Assessment of Factors Influencing Estimation of Lysine Availability in Cereal Products

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The availability of lysine in corn and rice proteins determined by growth and fecal analysis methods and the influence of the type of carbohydrate, amino acid pattern of the diet, and caloric content of the diet on these methods were examined. In general, the lysine of corn and rice proteins was highly available. Values obtained by the growth method were influenced by changes in the composition of the diet and the method of calculating availability. They were most reproducible and least influenced by other factors when availability was calculated as a function of lysine consumption rather than lysine level in the diet. The fecal analysis method gave somewhat lower values for corn products.

Two in vitro and three in vivo methods have been most commonly used for estimating the biological availability of amino acids in proteins. The values for availability obtained by these methods differ considerably and so far little effort has been made to distinguish between relative and quantitative values, to study the sources of error in the various methods, or to estimate the significance of the differences observed. Since an accurate estimate of the availability of amino acids is of practical importance, an assessment of the merits of the various methods is needed.

The in vitro methods are based on comparisons of the rates at which amino nitrogen or free amino acids are released from different proteins when they are incubated in vitro with proteolytic enzymes (*23*, *28*) and on measurement of the percentage of free epsilon-amino groups of lysine in different proteins by the fluorodinitrobenzene (FDNB) procedure (*4*, *5*). The former gives a relative rather than a quantitative measure of amino acid availability; the latter is a quantitative method, but only for lysine. The FDNB method is especially useful for estimating the effects of heat processing, which lowers particularly the availability of lysine by causing binding of epsilon-amino groups.

The in vivo methods are based on measurement of the increase in fecal excretion of a particular amino acid after feeding a test protein (*17*) and the ability of a protein of known amino acid composition to replace a specific amino acid in supporting growth or repletion of the intact animal (*32–34*) and in maintaining nitrogen balance in a mature subject (*18–21*).

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The in vivo methods can be used to measure the availability of any amino acid; however, the meaning of "availability" depends upon the method used. As determined by the fecal analysis method, it is a measure of the amount of unabsorbed amino acid. It is, therefore, a measure of digestibility applied to a specific amino acid and depends upon the digestibility of the protein and the presence of enzyme-resistant peptide bonds or enzyme-inhibiting substances in the sample under assay. The growth and nitrogen balance methods, on the other hand, assess not only digestibility but also the efficiency of utilization of the absorbed amino acids by the body.

In general, values obtained by the fecal analysis method are higher than those obtained by the growth method (*3*, *11*, *13*), particularly for poorly balanced proteins such as those of maize. Values as low as 50% for the availability of lysine from maize have been obtained using the growth method (*11*), compared to 89% by the fecal analysis method (*7*). For isoleucine in zein, a value of 30% was obtained using the growth method (*8*); by the fecal analysis method the isoleucine of maize was estimated to be 90% available (*7*). Although isolated zein is digested more slowly than zein in its natural form (*6*), it is unlikely that so great a difference is entirely a result of the isolation process. By contrast, in other experiments in which growth has been used as the criterion of availability, some values well in excess of 100% have been obtained (*22*, *27*).

The values obtained by the growth method should be influenced not only by the amount of amino acid lost in the feces but also by inefficient utilization due to delayed release of amino acids, and this may result in lower values, in some cases, than are obtained by the fecal analysis method. Growth is also influenced, however, by amino acid balance, protein level, type of carbohydrate, and calorie to protein ratio of the diet. Variations in any of these may affect values for availability obtained by the growth method; hence, some of the abnormally low or high values obtained by this method are probably the result of inadequate control over these factors. Values for availability obtained by the growth assay can also vary with the method used in calculating results (*3, 11*). Thus, like in vitro digestion methods, growth methods as described to date cannot be considered quantitative. From a survey of the literature, it seems evident that differences in the values reported by different authors must be due to factors that should, strictly speaking, be differentiated from "availability."

To appraise the significance of the effects of some of these factors, the availability of several indispensable amino acids in maize and rice proteins has been estimated by the growth and fecal analysis methods.

Materials and Methods

Samples and Experimental Rations. Corn gluten and "reconstituted" corn gluten samples were described earlier (*6*); the former was a commercial product, the latter an alcohol-extracted product supplemented with zein. Rice flour was prepared by grinding polished rice in a hammer mill. Rice protein was obtained by extracting rice flour with 0.05*N* NaOH (*26*). Values for the nitrogen content of corn gluten, "reconstituted" corn gluten, rice, and rice protein as determined by the Kjeldahl method were 7.02, 7.04, 1.00, and 13.50%, respectively. Values for lysine content of corn gluten, rice, and rice protein as determined by microbiological assay were, respectively, 0.880, 0.245, and 2.773%.

The basal diet consisted of a complete amino acid mixture (*3*) except for the amino acid under assay; 5% of salts; 4% of corn oil supplemented with vitamins A, D, and E; 0.15% of choline chloride; water-soluble vitamins (*14*); and dextrin to make up 100%. The amino acid mixture contained both dispensable and indispensable amino acids. Graded levels of the amino acid under test were added to portions of the basal diet to prepare the diets used for the standard curve. An improved amino acid diet has been devised since this work was completed (*29*) and although the diet cited above is adequate for comparative studies of the type described in this paper, the improved diet is to be preferred.

Methods. Male weanling Holtzman rats weighing 55 grams were used throughout and were housed individually in suspended wire-mesh cages. Before the experiment proper, all rats were fed for 4 days on a diet similar to the basal diet but containing a complete amino acid mixture. After this adaptation period, the animals were divided into equal-weight groups consisting of five rats each and allocated at random to the various diets. The experimental period was 14 days. Food consumption and weight gain were measured daily. From the fifth to the twelfth day, feces were collected.

At the termination of the experimental period the animals were killed and gastro-intestinal contents removed. The carcasses were dried for 48 hours at 75° C. and 48 hours at 105° C. The dried carcasses for each group were weighed, pooled, homogenized in water using a Waring Blendor, and then autoclaved for 2 hours in 20% HCl at 120° C. After this predigestion the suspension was made up to volume and aliquots were taken for nitrogen determination and for further acid hydrolysis to determine carcass amino acids.

The collected feces were homogenized and aliquots taken for nitrogen determination and acid hydrolysis. After 8 hours' autoclaving at 120° C. the hydrolyzates were filtered, adjusted to pH, and diluted to a range suitable for microbiological assay. All microbiological assays were carried out with *Leuconostoc mesenteroides* (*1*).

Determination of Availability by Growth Method. Five criteria were used to evaluate amino acid availability: change in weight, in empty weight (weight after removal of gastro-intestinal contents), water content, nitrogen content, and amino acid content of the carcass (amino acid under assay). Availability was calculated from the changes in each of these criteria by three methods (*3*). The available amino acid was estimated from the standard curve obtained in Method 1 by plotting the total change in the particular criterion against the per cent of amino acid in the diet; in Method 2, by plotting food efficiency against per cent amino acid in the diet; and in Method 3, by plotting the total change in criterion against the total amount of amino acid consumed. Per cent availability was calculated as follows:

$$\frac{\text{Amino acid utilized by animal as obtained from standard curve}}{\text{amino acid content of diet as determined by microbiological assay}} \times 100$$

Determination of Availability by Fecal Analysis Method. The availability of the amino acid under consideration was calculated from the difference between the amount consumed and that excreted in the feces. The amount excreted in the feces which is not derived from the supplement is taken into consideration in the calculation. This (metabolic amino acid) is estimated from the quantity of amino acid excreted by animals fed the basal diet which contains all amino acids except the one under assay. On the basis of the classical metabolic nitrogen determination (*24*) the metabolic amino acid is also taken to be dependent on the amount of food consumed and hence is adjusted for level of food intake. The calculation is as follows:

$$\frac{\text{Total intake of amino acid} - (\text{fecal excretion of amino acid} - \text{metabolic amino acid})}{\text{total intake of amino acid}} \times 100$$

Experimental and Results

Growth Method. In the study on the availability of lysine, corn gluten was substituted for starch in the experimental diets at three different levels, 35, 50, and 70%, and in one trial 4% of corn oil was included in the diet containing 35% of corn gluten. "Reconstituted" corn gluten, rice, and rice protein were substituted for starch in the experimental diets at levels of 35, 70, and 7.5%, respectively. Availability was also determined using a diet which contained 70% of rice supplemented with 0.1% of L-lysine·HCl.

Average values for food consumption and for five criteria of performance for groups of rats fed the standard diets and the experimental diets containing the components for which lysine availability was determined by the growth method are given in Table I.

Groups fed the basal amino acid diet lost weight, as did those receiving the smaller supplements of lysine. Groups fed the standard diets containing quantities of lysine that approximated the optimal requirements grew satisfactorily; a group receiving 1% L-lysine·HCl gained 38.6 grams per week.

In Table II are given values for percentage availability of lysine in the test samples as calculated by the three methods from the growth criteria in Table I.

Most of the values for availability of lysine in rice exceed 100%, as do several of those for corn gluten, in particular those obtained with the lowest level of supplementation. This indicates that rice used as a source of lysine stimulates the growth of rats more than an equivalent amount of free lysine. The value for rice protein was lower than that for rice, so the stimulating effect is apparently due to other components of rice, possibly the carbohydrate. Since the value for availability calculated from the standard curve obtained by plotting growth as a function of lysine consumed is lower than the values obtained by the other methods, this would suggest that increased food intake is responsible for much of the growth stimulation.

Table I. Performance of Rats Used in Lysine Availability Study
(14-day experimental period)

Addition to Basal Diet	Food Consumed, G.	Change in Weight, G.	Change in Empty Weight, G.	Carcass N Content, Mg.	Carcass H_2O Content, G.	Carcass Lysine Content, Mg.
Basal diet	53.4	−8.2	−9.1	1260	30.7	456
+ 0.1% lys·HCl	56.9	−4.0	−7.3	1242	32.9	522
+ 0.2% lys·HCl	67.0	0.8	−0.4	...	36.0	...
+ 0.3% lys·HCl	78.6	9.4	7.0	1440	40.5	680
+ 0.4% lys·HCl	94.1	17.2	14.5	1568	46.0	880
+ 0.5% lys·HCl	115.5	30.8	26.5	1810	54.7	1130
+ 35% corn gluten	82.4	11.2	7.9	1502	42.0	680
+ 50% corn gluten	79.5	15.6	10.9	1536	44.9	716
+ 70% corn gluten	90.2	29.4	23.4	1850	54.5	890
+ 35% reconst. corn gluten	86.6	11.0	7.4	1444	41.8	670
+ 35% corn gluten + 4% oil	75.0	7.0	4.8	1478	40.1	610
+ 70% rice	72.1	1.2	0.2	...	36.4	...
+ 7.5% rice protein	65.2	1.0	−0.6	1350	35.9	544
+ 70% rice + 0.1% lys·HCl	79.5	8.4	6.9	1428	41.6	640

Table II. Per Cent Availability of Lysine Calculated from Results of Growth Experiments

Criterion	Method of Calculation[a]	Corn Gluten 35%	50%	70%	35% (reconst.)	35% + 4% oil	Rice, 70%	Rice Protein, 7.5%	Rice, 70%, + Lysine ·HCl
Weight gain	1	105.5	88.4	78.7	103.9	87.6	119.8	97.1	105.9
	2	109.4	94.1		106.1	94.1	116.9	94.7	111.4
	3	110.0	98.9	99.4	104.8	95.2	107.3	95.6	111.6
Empty weight gain	1	101.2	81.9	77.1	97.4	87.6	122.8	96.1	110.7
	2	105.5	87.2	...	93.0	92.5	112.8	93.7	116.2
	3	100.4	87.9	94.5	89.8	90.8	113.8	93.3	109.3
Nitrogen content	1	110.3	86.0	84.4	97.4	103.9	...	108.0	105.0
	2	116.8	84.9		99.0	115.9	...	113.9	108.8
	3	110.2	93.7		88.8	116.8	...	113.9	104.6
H_2O content	1	107.0	89.5	81.9	105.5	92.5	119.8	93.7	118.1
	2	108.7	96.5	96.5	105.5	97.4	119.8	91.3	121.7
	3	108.2	100.2	103.6	102.9	99.5	113.8	95.5	123.2
Carcass lysine	1	97.4	74.4	64.1	94.1	68.2		69.2	
	2	99.0	74.4	64.9	98.0	75.5		74.0	
	3	92.9	74.3	70.5	84.4	67.9		62.5	

[a] 1. Using standard curve for change in criterion *vs.* % lysine in diet.
2. Using standard curve for change in criterion/100 grams food consumed *vs.* % lysine in diet.
3. Using standard curve for change in criterion *vs.* lysine consumed.

In general, lysine is highly available in all products. Its availability in a product varies considerably, depending upon the criterion of performance used, the method of calculation, and composition of the test diet.

Variation Due to Criteria of Performance Used to Determine Availability. Values for availability of lysine based on empty weight gain are in general lower than those based on live weight gain. Since the gastro-intestinal contents of rats fed the diets containing corn gluten were heavier at the termination of the experiment than the contents of the comparable groups receiving the standard diets, availability is overestimated if weight gain is used as the criterion of performance. The gastro-intestinal contents of rats fed on rice were lighter than those of the standard groups. By using the empty weight, gain inaccuracies due to differences in the amounts of gastrointestinal contents are eliminated.

The deposition of body protein as measured by carcass nitrogen was suggested by Calhoun, Hepburn, and Bradley (*3*) as the most reliable criterion for availability determination. In the present study values for availability as determined by carcass nitrogen gains were sometimes higher than corresponding values obtained by using empty weight gain as the criterion. The nitrogen gains for these groups (35% corn gluten, 35% corn gluten and 4% oil) were higher than for those receiving standard diets with an equivalent amount of lysine.

Except in a few instances, availability as determined from the water content of the rat was higher than the value obtained by the use of empty weight gain.

The use of lysine content of the carcass to determine availability resulted consistently in lower values. All values were below 100% and were of the same order as or below those determined by the fecal analysis method (Table VII).

Variation Due to Method of Estimating Utilized Lysine from Standard Curve. The availability values obtained by the first method of calculation are in general lower than those obtained by the second method—i.e., utilized lysine estimated from the standard curve based on per cent lysine in the diet *vs.* standard curve based on food efficiency. A similar effect was observed by Gupta *et al.* (*11*) and Calhoun *et al.* (*3*). The values are also much more influenced by the different levels of dietary protein than are those obtained by the other two methods of calculation. Although food intake is influenced mainly by the percentage of lysine in the diet in this type of experiment, the relationship can be altered by changes in other factors such as type of carbohydrate and protein to calorie ratio of the diet. A supplement that stimulated food intake more than the free amino acid would result in an overestimate of availability by method 1; a supplement that depressed food intake would result in an underestimate. From this and considerations discussed by Gupta *et al.* (*11*) it follows that the accuracy of availability determinations can be increased by taking food consumption into consideration.

Since both Methods 2 and 3 take food consumption into account, yet values for availability obtained by the two methods differ, the question arises as to which is the better method.

It can be accepted that when protein or an amino acid is limiting growth, growth tends to be a function of the protein or amino acid intake rather than of the food intake; this would suggest that Method 3 is the more reliable. In an earlier paper it was shown that Method 3 gave rise to the least variation (*15*). This was based on the fiducial limits calculated from the regression analysis of the values for utilizable lysine. Part of the problem centers around the sensitivity of the method. The more sensitive the method, the smaller is the difference required between treatments for detection of significance (*2*). In Table III are listed the degrees of freedom, F values, and probability that Method 3 is more sensitive than Method 2. These values are derived from individual weights of rats in different groups fed on graded levels of lysine, threonine, isoleucine, and valine. With the same number of animals the experiment with the highest F value can be taken as the most sensitive. Statistically significant differences between these F values can be tested by the method of Schumann and Bradley (*31*). In column 5 are given the probabilities that F_3 is greater than F_2 for the four animal trials. From these values it is evident that Method 3 is the most sensitive, so that less variation will occur in the estimation of the utilized amino acid and hence smaller differences between values for availability will be required for significance.

Variation Due to Composition of Experimental Diet. Results for groups receiving different levels of corn gluten (Table II) reveal that the value for availability tended to fall as the amount of supplement was raised. The effect was greatest when availability was calculated from the standard curve obtained by plotting the total change in criterion as a function of the per cent of amino acid in the diet—e.g., availability values calculated by this method from the results for change in empty weight gain for groups receiving 35 and 70% of corn gluten are 101 and 77%, respectively, a difference of 24%. If the standard curve was based on lysine intake, the values differed much less, being 100 and 94%, respectively, a difference of only 6%.

When changes in the various criteria are expressed per 100 grams of food consumed (food efficiency) and the standard curve is prepared by plotting these values

Table III. Sensitivity of Standard Curves Used to Determine Availability
(D.F. = 17)

Amino Acid	Calculation	F Value	Probability of $F_3 > F_2$
Lysine	2	590.0	0.100
	3	777.8	
Isoleucine	2	58.2	0.010–0.025
	3	228.2	
Threonine	2	162.2	0.025–0.050
	3	527.0	
Valine	2	23.1	0.025–0.050
	3	75.6	

against per cent of amino acid in the diet (Method 2) the effects of changes in the level of supplement on availability values are again larger.

The tendency for availability to decrease with increasing protein level as observed in the present study has been reported in several (*8*, *12*, *33*) but not all (*11*) studies of amino acid availability by the growth method. This may be related to the observations (*10*, *25*) that a large increase in protein content of the diet increases the lysine requirement of the animals. Depending upon the conditions, the influence of protein level may be of considerable importance in availability determinations and should be carefully controlled.

From Table IV it is evident that 6% of an amino acid mixture, lacking lysine, decreases the value for lysine utilization considerably. A diet containing 24% of the basal amino acid mixture requires extra lysine (group 4) in order to support a weight gain similar to that obtained with rats fed a diet containing 18% of the amino acid mixture (group 1). This need for additional lysine to prevent a fall in growth rate is due to the creation of an amino acid imbalance. On this basis a properly balanced supplementary amino acid mixture containing an adequate amount of lysine should result in a smaller apparent reduction in utilization of lysine present in the basal ration.

This is demonstrated in Table V. The availabilities were calculated from standards run concurrently with the experimental diets. Groups 1, 2, and 3 were given diets supplemented with 2, 4, and 6% of an amino acid mixture which contained 7.2% of L-lysine·HCl (as per cent of the amino acid mixture). Groups 4, 5, and 6 were supplemented with 2, 4, and 6% of the same amino acid mixture, except that lysine was present as only 1.8% of the amino acid mixture. Supplementation with more than 4% of the poorly balanced amino acid mixture resulted in a low value for availability. Since lysine is added in the free form, the term "utilization" is more appropriate than "availability" for this effect. The ratio of lysine to total amino acid content of the ration is therefore important in amino acid availability determinations. The relationship is complex, as pointed out by Fisher (*9*), and can be a problem in the determination of availability by the growth method. Although the value for availability may be low as a result of an imbalance, growth will be affected significantly only with the higher

Table V. Effect of Imbalance in Supplementary Amino Acid Mixture on Availability of Free Lysine

Group No.	Diet: Basal amino acid mixture, %	Diet: Supplementary amino acid mixture, %	Availability of Lysine, %
1	19[a]	2 A[b]	104
2	19	4 A	105
3	19	6 A	104
4	19	2 B[c]	96
5	19	4 B	91
6	19	6 B	80

[a] Basal amino acid mixture contained 1.4 g. L-lysine·HCl per 100 g. of mixture.
[b] Supplementary amino acid mixture A contained 7.2 g. L-lysine·HCl per 100 g. of mixture (balanced).
[c] Supplementary amino acid mixture B contained 1.8 g. L-lysine·HCl per 100 g. of mixture (poorly balanced).

levels of the lysine-free amino acid mixture. Hence, with small increments of protein the effect of protein level should be small if the limiting amino acid is not too low. Also, since this work was completed, it has become apparent that the main effect of an amino acid imbalance is on food intake; hence, when this is taken into account, the effect of poor amino acid balance should be minimized.

Another trend was evident when the values obtained with 35% of corn gluten with and without an additional 4% of oil (Table II, columns 1 and 5) were compared. The values for availability, with only one exception, fell as the calorie content of the diet was raised. The effect was smaller if the standard curve obtained by plotting total change in criterion as a function of total intake of lysine was used for estimating availability but was not eliminated. The inclusion of an extra 4% of corn oil in the diet depressed food intake, resulting in increased lysine requirement when expressed as a percentage of the diet (*30*). Williams and Grau (*35*) further demonstrated that feed efficiency decreased with increasing calorie content of a diet deficient in lysine. The present availability results are in agreement with these observations.

Table VI illustrates how growth of rats and hence availability can be influenced by a change in the type of carbohydrate in the experimental diet. The experiment was laid out in a randomized block design containing

Table IV. Effect of Amino Acid Imbalance on Availability of Free Lysine

Group No.	Diet: Basal amino acid mixture, %	Diet: Supplementary amino acid mixture free of lysine, %	Diet: L-Lysine·HCl, %	Average Gain, Grams/2 Weeks	Availability,[a] %
1	18	...	0.55	34.4 ± 3.1[b]	100
2	18	4	0.55	33.0 ± 4.2	95
3	18	6	0.55	23.4 ± 5.3	68
4	18	6	0.65	40.3 ± 2.8	...

[a] % utilization for groups 1, 2, and 3 calculated by taking weight gain of group 1 as 100% and expressing other weight gains as % of group 1.
[b] S. E. of mean.

Table VI. **Effect of Starch and Cellulose Levels in Diet on Growth and Food Efficiency**

Levels of Starch	Level of Cellulose 4%	8%	$\bar{X}$, G.	Analysis and Variance Source	D.F.	M.S.
	Weight Gain, G./14 Days					
No starch	19.2	23.2	21.2	Cellulose	1	160[a]
20% starch	17.2	27.6	22.4	Starch	3	187[a]
40% starch	20.0	25.0	22.5	Starch × cellulose	3	92
60% starch	22.2	32.8	27.5	Error	21	19
$\bar{X}$	19.6	27.1				
L. S. D. of means: within table, 8.7; for starch levels (8 observations), 6.1; for cellulose levels (16 observations), 4.3						
	Weight Gain, G./100 G. Food Consumed					
No starch	19.9	23.7	21.8	Cellulose	1	174[b]
20% starch	20.6	25.6	23.1	Starch	3	51
40% starch	24.4	22.1	23.2	Starch × cellulose	3	60
60% starch	22.4	33.5	27.4	Error	21	30.5
$\bar{X}$	21.8	26.3				
L. S. D. of means: within table, 8.1; for starch levels (8 observations), 5.7; for cellulose levels (16 observations), 3.4						

[a] Statistically significant at 1% level ($P \leq 0.01$).
[b] Statistically significant at 5% level ($P \leq 0.05$).

four blocks with eight rats per block. The composition of the experimental diets, in which lysine was the limiting amino acid (0.5% lysine·HCl as percentage of the diet), was identical with that described under methods except for the carbohydrate. Eight diets were prepared using combinations of four levels of maize starch (0, 20, 40, and 60%) and 2 levels of cellulose (4 and 8%), incorporated in the ration at the expense of glucose.

Analysis of variance of the interaction table computed from weight gains reveals that both increasing levels of starch and increasing levels of cellulose resulted in significant growth responses. If weight gain per 100 grams of food consumed is used in the computations, only the effect of increasing the fiber level in the diet was statistically significant. These data, which agree with earlier published work on the effect of various carbohydrates on growth of rats, demonstrate that availability values higher than 100% can be obtained by the growth method as a result of growth responses to factors other than the amino acid under assay.

Fecal Analysis Method. In Table VII are given availability values obtained by the growth method (mean of all values calculated by Method 3 from all criteria except carcass lysine) and true digestibility of protein in the experimental diets.

Although values for availability determined by the fecal analysis method are generally higher than those determined by the growth method (see introduction), in the present study the opposite was observed. If growth was stimulated by factors other than the lysine in the protein supplement, this would occur. The high value for lysine in rice is evidence of this. Availability values estimated by the fecal analysis method also show a slight decrease with increasing levels of protein in the diet but less than was observed for values obtained with the growth method. Whether this should be attributed to

Table VII. **Availability Values for Lysine Determined by Growth and Fecal Analysis Methods and True Digestibilities of Test Proteins**

Protein Source in Test Diet	Availability, % Growth method	Fecal analysis method	Digestibility of Protein
Corn gluten			
35%	107.2	86.4	98.8
50%	95.2	81.7	97.6
70%	99.1	80.2	98.1
35% (reconstituted)	96.5	70.5	97.0
35% + 4% oil	100.5	76.8	...
70% rice	111.6	100.0	102.1
7.5% rice protein	99.5	94.0	96.1

smaller variation between values as determined by the fecal analysis method is difficult to say on the basis of the present data, since the values have been calculated from pooled feces samples of five animals. Kuiken (*16*) reported that availability values determined by the fecal analysis method by independent trials with different animals varied by not more than 5%. A decrease in availability from 86 to 77 was also recorded in the availability of lysine in corn gluten with an increase in the calorie level of the diet. Lysine availability decreased from 86 to 70% in the reconstituted corn gluten sample. The true digestibilities were not influenced by these factors. They are close to 100% and in general agree better with the mean for availability of lysine, as assessed by the growth method, than with the values obtained by the fecal analysis method.

Discussion

Many factors can influence the measurement of availability by the growth method. In most published studies on availability of amino acids by growth methods little attention has been paid to growth-influencing factors other than the amino acid under assay.

The growth method in which the standard curve of weight gain *vs.* lysine consumed was used was subject to the least variation and the availability values were least influenced by increases in protein and calorie level in the experimental diet. Availability values calculated by this method should therefore give the nearest approximation of true availability. Averaging all values obtained by the different growth criteria using this calculation resulted in availabilities of lysine very close to the per cent digestibility.

The availability values obtained from carcass lysine content were affected most by increasing the protein, and calorie levels in the experimental diet and taking food intake into account in the calculation of availability did not reduce this effect. If the method is to be of value, the reasons for these discrepancies must be uncovered and ways of obviating them found.

The effect of increasing protein level on estimates of availability of an amino acid will depend on several factors. If a protein is severely unbalanced, as is the case with lysine and tryptophan in most cereal products, the estimate of availability may be low. If the amount of protein supplement in the experimental diet can be kept low, the effect of imbalance is not likely to be great. The effect of poor amino acid balance can also be minimized if food intake is taken into consideration in the calculation. The standard errors of measurements (Table IV) used for calculations of availability indicate that values for availability may differ considerably without being statistically different.

The growth method may also be influenced by the source of carbohydrate and the calorie content of the experimental diet, which can lead to either an under- or overestimation of availability. The calorie content of the test and standard diets should be equalized to permit accurate comparison of availability. The effect of calorie content has not generally been taken into consideration in availability studies and although the incorporation of 50% maize in a test diet raises the ether-extractable fraction by only 2%, this may contribute to the rather low values for availability of amino acids in maize determined by the growth method as compared with those of other cereals. The decrease in the values for availability of lysine with higher levels of corn gluten in the diet may partly be attributed to an increased energy level of the diet. Although ether extraction indicated a lipid content of only 2.2%, in a subsequent determination, in which a butyl alcohol extract was re-extracted with ether and chloroform, 8.0% of lipid material was obtained. The decrease in availability may therefore be due to a combination of both increases in total nitrogen and in energy level of the diet.

The type of carbohydrate and fiber content of the experimental ration can also influence significantly the availability of lysine as demonstrated in Table VI. If food consumption (weight gain per 100 grams consumed) was taken into consideration for the estimation of utilizable lysine, the effect of increasing levels of maize starch was eliminated. This is in agreement with the results obtained by Gupta *et al.* (*11*), who suggested that using weight gain per food consumed for availability determination was the more accurate method. The significant effect of fiber content in the diet persisted when food efficiency (gain per food consumed) was used to calculate the lysine utilized. Since an elevated fiber content is likely to result in a significant overestimation of availability of lysine, fiber content of the standard diets should be equal to those containing the protein under assay.

The lower availability values for lysine by the fecal analysis method rather than the growth method could be explained either by growth stimulation due to factors other than lysine in the products tested or by factors which influence the fecal analysis method. Although results obtained by the fecal analysis method are less subject to the effect of imbalances, other factors may influence the accuracy of the method. The estimation of metabolic lysine excretion is subject to the same limitations as estimation of metabolic nitrogen in the biological value and protein digestibility determinations. The method has also been criticized on account of possible influences of microbiological activity. If lysine is synthesized or destroyed by the intestinal microflora it would cause, respectively, lower or higher values for availability. Kuiken (*16*) was unable to detect differences in availability of the ten essential amino acids of cottonseed meal whether the ration was supplemented with 2% of sulfathiazole or not. More needs to be known about the microbiological activity in the intestinal tract to reach a final conclusion on this point.

From the work of Kuiken (*16*) it appears that there is less variation in the fecal analysis method than in the growth method, but more comparisons of the two methods with others under rigidly controlled conditions are needed.

Another limitation of the fecal analysis method may be encountered in estimating effects of food processing on amino acid availability. If heat damage results in the destruction of lysine or formation of a lysine-carbohydrate complex which is not acid-hydrolyzable, the lysine will not be recovered from the feces and hence availability will be overestimated. This error can, however, be avoided if total lysine is assessed in the product after heat treatment.

Differences in the ratio of available lysine to true digestibility illustrate that lysine is not necessarily lost to the body proportionally to protein. Digestibility of lysine—i.e., availability of lysine determined by the fecal analysis method—and digestibility of the protein are therefore apparently influenced by different factors.

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