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limit was 0.5 mg/kg). It was found that the AG level of abalone muscle was 40 mg/kg and its viscera, 69 mg/kg, and that of top-shell muscle was 200 mg/kg and its viscera, 224 mg/kg. In contrast, the AG levels of some processed foods were found to be fairly high, viz., salted-dried round herring contained 217 mg/kg, dried common filefish, 112 mg/kg, and salted steenstrap squid, as high as 650 mg/kg. The remaining processed foods contained almost no appreciable amount or very small amounts of AG.

AG has been isolated from a few invertebrate sources, notably from the sponge, *Geodia gigas*, and from several cephalopods (Baldwin, 1963). Present study revealed that relatively high concentrations of AG could be detected in fresh abalone and top-shell muscles ranging from 40 to 200 mg/kg. Both shellfish belong to gastropods which contain large amounts of arginine instead of creatine as phosphagen. The mode of AG formation from arginine in the shellfish remains unsolved; perhaps it might be formed by the action of an arginine decarboxylate in the muscle. It is shown that certain bacteria can decarboxylate arginine to form AG. Interestingly, the processed foods containing fairly high concentrations of AG did not give any sign of deterioration.

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Comparison of Chemical and Biological Methods for Determination of Thiamin in Foods

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The thiamin content of green beans was determined by the fluorometric thiochrome procedure and rat bioassay to determine the correlation between the chemical thiamin assay and the biologically available thiamin. Several methods of sample preparation for fluorometric analysis were examined to maximize the removal of interfering compounds. Preparative chromatography using Decalso ion-exchanger columns was found to provide lower apparent thiamin values than either direct analysis or preextraction with isobutanol of the sample extract of all rat bioassay diets analyzed. No significant difference was observed in the apparent thiamin content of green beans between the extract purification methods. These results indicate the necessity of preliminary testing of extract purification effects on each product assayed to ensure accuracy of routine chemical analysis. The rat bioassay was evaluated by growth, feed conversion efficiency, and urinary thiamin creatinine ratio. Estimates of available thiamin were equal by all three indicators. Comparison of the fluorometric and biological assay data indicated total bioavailability of thiamin in green beans.

Accurate chemical determination of the vitamin content of foods is of little value unless a correlation can be determined between the chemical assay value and the biologically available fraction of the vitamin in the food. Data on the bioavailability of vitamins is important for the evaluation of the adequacy of dietary intakes.

For the determination of total thiamin in foods, chemical procedures have been based on the oxidation of thiamin in a food extract to thiochrome, extraction of the thiochrome into isobutanol, and the subsequent fluorometric measurement of the thiochrome (Pyke, 1937).

Automated methods for thiamin analysis have been developed for foods (Khoury, 1966; Kirk, 1974; Pelletier and Madere, 1975, 1977) which improve the precision and shorten the analysis time. Chromatographic cleanup of the sample extract using the Decalso cation-exchange chromatographic procedure has been used for the removal of potentially interfering compounds from the sample extract (Freed, 1966; Strohecker and Henning, 1965; AOAC, 1975). Although previously reported to be a major source of imprecision in the thiamin assay (Jowett, 1940; Harris and Wang, 1941; Betchel and Hollenbeck, 1958), the Decalso purification procedure has been recently modified to greatly reduce this variability (Pippen and

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Table I. Composition of Diets (Percent by Weight)

Component	Stand-ard or basal diets	Test diets 3% bean	Test diets 5% bean
Casein (vitamin free, test)[a]	19.8	19.2	18.9
DL-Methionine, NRC[b]	0.2	0.2	0.2
Sucrose	60.5	58.4	56.9
Cellulose (Alphacel)[b]	9.4	9.1	8.9
Salt mix[c]	4.0	4.0	4.0
Vitamin mix[a,d]	1.1	1.1	1.1
Corn oil	5.0	5.0	5.0
Green beans (dry)		3.0	5.0

[a] ICN Pharmaceuticals, Inc. [b] Grand Island Biological Co. [c] Teklad Test Diets. Wesson modified Osborne-Mendel. $ZnCO_3$ added to provide 15 ppm Zn in basal and standard diets, 20 ppm Zn in bean diets. [d] Vitamin mix provided (per kg diet): vitamin A, 9900 units; vitamin D, 1100 units; α-tocopherol, 55 mg; choline chloride, 825 mg; menadione, 25 mg; niacin, 50 mg; riboflavin, 11 mg; pyridoxine hydrochloride, 11 mg; calcium pantothenate, 33 mg; biotin, 0.22 mg; folic acid, 0.99 mg; vitamin B_{12}, 0.015 mg.

Potter, 1975). Sample extract purification by extraction with isobutanol prior to thiochrome formation has also been shown to be effective in removing interfering compounds (Harris and Wang, 1941; Kirk, 1974; Defibaugh et al., 1978), providing an alternative to the Decalso procedure.

Numerous comparisons have been made between chemical and biological assays for determination of thiamin in foods. In these studies, we compared chemically determined values with and without extract purification (Decalso or isobutanol), with rat bioassay estimates for thiamin in green beans. Several biological parameters, i.e., rat growth, feed conversion efficiency, and urinary thiamin/creatinine ratio, were measured to determine their relative merits as bioassay indicators.

MATERIALS AND METHODS

Preparation of Rat Bioassay Diets. Green beans (U.S. Grade A Fancy; Kelley, Farquhar, and Co., Tacoma, Wash.) were obtained frozen. They were freeze-dried and finely ground before being blended into the diets or chemically analzyed. Diets were prepared as shown in Table I, thoroughly mixing the ingredients in a Hobart mixer. The composition of the standard diets was identical with the basal, except for the addition of a dry thiamin hydrochloride premix, which was prepared in casein, to a level of 0.1, 0.2, 0.3, and 0.4 μg of thiamin hydrochloride/gram diet. Test diets contained dry green beans as the only source of thiamin. Replacement of basal diet ingredients are shown in Table I was based on green bean proximate composition. Diets were stored in sealed containers at 2 °C until fed.

Bioassay Methods. Male weanling Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, Mich.) were randomly assigned to six groups of ten rats each. Rats were housed individually in metal cages with raised wire mesh floors. Water was supplied ad libitum. The basal diet was fed ad libitum to all rats for 10 days to induce a mild thiamin deficiency, as evidenced by cessation of growth. Standard (0.1, 0.2, 0.3, and 0.4 μg of thiamin hydrochloride/g) and test (3 and 5% bean) diets were fed to the respective groups, pair feeding all groups to the 0.1 μg B_1/g group for a 14-day assay period. Rats were weighed at the start of the assay period and after 7 and 14 days. Feed consumption was carefully measured. Spillage was low and, therefore, neglected. After the 14-day assay period, rats were transferred to individual metabolism cages in

Table II. Thiamin Chemical Assay[a]

Sample	μg of thiamin hydrochloride/g of sample — Direct analysis	Decalso cleanup	Differ-ence
Dry green beans	7.46 ± 0.14	7.38 ± 0.20	0.08
Diets			
Basal	1.09 ± 0.05	0.03 ± 0.02	1.06
Standard 0.1 μg/g	1.10 ± 0.03	0.18 ± 0.05	0.92
Standard 0.2 μg/g	1.23 ± 0.00	0.32 ± 0.00	0.91
Standard 0.3 μg/g	1.25 ± 0.06	0.46 ± 0.00	0.79
Standard 0.4 μg/g	1.39 ± 0.03	0.57 ± 0.02	0.82
Test 3% beans	1.01 ± 0.17	0.20 ± 0.00	0.81
Test 5% beans	1.17 ± 0.05	0.35 ± 0.00	0.82

[a] Mean and standard deviations. Data corrected for recovery of internal standards.

which urine was collected during a 22-h fast, water ad libitum. Urine was stored at -25 °C until analyzed. Urinary thiamin (Muiruri et al., 1974) and creatinine (Technicon Corp., 1969) were determined for calculation of the thiamin/creatinine excretion ratio.

Bioassay results were evaluated by comparison of the response of animals on test diets to the linear dose-response results of rats fed the standard diets. Linear regression techniques (Neter and Wasserman, 1974) were used for determination of linear dose-response relationships. Micrograms of thiamin hydrochloride/g of diet were plotted against rat growth for the assay period. The growth per gram feed consumed and urinary thiamin (μg)/mg of creatinine were also evaluated.

Chemical Thiamin Analysis. Samples of dry green beans and all rat diets were extracted and enzymatically treated (Mylase P, ICN Pharmaceuticals, Inc.) by AOAC methods (1975). Each sample was run in triplicate, with a 20 μg/100 mL internal thiamin standard added to one of the replicates of each sample prior to autoclaving. Decalso columns (thiochrome Decalso, Fisher Scientific Co.) were prepared and aliquots of all sample extracts were purified according to Freed (1966). Sample extracts, with and without Decalso cleanup, were then assayed for thiamin by the method of Kirk (1974). Apparent thiamin values for each sample were corrected for the mean recovery of the internal standards for each particular method.

RESULTS AND DISCUSSION

The results of the chemical thiamin assays (Table II) indicate that the effect of Decalso cleanup of the sample extract varies with sample composition. No difference was observed between apparent thiamin levels in dry green beans; however, all diets exhibited significantly lower values ($P < 0.001$) after Decalso treatment. The mean difference was 0.88 ± 0.10 μg/g for all diets assayed. This large divergence was not expected because of the diet composition, i.e., similarity to milk solids, since it had been observed that milk could be satisfactorily assayed without extract purification (Kirk, 1974). In agreement with the present results, Pelletier and Madere (1975) observed that dairy products were among several foods in which apparent thiamin values were lowered by Decalso chromatography.

Purification of the extracts by two extractions with two volumes of isobutanol was also investigated. While the percent recovery was significantly greater than that obtained in direct analysis, apparent thiamin values were approximately twice those obtained using Decalso purification. Therefore, in the bioassay diets, the isobutanol extraction did not completely remove compounds which interfere with the assay. These results suggest that compounds which were not soluble in isobutanol were

Table III. Recovery of Internal Standards[a,b]

Method	% recov	Coefficient of variation, %
Direct analysis	76.9	1.3
Decalso cleanup	59.3	9.4

[a] Twenty micrograms of thiamin added per 100 mL total volume for each sample. [b] Data represents eight samples.

oxidized to isobutanol-soluble species which interfered with the fluorometric assay of thiamin in the bioassay diets.

The recovery of the internal thiamin standards (Table III) was lower than reported for most other food products (Kirk, 1974), possibly because of the extensive browning during the autoclave extraction procedure. The lower recovery after Decalso cleanup corresponds closely to our typically observed value of approximately 75% recovery of thiamin standards from the columns. The consistency of recovery within each method justified the use of this data in calculation of thiamin in samples.

In the rat bioassay, all rats exhibited characteristic thiamin deficiency symptoms to varying degrees, and many lost weight during the assay period. The dietary thiamin concentrations used in this study were chosen on the basis of preliminary trials which showed a significant relationship between growth and dietary thiamin when pair feeding only at levels less than 0.4 μg/g. Pair feeding was required to compensate for the anorexia of thiamin deficiency and the preference of rats for diets containing green beans.

A linear relationship was observed between growth and concentration of dietary thiamin (Table IV). In spite of the pair feeding design, slight differences were observed in feed consumption between rats. Therefore, feed conversion efficiency was calculated, and this was found to be a linear function of dietary thiamin concentration. The ratio of urinary thiamin to creatinine has been recommended as an indicator of thiamin status in humans (Sauberlich et al., 1974), with values normally proportional to thiamin intake. In this bioassay, the ratio was a linear function of dietary thiamin; however, a negative slope was observed. No significant differences were found in 22-h urinary thiamin excretion values between dietary groups. Therefore, this negative slope was due to the dramatic effect of lean body mass on creatinine excretion (Arroyave and Wilson, 1961). Urinary thiamin excretion has been shown to exhibit very small changes in response to changes in dietary thiamin on low thiamin intakes (Sauberlich, et al., 1974). These factors would contribute to the observed negative correlation of thiamin/creatinine ratio with dietary thiamin concentration. Comparison of the linear regression parameters of these various dose–response curves (Table IV) indicates that growth and feed conversion efficiency were equally sensitive as indicators of thiamin status in this assay, with both being more precise than the urine thiamin/creatinine ratio. Fourteen-day growth and efficiency curves were only slightly more sensitive than 7-day curves. Therefore, a 7-day test period would suffice, providing a marked shortening of bioassay time compared to previous curative methods.

Calculation of available thiamin in green beans using the various dose–response curves indicates a mean value of 7.30 ± 2.46 μg/gram, as thiamin hydrochloride (Table V). This compares favorably with chemically determined thiamin values, indicating total availability of thiamin in this green bean sample. These data agree with previous reports based on bioassays which employed rat growth (Harris and Wang, 1941; Brown et al., 1943; Warnick et al., 1956; Day et al., 1957) and erythrocyte transketolase activity (Brin, 1964) as indicators of thiamin status that have indicated that thiamin is totally available in a wide variety of food products.

The cause of the disparity between chemically determined thiamin values for all diets with or without extract purification cannot be specifically identified. In view of the development of thiamin deficiency in all rats on these diets, along with the published thiamin requirement of 1.25 μg/g for growing rats (National Academy of Sciences, 1972), the thiamin values based on direct analysis appear to be invalid. The data obtained from analysis of Decalso purified extracts corresponds closely to the levels of thiamin added to the diets and the estimated basal value calculated from the microbiological assay of thiamin in the

Table IV. Bioassay Dose–Response Curves [Data Plotted as Biological Response (Y) vs. μg of Thiamin Hydrochloride per Gram Diet (X)]

Response parameter	Y intercept	Slope	Correlation coefficient
Growth, g			
1 week	-7.2	55.1	+0.6758
2 weeks	-33.3	95.9	+0.7139
Feed efficiency[a]			
1 week	-0.165	1.14	+0.6509
2 weeks	-0.461	1.27	+0.7064
Urinary mg of thiamin/mg of creatinine	11.8	-17.0	-0.5740

[a] (Gram of growth)/(gram of feed consumed).

Table V. Bioassay Estimates of Thiamin in Green Beans (Mean and Standard Deviation)

Response parameter	Replacement level[a] 3%	5%	Mean[a]
Growth			
1 week	5.33 ± 2.00	8.00 ± 2.20	6.67 ± 2.10
2 weeks	6.00 ± 2.67	8.60 ± 2.00	7.30 ± 2.34
Feed efficiency			
1 week	6.00 ± 2.33	7.40 ± 1.67	6.70 ± 2.00
2 weeks	6.00 ± 3.67	9.00 ± 0.80	7.50 ± 2.24
Urinary mg of thiamin/mg of creatinine	8.67 ± 5.67	8.00 ± 1.58	8.34 ± 3.63
Mean	6.40 ± 3.27	8.20 ± 1.65	7.30 ± 2.46

[a] As μg of thiamin hydrochloride/g of dry beans.

Polymerized L–Lysine–HCl

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"vitamin free" casein (0.14 µg/g of casein; ICN Pharmaceuticals, Inc.). These results confirm the necessity of preliminary testing of sample extract purification effects on the chemical thiamin determination to ensure accuracy during routine analysis.

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In Vitro Rumen Microbial Stability and in Vivo Availability of Polymerized L-Lysine-HCl

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The stability of a urea–L-lysine–HCl–formaldehyde polymer to in vitro rumen microbial degradation and the in vivo lysine availability of the polymer were determined. After incubation with rumen microorganisms for 24 or 48 h, from 51 to 59% of the polymerized L-lysine–HCl was recovered. In contrast, only about 11% of free L-lysine–HCl could be recovered after rumen fermentation. Rat growth studies indicated that essentially all of the L-lysine–HCl in the polymer was available.

Rumen bacteria and protozoa rapidly hydrolyze protein to amino acids, which are immediately deaminated to an organic acid and ammonia (Broderick, 1975). However, research within the past few years has shown that treatment of oilmeal and forage proteins with aldehydes and tannins can effectively reduce rumen microbial degradation of the treated protein and increase the quantity of protein reaching the lower gastrointestinal tract (Amos and Evans, 1976; Nishimuta et al., 1974; Axford and Evans, 1975; Chalupa, 1975). In many instances, increasing the quantity of dietary protein which is not degraded in the rumen has failed to increase animal growth or nitrogen retention. Part of this failure to increase these growth parameters may be due to deficiencies of methionine and lysine in the treated protein. These two amino acids have been shown to be deficient in microbial protein for growing lambs (Nimrick et al., 1970); responses have been shown from these amino acids by lactating cows (Schwab et al., 1976) and in growing steers to lysine (Boila and Devlin, 1972) and to methionine (Steinacker et al., 1970; Fenderson and Bergen, 1975). Since direct supplementation of

ruminant diets with a free amino acid is not effective in increasing the quantity of that amino acid available for absorption, due to deamination as cited earlier, alternate methods for supplementing the ruminant diet with amino acids are needed.

The objectives of this work were to determine the in vitro stability to rumen microbial activity and in vivo availability of the lysine present in a urea-formaldehyde-lysine polymer.

EXPERIMENTAL PROCEDURES

The polymerized lysine used in these studies was prepared by dissolving 100 g of urea (1.67 M) in 186 mL of 37% formaldehyde (2.29 M) at room temperature. After complete dissolution of the urea, 100 g of L-lysine–HCl (0.55 M) was added with constant stirring. The L-lysine–HCl–urea–formaldehyde mixture was allowed to react and harden at room temperature (usually 24–36 h). After hardening, the lysine polymer was ground to pass through a 1-mm Wiley mill screen, dried at 39 °C under reduced pressure (10 mmHg) for 24 h, and used in vitro rumen microbial stability and in vivo rat growth studies. Total nitrogen in the lysine polymer was determined by the Kjeldahl procedure (AOAC, 1970). Portions of the polymer were hydrolyzed in 6 N HCl (Wilkinson et al., 1968) and total lysine was determined according to Moore et al. (1958).

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