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I. INTRODUCTION

In assaying the relative nutritional values of proteins, workers have relied chiefly on indirect methods. Thus we have the classical nitrogen balance work of Mitchell (10), who in 1924 defined the "biological value" of a protein as the percentage of absorbed nitrogen retained in the body. Proteins were also evaluated according to their capacity to make young rats gain weight. "Protein efficiency" has been defined as gain in weight per gram of protein eaten, although Hegsted and Haffenreffer (4) have questioned the advantage of this term over simple weight gain.

Both these methods have been highly refined and are widely used at the present time. Even with the development of more specific methods, they will continue to be useful as confirmatory tools.

Recently methods based on a protein's ability to regenerate tissue in protein-depleted animals have come into use. These have the advantage of showing larger protein increases within shorter experimental periods than are possible in ordinary growth studies. Weight gain alone may be used, or the regeneration of plasma protein, liver protein, hemoglobin, and total carcass protein may be measured for more elaborate studies. In 1947 the originators of the "rat repletion" method (1)

THE DIGESTIVE AVAILABILITY OF LYSINE IN DIFFERENT PROTEINS

by

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stated: "It is suggested that ultimately it may become necessary to evaluate proteins in terms of the limiting action of each indispensable amino acid in the test protein rather than to attempt to evaluate amino acid levels indirectly by current methods."

The following year Kuiken and Lyman (8) determined the availability of the ten indispensable amino acids in roast beef, cottonseed flour, peanut flour, and wheat flour. The amino acids were determined microbiologically in the foods and rat feces. So-called "metabolic" or non-dietary amino acid excretion levels were determined by using a low protein (4%) egg ration as a standard. This was the first systematic attempt to evaluate proteins directly in terms of the availability of the individual amino acids to the animal.

Using a similar technique, the present study investigates the digestive availability of lysine in twelve different foods. Lysine was selected because it is a limiting amino acid in several common proteins, because of its importance to the poultry industry, and because growth studies have shown lysine availability is affected by heat.

II. METHODS

A. THE STANDARD

An important prerequisite of an availability experiment is the satisfactory determination of the metabolic or endogenous excretion of the amino acid being studied. A ration containing 6% defatted whole egg (4% protein) was used by Kuiken and Lyman (8,9) for this purpose.

Since whole egg protein was not obtainable at the time this work was undertaken, 6% casein (5% protein) was used instead. Geiger (3) has recently demonstrated that casein levels higher than this are 99% absorbed six hours after their injestion by rats, so the use of casein as a standard seemed warranted.

B. COMPOSITION OF DIETS AND FEEDING

Like the standard, all the test foods were fed at a 5% protein level (6.25 X N). 50% of each diet consisted of the protein-free ration developed by Frost and Sandy (2):

Cerelose	3320g.
Wesson oil	184
Crisco	168
Salt mixture	160

Agar	56g.
Cod Liver Oil	56
Choline Chloride	6
Inositol	4
Thiamine HCl	0.0240
Riboflavin	0.0480
Pyridoxine HCl	0.0240
p-aminobenzoic acid	0.0240
Biotin	0.0016
Folic Acid	0.0240
Vitamin B-12	0.0002
2-methyl naphtho-quinone	0.0160
d-Ca pantothenate	0.2000
Nicotinamide	0.1480
$CoCl_2 \cdot 6H_2O$	0.0080
$ZnSO_4 \cdot 7H_2O$	0.1600

Cerelose was then added to make up 100%. The compositions of three typical diets were as follows:

Diet I	Casein	60g.
	Cerelose	440
	Protein-free ration	500
Diet VI	Wheat flour	310
	Cerelose	190
	Protein-free ration	500

Diet XII	Cottonseed meal	120 g.
	Cerelose	380
	Protein-free ration	500

Each diet was thoroughly mixed, then weighed into test tubes in 10 gram portions.

Experiments with feces markers (Fe_2O_3 and lampblack) fed at various levels gave erratic and unpredictable results. In some cases, after the marked ration had been fed for one day, the marker appeared in the feces, disappeared, then reappeared several days later. To avoid the use of markers, the following system was employed: Groups of five young male rats (Sprague-Dawley, 85-100 grams) were fed 10 grams of the test diet at nine o'clock each morning for a period of ten days. After the first three days, feces collections were made daily until the end of the ten-day period. Since the diets were completely eaten by the rats, it was assumed that each 7-day feces collection represented 70 grams of diet.

C. DETERMINATION OF LYSINE

1. Hydrolysis

The feces were refrigerated until the end of the collection period, then dried 16 hours at 65° C., separated from hair, weighed, and refluxed with 20% HCl for 24 hours. To avoid sampling errors, the entire

sample was hydrolized. Five gram portions of each test food were similarly hydrolized.

After most of the HCl had been driven off, the hydrolysates were buffered and neutralized with NaOH to a pH of 6.9. The hydrolysates were then filtered, diluted to 500 cc., and refrigerated under toluene.

2. The Microbiological Determination

The hydrolysates were assayed for lysine by the microbiological method of Henderson and Snell (5), as modified by Kemmerer (6). The organism used was Leuconostoc mesenteroides P-60.

For convenience, solutions of compounds needed in the assay medium were made up in advance. First was a solution containing the necessary amino acids with the exception of lysine:

DL-alanine	2.0000g.
DL-aspartic acid	2.0000
L-glutamic acid	2.0000
DL-serine	0.4000
L-proline	0.2000
L-tyrosine	0.2000
L-cystine	0.2000
glycine	0.2000
L-arginine	0.4000
L-histidine	0.2000

DL-isoleucine	0.4000
DL-leucine	0.4000
DL-methionine	0.4000
DL-phenylalanine	0.4000
DL-threonine	0.4000
DL-tryptophane	0.4000
DL-valine	0.4000

in 500 cc.. A "Salts C" solution contained:

$MgSO_4$	4.86g.
$FeSO_4 \cdot 7H_2O$	0.50
NaCl	0.50
$MnSO_4 \cdot 4H_2O$	1.61

in 250 cc.. 100 mg. of adenine, guanine, and uracil respectively were dissolved and made up to 100cc.. 100 mg. of xanthine were dissolved separately in 100 cc.. A vitamin solution was prepared by first dissolving

100 mg.	Pyridoxine HCl
100	p-aminobenzoic acid
5	Biotin
5	Folic acid

in 250 cc., then adding 10 cc. of this to

20 mg.	Thiamine HCl
20	Niacin
20	Ca pantothenate

and making up to 200cc.. Riboflavin was kept

separately, 10 mg. per 100cc.. The assay medium was then made up by adding

10 cc.	Salts C
5	Vitamins
5	Riboflavin
5	Xanthine
5	Adenine-guanine-uracil
125	Amino acids (-lysine)

to

10 g.	Glucose
10	Na Citrate
0.5	Na Acetate
1.5	NH_4Cl
2.5	$K_2HPO_4 \cdot 3H_2O$

The pH was adjusted to 6.9 and the volume made up to 250 cc..

To one cc. of assay medium in each Wasserman tube (13 mm. by 100 mm.) a dilution of one of the hydrolysates was added. Dilutions of 0.4, 0.6, 0.8, and 1.0 cc. were made, the difference being made up with distilled water. Each dilution was run in duplicate, so that for the analysis of each hydrolysate 8 tubes were required. An automatic pipette was used in making the dilutions.

The test organism was carried by monthly transfers

in an enriched basal medium. One drop of a saline suspension of the organism was added to each tube with a sterile pipette, and the cultures were incubated for four days at 37° C..

Lysine hydrochloride was used as the standard, curves being drawn from the titration values of 10 gamma, 20 gamma, 30 gamma, 40 gamma, and 50 gamma dilutions. Since absolute lysine contents were not required in this experiment, all the lysine values quoted, except in Table II, represent lysine hydrochloride.

3. Titration

After the four-day incubation, the lactic acid produced in each culture was titrated to neutrality with 0.04 N NaOH, using a pH meter and an automatic stirrer. From the standard curves, the gammas of lysine corresponding to the cc. NaOH required for each dilution were obtained.

D. CALCULATION OF DATA

Lysine availability was calculated as outlined below for white flour:

Lysine recovered	0.0510 g.
Non-dietary lysine	0.0270
Net lysine excreted	0.0240
Lysine consumed	0.0839
Net lysine excreted	0.0240
Lysine absorbed	0.0599

$$\frac{\text{Lysine absorbed}}{\text{Lysine consumed}} = \frac{0.0599}{0.0839} \times 100 = 71\% \text{ Availability}$$

III. RESULTS

TABLE I

LYSINE AVAILABILITY IN TWELVE FOODS

Food	No. Rats	G. Lysine Consumed	G. Lysine Recovered Range	G. Lysine Recovered Average	% Availability
Casein	4	0.3855	.022-.030	0.0270	100
Gelatin	5	0.1323	.022-.055	0.0280	99
Cheese	4	0.3288	.030-.033	0.0320	98
Tuna	5	0.3364	.011-.021	0.0160	100
Starlac	3	0.3753	.084-.123	0.0940	82
Wheat flour	4	0.0977	.051-.065	0.0580	68
Wheat bread	5	0.0958	.054-.075	0.0610	65
White f.	5	0.0839	.045-.057	0.0510	71
White b.	4	0.0970	.049-.075	0.0620	63
Beans	4	0.3307	.182-.247	0.2190	42
Cotton-seed meal	5	0.1386	.063-.081	0.0710	68
Rye flour	5	0.1638	.060-.092	0.0700	74

TABLE II

PERCENTAGE LYSINE IN FOODS TESTED

Food	% Lysine
Casein	6.6863
Gelatin	2.7536
Cheese	1.9116
Tuna	2.2438
Starlac	2.6036
Wheat flour	0.3278
Wheat bread	0.3278
White flour	0.2182
White bread	0.3058
Beans	1.3766
Cottonseed meal	1.2020
Rye flour	0.3642

IV. DISCUSSION OF RESULTS

For the most part, these results are substantially what one would expect. Kuiken and Lyman (8) found the lysine in roast beef 100% available and 65% available in cottonseed flour. Here, lysine was 100% available in canned tuna fish and 68% available in cottonseed flour.

The lysine in Starlac was found to be less available than the lysine in casein or American cheese. It has been shown that heating lysine in the presence of carbohydrate lowers its availability (11). The drying process used in the manufacture of Starlac apparently accounts for this diminished availability. Similarly, the lysine in white and whole wheat breads (63% and 65%) was found to be slightly less available than the lysine in white and whole wheat flours (71% and 68%).

That less than half the lysine in beans (pink, uncooked, finely ground) should be available was surprising. Beans are proverbial for causing flatulence, and the feces output of the rats on this ration was double the average, suggesting a marked laxative effect. Possibly this increased peristaltic activity interfered with the hydrolysis and absorption of the lysine. Trypsin inhibitors have been found in legumes, soybeans, and lima beans (7). The presence of such an inhibitor

in pink beans would further reduce the lysine availability.

Availability of lysine in grains clustered around 70%: wheat flour 68%, rye flour 74%, white flour 71%. This is in disagreement with Kuiken and Lyman (8), who found lysine 92.8% available in wheat flour.

These workers permitted ad lib feeding, so that different amounts of carbohydrate, fat, and vitamins were ingested by each rat. They also pooled the feces of each group of five, in order to reduce the number of analyses. The extent of individual variation is therefore not known.

The errors inherent in availability experiments should be borne in mind when studying the results. It has been assumed that all the rats eating 70 grams of a 6% casein diet per week will excrete 0.0270 grams of lysine, which is obviously not true. The average lysine excretion for rats on the tuna fish diet was actually below that of the casein standard, although the availability is given as 100%.

Fluctuations in non-dietary lysine excretion constitute the chief source of error in this experiment. Perhaps by alternating the test diets with the standard diet, individual values for non-dietary lysine excretion of each rat can be obtained. When this is not done and when average values of amino acids in the feces are given,

the extent of individual variation must also be given, lest one credit the method with a precision it does not possess.

Further experiments with a larger number of animals will have to be made in order to obtain conclusive results.

V. SUMMARY

1. Analyses of the lysine consumption and excretion of rats indicate that the lysine in tuna fish, gelatin, and cheese is completely available, and only 82% available in Starlac.

2. The lysine in rye flour, white flour, whole wheat flour, and cottonseed flour was found to range from 68% to 74% available.

3. Less than half the lysine in uncooked pink beans was found to be available to rats.

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