Influence of Various Levels of Lysine Intake on Weight Gain and Body Composition of Rats

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When rats were fed a diet containing a low level of plant protein supplemented with an excessive amount of lysine, growth retardation often occurred as a result of amino acid imbalance. In this study, rats were fed various levels of lysine in 18% of wheat gluten diet. The basal diet contained no supplemental lysine, while the other diets contained the following percentages of supplemental L-lysine: 0.20, 0.4, 0.8, 1.6, and 3.2. Body weight, serum protein, serum albumin, and bone calcium concentrations increased as the amount of lysine in the diet was increased and reached maximum at the 0.4% level of lysine. Lysine supplementation increased urinary lysine excretion most prominently. The concentrations of some other urinary amino acids were also changed but to a lesser degree. Liver glycogen and liver fat were reduced when lysine was included in the diets.

Lysine is low in protein of plant origin. Supplementation with lysine to improve the nutritive value of plant proteins has become the subject of many investigations (Sure, 1954; Westerman *et al.*, 1957). The beneficial effects of lysine supplementation depends upon the quantity and quality of protein in the diet. With a slight excess of lysine added to a diet containing a low level of inadequate protein, growth retardation in experimental animals often occurs as a result of amino acid imbalance (Rosenberg, 1959).

The purpose of this experiment was to study the changes in body composition of rats at various levels of lysine intake and the possible adverse effect of feeding a high lysine diet which contains a level of protein sufficiently high to support maximal growth.

METHOD

Sixty weanling albino rats of the Sprague-Dawley strain were divided into six groups with ten animals in each group. They were fed six experimental diets when they were three weeks old and weighed 60 to 70 grams. Food and water were supplied *ad libitum*. Body weight and food consumption were recorded weekly.

The six experimental diets consisted of the following ingredients in per cent, with the addition of varied amounts of L-lysine·HCl and cornstarch: wheat gluten 18, Crisco 8, salt mix (USP 14) 4, cod liver oil (USP) 2, and vitamin mix in sucrose 1. The basal diet contained no added L-lysine; the other five diets had L-lysine·HCl added in the following percentages: 0.25, 0.5, 1.0, 2.0, and 4.0. The basal diet contained 67% cornstarch, while the percentages of cornstarch in the other five diets were: 66.75, 66.5, 66, 65, and 63 to compensate for the L-lysine·HCl added. The vitamin mix in sucrose included all the vitamins known to be essential to rats, as described previously (Chang, 1962).

To convert the level of L-lysine·HCl to L-lysine, the levels stated above were multiplied by a factor of 0.8 This gives the exact amount of L-lysine added, that is 0.25 × 0.8 or 0.20, 0.5 × 0.8 or 0.4, 1.0 × 0.8 or 0.8, 2.0 × 0.8 or 1.6, and 4.0 × 0.8 or 3.2% L-lysine added. The figure indicating the exact amount of L-lysine added is used throughout the paper.

At the end of six weeks, the animals were placed in metabolic cages; urine and fecal samples were collected from the 10 rats in each group for a period of five days for the determination of total urinary and fecal amino acids, lysine, methionine, leucine, and valine by the microbiological method (Dunn *et al.*, 1949). The method for the collection and preparation of urine and fecal samples was essentially as described in a previous paper (Chang, 1962).

At the end of eight weeks, the animals were anesthetized by the injection of sodium amytal; blood samples were drawn from the rats by heart puncture for the individual analysis of total serum protein and serum protein fractions. The livers were removed and analyzed for total protein, fat, and glycogen. Total serum and liver protein were determined by the micro-Kjeldahl method (A.O.A.C., 1960). Liver fat was determined according to the method of A.O.A.C. (1960). Liver glycogen was determined by the method of Seifter *et al.* (1950). Serum proteins were separated into five fractions by paper electrophoresis (Chang and Varnell, 1966).

For the determination of calcium in rat bones, the two femurs from each rat were removed, weighed, and dried to constant weight. Incineration was carried out in a muffle furnace at a temperature of 540° C. The ash thus obtained was weighed and dissolved in dilute HCl for the determination of calcium by the method of A.O.A.C. (1960). All data were analyzed statistically by analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Growth. The growth responses of rats and dietary levels of lysine are shown in Table I. The body weight increased as the level of lysine in the diet increased and reached a maximum at the 0.4% level. Since the lysine

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Table I. Growth and Protein Efficiency Ratios for Rats at Various Levels of Lysine Intake

Diets	Initial Wt., G.	Final Wt., G.	Total Wt. Gain,[a] G.	PER[b]
1 Basal[c]	61	108	47[a]	0.63[e]
2 Basal + 0.2% L-lysine	60	217	157[b]	1.32[f]
3 Basal + 0.4% L-lysine	59	245	186[c]	1.55[g]
4 Basal + 0.8% L-lysine	62	245	183[c]	1.47[g]
5 Basal + 1.6% L-lysine	61	248	187[c]	1.63[g]
6 Basal + 3.2% L-lysine	59	235	176[c]	1.60[g]

[a] Means having the same superscript are not significantly different ($P \leq 0.05$).
[b] Protein efficiency ratio calculated during six weeks experiment.
[c] Basal diet containing 18% gluten without addition of L-lysine.

content from 18% of wheat gluten in diets was 0.25% as analyzed in this laboratory, the minimum lysine requirement for rats, expressed as percentage of diet, for optimal growth under this experimental condition was then estimated to be 0.25% plus 0.4 or 0.65%. No adverse effects in growth were observed when the rats were fed diets containing 0.8, 1.6, or 3.2% of L-lysine in this study.

Total Serum Protein and Serum Protein Fractions. The effect of lysine intake on total serum protein concentrations and serum protein components is shown in Table II. Rats fed a diet containing 18% wheat gluten, without lysine supplementation, showed the lowest total serum protein, serum albumin, and alpha$_1$ globulin concentrations. Addition of lysine to the diet increased total serum protein, serum albumin, and alpha$_1$ globulin concentrations, and reached a maximum value when the supplemented lysine level was 0.4%. Increase of the lysine to 0.8, 1.6, or 3.2% caused no further alteration in total serum protein and serum protein components.

Excretion of Urinary and Fecal Amino Acids. The excretion of urinary and fecal amino acids—lysine, leucine, methionine, and valine—by rats fed the six experimental diets is shown in Table III. Urinary amino acids excreted by rats were the lowest in the unsupplemented group. Supplementation of 0.20 or 0.4% of L-lysine to the diet increased the excretion of the amino acids significantly. Supplementation of L-lysine to the diet at levels higher than 0.4% caused no additional increase in the excretion of leucine, methionine, and valine, but lysine became higher when the levels of lysine in the diet were increased. The sharp change in urinary excretion of lysine indicates that the renal threshold of lysine had been exceeded before the tissue concentration of lysine became high enough to interfere with the essential metabolic functions of the rat. Such an assumption may help to explain why lysine is less toxic than the other amino acids. The amount of fecal lysine excreted by the rats was not significantly different among the various experimental groups regardless of the amount of lysine added. Evidently, the added lysine in the diets was completely absorbed.

Liver Components. The weight, protein, fat, and glycogen in the livers of rats fed the various experimental diets are shown in Table IV. Higher fat, lower weight, and lower protein were observed in the livers of the unsupplemented rats. Supplementation of lysine increased the weight and protein and reduced fat concentrations in the livers of all groups. These findings were anticipated, as the wheat gluten used in the diets was an inadequate protein, and feeding rats with an inadequate protein results in an increase of liver fat and a decrease of liver protein (Guggenheim and Buechler-Czaczkes, 1950;

Table II. Serum Protein and Serum Protein Fractions in Rats at Various Levels of Lysine Intake

Diets[a]	Serum[b] Protein, %	Albumin,[c] G.	α_1 Globulin,[c] G.	α_2 Globulin,[c] G.	β Globulin,[c] G.	γ Globulin,[c] G.
1	4.13[d]	1.772[d]	0.561[f]	0.506[i]	0.779[k]	0.507[l]
2	5.27[b]	2.705[e]	0.787[g]	0.560[i]	0.798[k]	0.425[l]
3	5.99[e]	2.860[e]	1.090[h]	0.710[i]	0.905[k]	0.421[l]
4	5.47[c]	2.282[e]	1.044[h]	0.841[j]	0.887[k]	0.435[l]
5	5.56[c]	2.567[e]	0.978[h]	0.761[j]	0.802[k]	0.460[l]
6	5.37[c]	2.463[e]	1.028[h]	0.585[i]	0.849[k]	0.445[l]

[a] Dietary groups, see Table I.
[b] Grams of protein per 100 ml. of serum.
[c] Grams of serum protein components per 100 ml. of serum.
[d] Means having the same superscript are not significantly different ($P \leq 0.05$).

Table III. Excretion of Total Amino Acids by Rats at Various Levels of Lysine Intake[a]

	Urine[c]				Feces[c]			
Diets[b]	Lysine, Mg.	Meth., Mg.	Leucine, Mg.	Valine, Mg.	Lysine, Mg.	Meth., Mg.	Leucine, Mg.	Valine, Mg.
1	1.94[a]	0.82[e]	4.31[h]	2.68[k]	33.20[n]	10.79[o]	31.20[p]	35.20[r]
2	15.42[b]	1.53[f]	9.70[i]	5.01[l]	39.80[n]	9.10[o]	42.50[q]	32.60[r]
3	20.21[b]	3.01[g]	22.33[j]	7.68[m]	35.89[n]	11.54[o]	35.14[p]	39.14[r]
4	38.26[c]	3.29[g]	19.41[j]	7.52[m]	24.36[n]	9.49[o]	39.96[q]	27.38[s]
5	56.00[d]	3.04[g]	18.52[j]	8.26[m]	30.20[n]	10.97[o]	44.56[q]	31.14[r]
6	191.40[e]	2.30[g]	21.80[j]	6.09[m]	30.50[n]	8.91[o]	38.19[q]	29.37[s]

[a] Average of five-day collection from 10 rats in each group.
[b] Dietary groups, see Table I.
[c] Means having the same superscript are not significantly different ($P \leq 0.05$).

Table IV. Liver Composition of Rats at Various Levels of Lysine Intake

Diets, see Table I	Liver[a] Wt., G.	Liver[b] Protein, %	Liver[c] Fat, %	Liver[d] Glycogen, %
1	5.326[a]	12.99[d]	11.92[g]	7.76[j]
2	9.734[b]	15.78[e]	7.72[h]	5.69[k]
3	11.315[c]	18.35[f]	7.66[h]	5.47[k]
4	11.381[c]	16.02[e]	10.55[i]	4.86[k]
5	11.452[c]	18.78[f]	10.31[i]	6.15[k]
6	11.877[c]	16.44[e]	7.70[h]	5.77[k]

[a] Means having the same superscript are not significantly different ($P \leq 0.05$).
[b] Grams of protein per 100 grams of fresh tissue.
[c] Grams of fat per 100 grams of dried tissue.
[d] Grams of glycogen per 100 grams of fresh tissue.

Harper *et al.*, 1955). The glycogen concentration in the liver of rats in the unsupplemented group was the highest; addition of lysine to the diet reduced the glycogen concentration in rat livers in all groups. These results were in accord with data reported by Szepesi and Freedland (1968) who found that glycogen concentration in rat livers was affected by the protein content of the diet. A protein free diet caused high liver glycogen concentration, while high protein diet reduced liver glycogen markedly.

Bone Calcification. The effect of lysine supplementation on bone calcification in rats is shown in Table V. Rats fed diets supplemented with various amounts of lysine showed varied amounts of calcium in their femurs. The weight of femurs, weight of ash, calcium content in ash, and per cent of calcium in femurs were consistently smaller in the unsupplemented group than in the lysine supplemented groups. The enhanced bone calcification, however, did not increase linearly with the amounts of lysine added. Maximum bone calcification was reached at the 0.4% level. A level of 0.8 or 1.6% lysine added to the diets was no more efficient in enhancing bone calcification than a level of 0.4%. When 3.2% lysine was added to the diet, the per cent of calcium in bone tended to be less.

Many investigators have shown that calcium utilization is reduced when the dietary protein is low in quality or quantity (Frandsen *et al.*, 1954). Poor calcium utilization

Table V. Calcium Content in the Femur of Rats at Various Levels of Lysine Intake

Diet[a]	Weight of Femur: Fresh Wt., G.	Weight of Femur: Dried Wt., G.	Wt. of Ash, G.	Wt. of Calcium in Femur, Mg.	Calcium in Fresh Femur, %[b,c]
1	0.931	0.607	0.315	111.3	12.18[a]
2	1.560	1.054	0.615	212.0	13.70[b]
3	1.674	1.151	0.656	240.7	14.46[c]
4	1.646	1.135	0.644	232.7	14.16[c]
5	1.659	1.069	0.606	232.1	14.30[c]
6	1.610	1.058	0.622	211.5	13.15[b]

[a] Dietary groups, see Table I.
[b] Grams of calcium per 100 grams of fresh femur.
[c] Means having the same superscript are not significantly different ($P \leq 0.05$).

in animals has also been found in diets deficient in one or more of the essential amino acids (Bavetta *et al.*, 1954). In studies of the influence of lysine on gastrointestinal absorption of calcium[45] by rats, Wasserman *et al.* (1957) found that L-lysine was most potent in promotion of calcium[45] absorption. The better utilization of calcium in the lysine supplemented groups, as described in this paper, could be due to two factors: Lysine promotes calcium utilization, and lysine improves the nutritive value of wheat gluten, thus enhancing calcium utilization.

While numerous studies have been undertaken on the addition of lysine in fortification of foods and feedstuffs, information about the tolerance of animals for lysine in food would seem to be important, both from a fundamental and a practical viewpoint. Data obtained in this paper suggest that the quantity of lysine included in a gluten based rat diet may be modified considerably without any marked effect, provided the level of protein is sufficiently high. In human studies, Clark *et al.* (1966) studied the influence of various amounts of lysine intake on nitrogen retention and found that when excessive amounts of lysine were added to diets containing adequate amounts of protein, no alteration in nitrogen retention in human subjects was found.

As reported in this paper, lysine deficiency results in slow growth, lower levels of serum protein and serum protein components, higher liver fat and lower liver weight, and poor bone calcification. Such biological changes in rats are also found in cases of protein deficiency, as shown in the authors' previous investigations. It would seem, therefore, that symptoms or biological changes that occur as a result of lysine deficiency are similar to those occurring in a protein deficiency state.

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