The results from the analysis of zinc (45 market baskets), iron (35 market baskets), calcium (35 market baskets), and phosphorus (20 market baskets) are found in Table III. The figures represent the mean values of the total diet market basket composites.

The efficiency of the method was determined by adding known amounts of zinc, iron, calcium, and phosphorus to the composites representing the total diet market baskets and then calculating the percent recoveries. The recoveries obtained indicate that there is good precision in determining these trace elements by the proposed methodology in food products. These results are found in Tables IV–VII.

It has not been our objective to determine the nutritional significance of the data we have obtained from the market baskets analyzed and given in this report. Our objectives have been to develop an analytical method that would give good precision and to give us an idea of how much zinc, iron, calcium, and phosphorus one would expect to find in the total diet market basket.

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Dinitrophenylation of the Compounded Chick Starter Rations for the Estimation of Available Lysine

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Available lysine of compounded starter rations of poultry was estimated by treating the rations with 2,4-dinitrofluorobenzene followed by acid hydrolysis. The hydrolysate was purified by passing through a Sephadex G-50 column and the color was read at 390 mμ using a DBG Beckman spectrophotometer. As the chemically estimated values conformed with the biological results, the present method will be useful for the assessment of compounded poultry rations.

It is not possible to estimate the nutritional availability of lysine units whose ε-amino groups are chemically bound in the heat-processed high carbohydrate rich feeds simply by acid hydrolysis. The process no doubt overvalues the feeds with respect to this particular amino acid content and thus the assessment of compounded poultry rations by chemical methods becomes erroneous. Carpenter (1960) estimated available lysine of foods with the help of 2,4-dinitrofluorobenzene which underwent Sanger reaction (1945) with ε-amino groups. The interfering components were separated by Rao et al. (1963) who used a column chromatographic technique for the estimation of ε-dinitrophenyllysine in oilseed meals, but no attempt was made by them to determine the available lysine content in a compounded ration. This paper describes a simple method for the estimation of available lysine in chick starter rations by applying Sanger reaction and then purifying the acid hydrolysate of the reacted product with the help of a Sephadex G-50 column.

EXPERIMENTAL PROCEDURE

Formulation of the Compounded Starter Rations. First, four starter rations designated as A, A_1, B, and B_1 were prepared by mixing the processed ingredients in requisite amounts. The protein content of the rations in each group was kept more or less constant but the amount of lysine-enriched materials was changed to differentiate the rations in lysine content.

Another set of experiments was conducted by adding known amounts of synthetic L-lysine hydrochloride to the starter ration C which was deficient in lysine content. Thus, four more rations designated as C_1, C_2, C_3, and C_4 which were different in added amounts of lysine were prepared. All other ingredients of these rations were the same as in ration C.

Reaction with 2,4-Dinitrofluorobenzene. To 2 to 3 g of finely ground fat-free material, 30 ml of 50% sodium bicarbonate solution was added with gentle stirring. The mixture was left undisturbed for 15 to 20 min and 1.5 ml of 2,4-dinitrofluorobenzene in 40 ml of ethanol was added. After preliminary stirring the contents were shaken in the dark for 2 h on a mechanical shaker with slow motion. An air stream was passed to remove alcohol and a major portion of water and the residue was taken up for acid hydrolysis.

Preparation of the Acid Hydrolysate. To the residue 200–250 ml of 6 N HCl was added and the mixture was refluxed for 24 h. When hydrolysis was over, the mixture was cooled and filtered cautiously through Whatman filter paper (No. 41). The residue was washed repeatedly with cold distilled water. The filtrate and the washings were mixed together and evaporated to dryness under reduced pressure. The dried residue was again dissolved in cold distilled water and made up to a definite volume.

Purification of the Hydrolysate. A 4-ml aliquot of the prepared acid hydrolysate was extracted 5 times with 25-ml portions of peroxide-free ether and the aqueous phase of the hydrolysate was evaporated to dryness under reduced pressure. The residue was dissolved in 4 ml of 0.3 N hydrochloric acid and introduced into a Sephadex G-50 column, 90 × 1.5 cm, previously equilibrated with 0.3 N hydrochloric acid. The yellow band was collected in one portion by eluting with 0.3 N hydrochloric acid at a flow rate of 35 ml/h. The collected fraction was again evap-

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Table I. Composition of the Chick Starter Rations

Ingredients	Amount added: Group 1 A	Group 1 A_1	Group II B	Group II B_1	Group III, C
Yellow maize	30.0	30.0			
Bajra (millet)			30.5	30.5	
Jawar (sorghum grain)					26.0
Barley	8.0	5.0			
Rice polish	10.0	10.0	22.0	14.0	
Wheat bran					15.0
Mustard cake			10.0	25.0	
Groundnut cake	16.0	13.0	8.0	16.0	
Sesame cake	10.0	28.0			45.0
Meat meal			15.0		
Fish meal	12.0		2.0	2.0	
Molasses	10.0	10.0	9.0	9.0	10.0
Dicalcium phosphate	1.5	1.5	1.0	1.0	1.5
Limestone	1.0	1.0			1.0
Calcium carbonate			1.0	1.0	
Common salt	0.5	0.5	0.5	0.5	0.5
Vitamin premix[a]	1.0	1.0	1.0	1.0	1.0
	100.0	100.0	100.0	100.0	100.0

[a] Composition of the vitamin premix: 1.30 g of dry mineral-stable vitamin A (325 000 I.U. of vitamin A per g), 350 mg of dry mineral-stable vitamin D_3 (200 000 I.U. of vitamin D_3), and 450 mg of riboflavin were properly mixed with the ration per kg of premix. Antibiotics and minerals: tetracyclin at the rate of 12 g/ton of ration and 20 g of manganese sulfate per 100 kg of ration were added.

orated to dryness under reduced pressure and the dried residue was taken up with 4 ml of water.

Determination of ϵ-Dinitrophenyllysine. Using a DBG Beckman spectrophotometer the absorbance of the purified yellow solution was measured at 390 mμ against a water blank. Solutions of pure ϵ-dinitrophenyllysine in different concentrations were also prepared and the absorbances were read at the same wavelength. A standard curve was drawn from which the available lysine contents of the rations were determined easily.

Biological Experiments. The experiments were conducted with a total of 324 White Leghorn day-old chicks. Before starting the experimental trial all the preliminary precautions were taken to prevent any epidemic disease and the selected chicks, after weighing individually, were wing banded. These were then randomly distributed into 27 batches of 12 each and each experimental ration tested in triplicate. Water and feed were provided ad libitum. All the chicks were kept in an electrically heated battery brooder and 16 h of light was given daily.

The chicks were group weighed at weekly intervals and the average gain in weight at 8 weeks of age was calculated. The feed consumption by each group was also recorded at the time of weighing the chicks.

RESULTS AND DISCUSSION

Compositions of the rations of three groups are tabulated in Table I. Protein contents of the rations were 21.4, 20.9, 22.6, 22.0, and 20.4%, respectively. It can be seen from these data that both the rations in groups 1 and 2 were more or less isoproteinic. Available lysine contents of the rations, estimated by the present method, are shown in Table II. The results revealed that the values were low when lysine-rich ingredients were fully absent or present in a small amount. The method of Rao et al. (1963) was

Table II. Available Lysine Contents of the Rations

Group	Ration	% protein[a,b]	% total Lys[a]	% added L-Lys hydrochloride	% available Lys[a]: Method of Rao et al. (1963)	% available Lys[a]: Present method
I	A	21.4	1.06		0.90	0.87
	A_1	20.9	0.64		0.55	0.54
II	B	22.6	1.12		0.89	0.93
	B_1	22.0	0.68		0.53	0.51
	C	20.4	0.40		0.33	0.32
	C_1	20.4	0.40	0.20	0.53	0.51
III	C_2	20.4	0.40	0.35	0.64	0.68
	C_3	20.4	0.40	0.50	0.75	0.81
	C_4	20.4	0.40	0.65	0.91	0.95

[a] Values are on a moisture-free basis. [b] Determined by the micro-Kjeldahl method.

Table III. Recovery of ϵ-Dinitrophenyllysine Added to the Hydrolysates of the Rations

Ration	ϵ-Dinitrophenyllysine: Added, μg	Recovered, μg	Recovery, %	Mean recovery, %
A	40	39.6	99.1	
A_1	60	59.4	99.0	
B	80	79.3	99.1	99.1
B_1	100	99.3	99.3	
C	150	148.7	99.1	

Table IV. Average Gain in Weight of Chicks at 8 Weeks of Age and Feed Efficiency

Group	Ration	Av gain in wt of chicks at 8 weeks, g	Feed efficiency
I	A	564[a]	2.8
	A_1	477[a]	3.1
II	B	596[b]	3.3
	B_1	495[b]	3.4
III	C	411[c]	4.5
	C_1	482[c]	3.8
	C_2	525[c]	3.5
	C_3	580[c]	3.2
	C_4	620[c]	3.0

[a-c] Treatments are significantly ($P < 0.05$) different.

also carried out to estimate the available lysine contents of the rations and it was observed that the estimated values obtained by both the methods were very close. The specificity of the present procedure was also checked by carrying out recovery experiments. Known amounts of pure ϵ-dinitrophenyllysine were added to the acid hydrolysate prior to ether treatment and estimated following the same procedure as described before. Table III shows that the mean recovery percentage of the ϵ-dinitrophenyllysine was found to be 99.1%. The total lysine content of the experimental rations was determined by the method of Moore et al. (1958).

The acid hydrolysate was extracted with ether to remove ether-soluble dinitrophenylated amino acids. A Sephadex G-50 column was used to separate free amino acids. The color of the yellow solution was read at 390 mμ following the observation of Selim (1965) who showed that the other amino acids which possessed reactive ϵ-amino groups did not show absorbance to any notable degree at this particular wavelength.

The biological results are tabulated in Table IV. It can be seen from the furnished data that the average gain in

weight of chicks at 8 weeks of age for rations A and B was significantly higher ($P < 0.05$) than those of A_1 and B_1, respectively. The available lysine values when estimated by the present chemical method were also higher in rations A and B. The average gains in weight of the chicks fed with rations C, C_1, C_2, C_3, and C_4 were also significantly different ($P < 0.05$). It was observed from the furnished data that the gain in weight of the chicks increased with the increase of lysine availability. The results showed that the chemically estimated values conformed well with the biologically determined values and thus the present method was very useful for the assessment of compounded poultry rations. Moreover, the present colorimetric method is to some extent more rapid than the method of Rao et al. (1963) because it would then not be necessary to separate dinitrophenylated and free amino acids by ion exchange column chromatography for the estimation of available lysine in the compounded rations.

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Synthesis and Mass Spectrometry of Isotopically Labeled Isopropyl 2-(4-Thiazolyl)-5-benzimidazolecarbamate (Cambendazole)

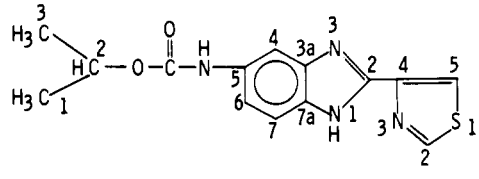
Robert L. Ellsworth,* Holly E. Mertel, and William J. A. VandenHeuvel

The synthesis of a number of heavy atom and radioactively labeled cambendazoles [isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate] for use in metabolism and residue studies is described. Availability of the former has facilitated study of the mass spectrometric fragmentation of this drug. The feasibility of establishing specific activities of the ^{14}C-labeled cambendazoles by mass spectrometry has been demonstrated.

Metabolism and tissue residue studies are integral components in the development of a new animal health drug for use in food-producing animals. Such investigations are facilitated by the use of isotopically labeled drugs, usually with radioactive, but occasionally with stable or heavy isotopes. The former are indispensable for determining levels of drug-related material in tissue, while the latter can be helpful in establishing the structure of metabolites and their mechanism of formation. We have recently prepared cambendazole, isopropyl 2-(4-thiazolyl)-5-benzimidazolecarbamate, an anthelmintic agent (Hoff et al., 1970), with both stable and radioactive labels in a variety of positions. The compounds discussed in this report are presented in Table I.

Cambendazole in which the thiazole ring nitrogen is enriched with ^{15}N was used in metabolic studies to determine the source of the nitrogen atom (body pool or thiazole N) in 2-carboxamido-5-isopropoxycarbonylaminobenzimidazole (Ib), a urinary metabolite of the drug (VandenHeuvel et al., 1974). Cambendazole enriched with ^{13}C in the C-2 (imidazole ring) position was used in an in vitro metabolism study (Wolf et al., 1974). A number of radiolabeled species of cambendazole have been prepared for use in tissue residue studies to partially characterize the nature of the residue. The availability of the heavy atom labeled cambendazoles has facilitated study of the mass spectrometric fragmentation of this drug. Furthermore, we have examined several of the ^{14}C-labeled preparations by mass spectrometry to determine the role this technique might play in establishing specific activities of labeled compounds independent of radioactivity measurements.

Table I. Isotopically Labeled Cambendazoles



Label type	Label position	% enrichment or sp act.
^{13}C	Thiazole C-4	57%
^{15}N	Thiazole N-3	33%
^{14}C	Benzimidazole C-2	2.81 mCi/mmol
^{14}C	Benzimidazole C-3a,4, 5,6,7,7a (*ar-U-*^{14}C)	2.84 mCi/mmol 9.00 mCi/mmol 114.16 mCi/mmol
^{14}C	Carbamoyl C=O	1.93 mCi/mmol

RESULTS AND DISCUSSION

The radioactive starting material for the compounds labeled in the benzenoid ring with carbon-14 was aniline-^{14}C. The process (Schmid et al., 1966) used by our supplier to prepare this substance from barium carbonate-^{14}C consisted of reduction to barium carbide-^{14}C,

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