poults fed rations containing practical feed ingredients. However, the occurrence of this deficiency in commercial flocks was either not recognized or not reported until recently. It had been generally believed that the feedstuffs in use, combined with biotin arising from intestinal synthesis, supplied sufficient biotin to meet the poults' requirement. Recently, however, the occurrence of biotin deficiency in commercial flocks has been reported (Brown, 1966; Wilson, 1967; Richardson and Wilgus, 1967; Johnson, 1967). Marusich et al. (1970) encountered biotin deficiency symptoms in poults fed a commercial ration in the laboratory. Apparent biotin deficiencies in swine under commercial conditions have also been reported (Adams et al., 1967; Cunha et al., 1968). As a consequence of these findings, a reevaluation of the biotin content of feedstuffs was desirable, particularly since the available published data cover only a limited number of feedstuffs and some of the results were obtained by methods whose validity could be questioned. The present study was undertaken to provide more comprehensive data on the biotin content of a variety of feedstuffs. Biotin determinations were made by microbiological assay using *Lactobacillus plantarum* (*arabinosus* 17-5, ATCC no. 8014), the test organism considered to yield the most reliable results.

For the preparation of extracts for microbiological assay, no single hydrolytic procedure is universally effective for maximum liberation of bound biotin. Table I summarizes the results of various acid extraction procedures employed by a number of investigators. These studies indicate that stronger acid concentrations are required to liberate bound biotin from animal tissues than from plant tissues. In the extraction of plant tissues, biotin is less stable in relation to autoclaving time and acid concentration than in extraction from animal tissues.

METHODS

The microbiological assay procedure for biotin was that of Wright and Skeggs (1944) with the exception that the test organism was grown on the liver–tryptone agar of Nymon and Gortner (1946). Inocula were prepared from stab cultures transferred the previous day.

In view of the demonstrated effects of acid concentration and conditions of hydrolysis on yields of biotin from different materials, two hydrolytic procedures were used in the present study, namely, autoclaving for 2 hr at 121° with either 2 *N* or 6 *N* H_2SO_4. In each case, 20 ml of acid was used per gram of sample. Similar conditions were used for extraction of a number of samples with water as a means of estimating the content of free biotin. Recovery tests were

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Table I. Acid Extraction Procedures Employed by Various Investigators for Biotin Assay

Investigator	Extractant	Extraction conditions	Product	Conditions for max liberation	Comments
Thompson et al. (1941)	6 *N* NaOH 6 *N* HCl 6 *N* H_2SO_4 H_2O Autolysis Enzyme	Autoclaving 1–10 hr at 121° Cold or hot 24 hr at 37° Digestion with clarase or caroid at 37°	Various	6 *N* acid	Alkaline hydrolysis gradually destroys biotin
Lampen et al. (1942)	1,2,4,and 7 *N* H_2SO_4	Autoclaving	Various Liver Yeast Milk	2 *N*/2 hr 4 or 7 *N*/1 or 4 hr 4 *N*/1 hr (some loss in 2–3 hr) 1 *N*/1 hr	Losses with 4 *N* in many crude products; no losses with 1 *N* and in only a few cases with 2 *N*; optimum extraction usually 2 *N* for 2 hr
Cheldelin et al. (1942)	1 and 6 *N* H_2SO_4	Autoclaving	Degerminated blackeye peas Egg Whole wheat	1 *N*/30 min 6 *N*/2 hr 1 *N*/30 min	
Schweigert et al. (1943)	2,4, and 6 *N* H_2SO_4	Autoclaving	Meats	4 or 6 *N*/2 hr (loss in longer periods)	Autoclaving with 6 *N* H_2SO_4 for 2 hr adopted for assay of meat products; recoveries satisfactory
Wright and Skeggs (1944)	6 *N* H_2SO_4	Autoclaving 1 hr at 15 lb			*L. casei* yielded higher values than *L. plantarum* for autolyzed or enzyme-treated products; after acid hydrolysis both organisms gave equivalent results with 100% recovery of added biotin
Bowden and Peterson (1949)	2,4,9, and 18 *N* H_2SO_4	Autoclaving 2 hr/120°	Liver Fibrin Casein	9 *N* using *L. plantarum* 9 *N* using *L. plantarum* 9 *N* using *L. plantarum*	Results with *L. casei* and *S. cerevisiae* somewhat different
Calhoun et al. (1958a)	0.5,1,2, and 6 *N* H_2SO_4	30,60, or 120 min at 20 lb	Wheat or flour	0.5,1, and 2 *N* superior to 6 *N*	
Janicki and Trojanowska (1970)	4 and 6 *N* H_2SO_4	60 or 120 min at 121°	Wheat and rye		Increasing hydrolysis time from 60 to 120 min or increasing the acid concentration from 4 to 6 *N* destroyed biotin
Trojanowska (1971)	1–6 *N* H_2SO_4 or HCl	Hydrolysis 0.5,1, or 2 hr	Various	Plant tissue 1–3 *N*, 0.5–1 hr Animal tissue 3–6 *N*, 2 hr	

made in which simple solutions containing 0.01 μg of biotin per ml in 2 *N* and 6 *N* H_2SO_4 were autoclaved for 2 hr at 121°.

RESULTS

Comparative Effectiveness of Extraction with 2 *N* H_2SO_4 or 6 *N* H_2SO_4. Table II lists a number of plant and animal feedstuffs for which the relative effect of the two acid concentrations on biotin assays was determined. As expected, more efficient liberation of biotin was obtained from feedstuffs of plant origin with 2 *N* acid and from feedstuffs of animal origin with 6 *N* acid. The relatively close agreement of duplicate assays with both acid concentrations indicates that the sizable differences in results with 2

Table II. Comparison of 2 *N* H_2SO_4 and 6 *N* H_2SO_4 Extraction Procedures for Biotin

Feedstuffs yielding higher results with 2 *N* H_2SO_4	Biotin content, μg/g		
	2 *N* H_2SO_4	6 *N* H_2SO_4	$2N/6N \times 100$, %
Alfalfa	0.65	0.38	171
Corn	0.100	0.071	141[a]
	0.083	0.057	146[a]
Corn extractives and residues, dried	0.43	0.37	116
Milo	0.238	0.154	155[a]
	0.230	0.150	153[a]
Wheat, soft	0.123	0.088	140[a]
	0.098	0.058	169
hard	0.111	0.081	137
Peanut meal	1.79	1.57	114
Safflower seed, solvent extracted	1.55	1.29	120[a]
Streptomyces meal and solubles, dried	2.08	1.72	121[a]
Feedstuffs yielding lower results with 2 *N* H_2SO_4			
Herring meal	0.35	0.41	85[b]
Blood fibrin	0.29	0.33	88
Whey–yeast product	1.13	1.63	69[b]
Crab meal	0.26	0 31	84[b]
Meat and bone meal, 1	0.16	0.17	94[b]
2	0.17	0.21	81[b]
3	0.24	0.31	77[b]
Poultry by-product meal	0.36	0.47	77[b]
Liver, dried	5.3	6.1	87[b]
Tuna meal	0.19	0.22	86[b]
Brewers' dried yeast[c]	1.30	1.46	89

[a] These seven feedstuffs were assayed in duplicate and the 2 *N* results averaged 139% of the 6 *N* values. The average differences between the duplicates (%) ± standard deviation were: 2 *N* acid, 4.0 ± 1.5; 6 *N* acid, 3.0 ± 1.9. [b] These nine feedstuffs were assayed in duplicate and the 2 *N* results averaged 82% of the 6 *N* values. The average differences between the duplicates (%) ± standard deviation were: 2 *N* duplicates, 5.1 ± 2.3; 6 *N* duplicates, 3.1 ± 2.6. [c] Two other samples of Brewers' yeast showed no difference in results by the two extraction procedures.

N and 6 *N* acid are statistically significant. Of three yeast samples tested, two gave similar results with both extraction methods and one yielded higher results with 6 *N* acid. On the basis of the data in Table II, all subsequent samples of plant origin were extracted with 2 *N* acid and those of animal origin with 6 *N* acid. Recoveries on autoclaving of simple solutions of biotin were 100% for 2 *N* acid and 94% for 6 *N* acid.

Biotin Content of Feedstuffs. In Table III the results of the present study are summarized and compared to data reported in the compilations of Scott (1968) and the National Research Council (1969) as well as to the original data of Calhoun et al. (1958b) and Jensen (1967). To facilitate the evaluation of these feedstuffs as sources of biotin, they are listed in Table IV according to the following categories based on the biotin content in micrograms per gram: excellent (0.8 or greater); good (0.4 to 0.79); fair (0.20 to 0.39); and poor (less than 0.2).

DISCUSSION

Many of the average results of this study are somewhat higher than comparable literature values. For feedstuffs of plant origin, the differences may be due to the greater efficiency of extraction of biotin by 2 *N* as compared to 6 *N* acid and/or to the destruction of biotin by the higher acid concentration. In the limited comparisons for feedstuffs of animal origin, the results for fish meals are considerably higher than those reported in the literature. These differences may stem from actual differences in biotin content and/or from differences in assay methodology.

Anderson and Warnick (1970), using a chick bioassay, estimated the biotin content of 23 feedstuffs which had been assayed microbiologically in the present study. Although these authors state that a number of factors made accurate estimation of the biotin content of the feedstuffs by the chick test difficult, generally the microbiological and chick assays showed agreement as to whether the various feedstuffs were rich sources or poor sources of biotin. About 40% of the results agreed within ±20% and about 40% were considerably higher by microbiological assay. In view of difficulties encountered in the chick assays, the significance of the differences in results by the two methods is uncertain but incomplete bioavailability to the chick is likely an important factor.

Data reported by Lampen et al. (1942) indicate that feedstuffs of plant origin contain a higher ratio of free biotin (biotin available to the test organism after water extraction) to total biotin than feedstuffs of animal origin. This finding has been confirmed by determinations of the relative amount of free biotin in a number of feedstuffs of different origins as shown in Table V.

The feedstuffs of plant origin contain a higher percentage of free biotin than those of animal or yeast origin with the exception of the poultry by-product meal. These results suggest that the lower values obtained for feedstuffs of plant origin after extraction with 6 *N* H_2SO_4 may be associated with destruction of some of the biotin present. In the case of alfalfa, this seems definitely indicated since the result after 6 *N* acid extraction was about 25% lower than after water extraction.

Table III. Biotin Content of Various Feedstuffs, μg/g

Feedstuff	Present study			Lit. values
	No. of samples	Av	Range	
Alfalfa meal, dehydrated	7	0.49	0.33–0.69	0.33, Scott (1968)
Barley	3	0.11	0.09–0.13	0.13, Wagstaff et al. (1961) 0.20, Jensen (1967) 0.17, Scott (1968) 0.20 (99[a]), NRC (1969)
Barley, soaked	1	0.14		
Blood fibrin	6	0.37	0.20–0.59	
Brewers' dried grains	2	0.28	0.26–0.29	
Cane molasses	1	0.69		
Casein	6	0.08	0.04–0.14	
Cerelose	1	0.005		
Corn, yellow	11	0.11	0.06–0.15	0.08, Jensen (1967) 0.08, Scott (1968) 0.06 (21[a]), NRC (1969)
Corn extractives and residues, dried	3	0.50	0.46–0.53	
Corn gluten meal	2	0.35	0.28–0.41	0.19, Jensen (1967) 0.15, Scott (1968)
Cottonseed meal	2	0.46	0.45–0.47	0.08, Jensen (1967) 0.10, Scott (1968)
41% protein	1	0.46		
46% protein	1	0.63		
50% protein	2	0.67	0.67	
60% protein	1	0.71		
Crab meal	1	0.31		
Distillers' dried grains with solubles	5	0.33	0.30–0.36	
Distillers' dried solubles	2	0.45	0.44–0.45	1.10, Scott (1968)
Fish meal				
Hake	1	0.55		
Herring	6	0.42	0.31–0.63	0.15, Jensen (1967)
South American	4	0.38	0.28–0.43	
Tuna	1	0.21		
Unspecified	5	0.46	0.42–0.55	0.20, Scott (1968)
Gelatin	5	0.010	0.005–0.017	
Grape pomace	1	0.24		
Meat meal	1	0.26		
Meat and bone meal, 50%	7	0.19	0.13–0.31	0.15, Jensen (1967) 0.07, Scott (1968)
Milo	6	0.24	0.18–0.28	0.15, Jensen (1967) 0.18, Scott (1968)
Oats	2	0.19	0.11–0.27	0.15, Jensen (1967) 0.31 (99[a]), NRC (1969)
Peanut meal	1	1.76		0.39, Scott (1968)
Poultry by-product meal	2	0.48	0.47–0.48	
Rice bran	1	0.38		
Safflower seed, solvent extracted	6	1.45	0.77–2.00	
Sesame meal	1	0.34		
Soybean meal, solvent extracted	8	0.40	0.32–0.46	0.25, Jensen (1967) 0.31, Jensen (1967) 0.32, Scott (1968)
Soy protein, isolated (C-1)	3	0.49	0.41–0.57	
Streptomyces meal and solubles	1	2.15		
Sucrose, commercial	1	0.005		
Whale meal	1	0.10		

Table III (*Continued*)

Feedstuff	Present study: No. of samples	Present study: Av	Present study: Range	Lit. values
Wheat	3	0.11	0.10–0.13	0.10 (84[a]), NRC (1969) 0.08, Jensen (1967) 0.08, Scott (1968) 0.11, Calhoun (1958b)
hard	2	0.12	0.11–0.13	
soft	3	0.14	0.12–0.15	
soft soaked	1	0.12		
Wheat bran	1	0.49		0.44, Calhoun (1958b)
Wheat farina	7	0.031	0.008–0.057	0.010, Calhoun (1958b)
Wheat germ toasted	2	0.26	0.20–0.31	0.17, Calhoun (1958b)
Wheat gluten	1	0.22		
Wheat middlings	1	0.37		0.35, Calhoun (1958b)
Wheat mill run	1	0.32		
Whey, dried partially delactosed	2	0.27	0.26–0.28	
Whey–yeast product	2	1.92	1.63–2.20	
Yeast, brewers' dried	4	1.18	0.87–1.52	0.75, Jensen (1967) 1.30, Scott (1968)

[a] Coefficient of variability.

Table IV. Relative Value of Feedstuffs as Sources of Biotin

Excellent (0.8 or greater)[a]	Good (0.40 to 0.79)[a]	Fair (0.20 to 0.39)[a]	Poor (Less than 0.2)[a]
Peanut meal	Alfalfa meal	Blood fibrin	Barley
Safflower seed meal	Cane molasses	Brewers' dried grains	Casein
Streptomyces meal and solubles	Cottonseed meal	Corn gluten meal	Cerelose
Brewers' yeast	Distillers' dried solubles	Crab meal	Corn
Whey–yeast product	Fish meal	Distillers' dried grains with solubles	Farina
Dried liver	Poultry by-product meal	Fish meal	Whale meal
	Soybean meal	Grape pomace	Gelatin
	Wheat bran	Meat meal	Meat and bone meal
		Milo	Oats
		Rice bran	Sucrose, commercial
		Sesame meal	Wheat
		Toasted wheat germ	
		Wheat gluten	
		Wheat middlings	
		Wheat mill run	
		Whey, dried	

[a] Biotin contents shown in parentheses represent micrograms/gram.

Of the feedstuffs assayed, peanut meal, safflower meal, dried liver, streptomyces meal and solubles, brewers' dried yeast, and a whey–yeast product have the highest biotin content. Ether extraction of acid extracts of peanut meal and safflower meal did not change the biotin results, indicating that the assay values for these meals were not affected by fatty acid stimulation of the test organism.

Jensen and Martinson (1969) have commented that they had difficulty in obtaining consistently reliable results with the microbiological assay for biotin. This laboratory also has encountered occasional difficulties despite scrupulous glassware cleaning (machine washing followed by chromic acid cleaning and finally heating the glassware at 400°F for 2 hr.). Based on our experience, biotin assays of feedstuffs should be conducted in an area in which the chance of contamination with traces of biotin is minimal.

Assay of Mixed Feeds. As shown in Table II, more efficient liberation of biotin is effected with 2 *N* H_2SO_4 for feedstuffs of plant origin and with 6 *N* H_2SO_4 for feedstuffs of animal origin. Since practical poultry rations are composed mainly of plant materials, extraction of a composite poultry feed with 2 *N* acid should yield higher biotin values than extraction with 6 *N* acid. Assays of complete rations based on corn, milo, or soaked wheat and containing fish meal were carried out with both acid concentrations and yielded higher results with 2 *N* acid.

Availability of Biotin in Feeds. Although the values in Table III represent the total amounts of biotin found in feedstuffs, they do not necessarily indicate the amounts available to the animal. Incomplete availability of biotin in practical turkey starting rations was reported by Patrick et al. (1942). Wagstaff et al. (1961) found that barley and

Table V. Free and Total Biotin in Feedstuffs of Plant and Animal Origin

	Free biotin, μg/g	Total biotin, μg/g	(Free biotin/total biotin) × 100, %
Alfalfa	0.52	0.65	80
Safflower meal	0.96	1.56	62
Milo	0.075	0.23	33
Corn	0.018	0.08	23
Casein	0.014	0.050	28
Wheat, soft	0.035	0.098	36
Soybean meal	0.10	0.44	23
Brewers' dried yeast	0.200	1.36	15
Herring meal	0.050	0.45	11
Meat and bone meal	0.030	0.20	15
Poultry by-product meal	0.14	0.48	29

wheat contained less available biotin for the chick than did corn, milo, or oats. Only one-third of the biotin in barley was available to the chick. Scott (1968) has stated that in most instances approximately one-half of the microbiologically determined biotin in a feedstuff may be unavailable to chickens and turkeys. Further evidence for the variations in the biological availability of biotin in various feedstuffs to chicks was presented by Anderson and Warnick (1970). They reported that wheat, barley, milo, fish meals, and meat and bone meal provided chicks with less biotin than was found in these feedstuffs by microbiological assay.

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