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POLY-1-LYSINE, A NUTRITIONAL SOURCE OF LYSINE[1]

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ABSTRACT

Poly-1-lysine was tested as a nutritional source of lysine for weanling rats. This synthetic amino acid polymer was as effective as 1-lysine·HCl in meeting the lysine requirement of rats as evidenced by nearly equivalent values in terms of growth, feed to gain ratio, PER, BV, NPU, and TPD. Amino acid deficiencies in human or animal diets may be corrected in the future with synthetic amino acid polymers.

INTRODUCTION

Protein is believed by most nutritionists to be the key critical ingredient in world nutrition, however, protein quality is more important than protein quantity for the young growing animal. The absence or limiting deficiency of a single indispensable amino acid severely reduces the bioavailability and utilization of the total protein (1). Although cereal grains are primarily consumed for calories, they represent a major source of protein for a large segment of the world population (2). Of the nine or ten essential amino acids, lysine is the first limiting in most cereal proteins (3). Numerous attempts have been made by plant breeders through selection and mutation to increase the lysine content of maize (4), sorghum (5), and barley (6). Several high-lysine cultivars have been discovered or created in this manner in these cereals and they possess a definite nutritional potential. In most instances the high-lysine strains exhibit one or more limiting characteristics, such as low bioavailability of protein and poor yield associated with shrunken kernels. Even the highest lysine-containing mutant known (Bomi Risø Mutant 1508 barley, 5.65 g lysine/16 g N) does not contain sufficient available lysine and other indispensible amino acids to meet the total needs of young growing animals (7). The common solution to the lysine limitation problem is to balance the diet with high lysine protein sources such as soybeans, meats, fish or supplementation with the synthetic free 1-amino acid.

It is quite possible that in the very near future genetically altered microorganisms will be used to synthesize polypeptides with any

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given amino acid content (8). Single cell protein is not a new concept for improving quantity and quality of protein (9), however, genetic engineering of single cell microbes has not yet been used to produce large amounts of prescribed and/or unnatural polypeptides such as polymers of pure lysine, i.e., poly-l-lysine. The purpose of this study was to explore the value of a synthetic polymer composed entirely of l-lysine as a source of this essential amino acid by determining its bioavailability and acceptability for young growing rats.

MATERIAL AND METHODS

L-amino acids and a lysine polymer (poly-l-lysine - TYPE VI Mol wt. 4,000-15,000) were obtained from Sigma Chemical Company. The poly-l-lysine contained approximately 84 lysine units and approximately 35% bromine which was removed by ultra filtration through a UM-10 Diaflo membrane (Amicon Corporation, Lexington, Mass). This step excluded any peptide below ca 10,000 MW. Thus purified, the poly-l-lysine was lyophilized and analyzed as 84.0% l-lysine.

Two amino acid mixtures were prepared to meet the requirements of weanling rats (10) using l-lysine·HCl (mixture no. 1) and poly-l-lysine (mixture no. 2) as lysine sources (Table I). A third amino acid mixture was prepared (mixture no. 3) identical to mixture no. 1 except that l-lysine·HCl was omitted and an equivalent quantity by weight of glutamic acid was substituted (Table I). Three synthetic diets (Diets 1, 2 and 3) were prepared using the above three amino acid mixtures (Table II). Two semi-purified diets were also prepared using either high-nitrogen casein obtained from ICN Nutritional Biochemicals (Diet 4) or soybean meal (Diet 5) as sources of amino acids (Table II). These two proteins were added to the diets as levels calculated to meet the lysine requirement of weanling rats (10). Calculations for amino acid levels furnished by casein and soybean meal diets shown in Table I were from previous analysis of these proteins in our laboratory. L-glutamic acid, l-methionine and l-phenylalanine were calculated as deficient in the semi-purified diets and free amino acids were added to equal the levels of these amino acids provided in Diets 1, 2 and 3. Diets were analyzed for Kjeldahl protein and lysine (Table III) (AAA Laboratory, 6206 89th Avenue S.E., Mercer, WA 98040).

Two weanling female rats weighing approximately 70g were assigned to each of the five diets. The average weight of the 10 rats was 70.40 $\pm$ .66g. The cost of poly-l-lysine precluded the use of more than two rats per diet in this pilot study. They were placed in metabolism cages equipped for individual feeding and separate collection of feces and urine. Each rat was provided a maximum of 10g daily of each assigned diet for nine days. Feed intake and rat weights were recorded daily. The first five days were considered an adjustment period prior to measuring nitrogen utilization. On days six through nine, feces and urine were collected separately and quantitatively in 5% sulfuric acid. Fecal and urine collections were prepared and analyzed for total nitrogen as described by Eggum (11) and a true digestible protein (TDP) biological value (BV) and net protein utilization (NPU) calculated (12). Endogenous urinary nitrogen and metabolic fecal nitrogen used in these calculations were previously determined in our laboratory with similar

TABLE I. Amino acid requirements of the weanling rat[a] and amino acids provided in mixtures for purified diets and by casein and soybean meal in semi-purified diets.

		Amino acid mixtures provided by diets				
		1	2	3	4	5
Amino acid %	Amino acid requirements %	L-lysine	Poly-l-lysine	No l-lysine	Casein	Soybean meal
L-arginine	.67	.67[b]	.67[b]	.67[b]	.70	1.00
L-asparagine	.44	.44[c]	.44[c]	.44[c]	.89	1.66
L-glutamic acid	4.40	4.40	4.40	5.65[f]	4.40[g]	4.40[h]
L-histidine	.33	.33	.33	.33	.32	.38
L-isoleucine	.61	.61	.61	.61	.62	.98
L-leucine	.83	.83	.83	.83	1.13	1.26
L-lysine	1.00	1.00[d]	1.00[e]	---	1.00	1.00
L-methionine	.67	.67	.67	.67	.67[g]	.67[h]
L-phenylalanine	.89	.89	.89	.89	.89[g]	.89[h]
L-proline	.44	.44	.44	.44	1.15	.97
L-threonine	.56	.56	.56	.56	.69	.59
L-tryptophan	.17	.17	.17	.17	.18	.20
L-valine	.67	.67	.67	.67	.83	.77
L-alanine	.20	.20	.20	.20	.41	.66
L-glycine	.20	.20	.20	.20	.21	.83
L-serine	.20	.20	.20	.20	.71	.70
TOTALS	12.28	12.28	12.28	12.53	14.80	16.96

[a]Requirements of the weanling rat according to the National Research Council (1972).
[b]Added as l-arginine·HCl and increased by 17.3% above the requirement.
[c]Added as l-asparagine·H_2O and increased by 12.0% above the requirement.
[d]Added as l-lysine·HCl and increased by 25.0% above the requirement.
[e]Added as a polypeptide at 118% of l-lysine by weight.
[f]Glutamic acid level increased proportionately by weight of l-lysine·HCl removed.
[g]1.53, .35 and .30 g of l-glutamic acid, l-methionine and l-phenylalanine, respectively added per 100 g of diet 4.
[h]1.78, .42, .16 g of l-glutamic acid, l-methionine and l-phenylalanine, respectively added per 100 g of diet 5.

TABLE II. Purified and semi-purified diets for weanling rats.

Diet ingredient, %	Diet number				
	1	2	3	4	5
Amino acid mixture	12.73	12.66	12.73	2.18	2.36
Maize starch	72.22	72.29	72.22	68.09	48.09
Maize oil	5.00	5.00	5.00	5.00	5.00
Alphacel[a]	5.00	5.00	5.00	5.00	5.00
Vitamin mixture[b]	2.00	2.00	2.00	2.00	2.00
Mineral mixture[c]	2.00	2.00	2.00	2.00	2.00
Calcium carbonate	.80	.80	.80	.80	.80
Antibiotic[d]	.25	.25	.25	.25	.25
Casein[e]	---	---	---	14.68	---
Soybean meal[f]	---	---	---	---	34.50
TOTALS	100.00	100.00	100.00	100.00	100.00

[a]Cellulose, ICN Nutritional Biochemicals, Cleveland, Ohio.
[b]Vitamin fortification mixture, ICN Nutritional Biochemicals, Cleveland, Ohio.
[c]Bernhardt-Tormarelli salt mixture, modified, ICN Nutritional Biochemicals, Cleveland, Ohio.
[d]Furnished 110.2, 110.2 and 55.1 mg of chloratetracycline, sulfamethazine and penicillin per kg of diet.
[e]Purified, high-nitrogen casein, ICN Nutritional Biochemicals, Cleveland, Ohio.
[f]Solvent extracted, 44% protein soybean meal.

TABLE III. Protein and lysine composition of diets by analysis, as fed basis.

Item, %	Diet				
	L-lysine	Poly-l-lysine	No lysine	Casein	Soybean meal
	1	2	3	4	5
Protein[a]	10.4	10.3	10.1	14.2	16.5
L-lysine	1.29	.65	.00	1.33	.94

[a](N x 6.25)

rats (7). Protein efficiency ratios (PER) and feed to gain ratios were also calculated (11).

Following the final excreta collection and weighing on day nine, one rat fed the diet without lysine (Diet 3) was transferred to the diet supplemented with l-lysine·HCl (Diet 1) and the other was transferred to the poly-l-lysine diet (Diet 2). Diets were fed *ad libitum* to these two rats in individual cages for seven days during which daily weights and feed consumption were recorded.

RESULTS AND DISCUSSION

Protein (N x 6.25) and lysine levels of diets as determined by analyses are shown in Table III. Protein levels in the purified Diets 1, 2 and 3 were essentially the same and lower than the casein and soybean meal supplemented diets. Lysine analysis did not correspond completely with the predicted values shown in Table I. This may be explained in part by poor sampling. The poly-l-lysine was of a particle size and type that did not blend evenly and readily in the diet and the small non-representative sample taken for analysis could have resulted in the lower value (.65%) than the predicted level of 1.0%. Analysis of the peptide showed 84.0% l-lysine, thus when added to the diet at 1.18% (Table I) it would theoretically provide .99% l-lysine. We feel certain that the predicted values for amino acids as shown in Table I are accurate and can be relied upon in interpreting the biological data shown in Table IV and Figures I and II.

TABLE IV. Effect of source of lysine on growth and nitrogen utilization by young growing rats, 9 day period.

Item	Diet				
	L-lysine	Poly-l-lysine	No lysine	Casein	Soybean meal
	1	2	3	4	5
Initial weight, g	70.75	70.50	70.25	70.25	69.75
Final weight, g	95.50	94.50	58.50	100.50	102.00
Gain, g	24.75	24.00	-11.75	30.25	32.25
Feed consumed, g	88.0	87.25	57.00	89.25	88.25
Feed to gain ratio	3.56	3.70	-4.85	2.97	2.78
Protein efficiency ratio	2.71	2.67	-2.05	2.39	2.18
True protein digestibility, %	99.88	101.13	91.79	94.69	82.71
Biological value, %	92.82	89.30	53.58	83.10	80.04
Net protein utilization, %	92.71	90.31	49.18	78.69	66.20

Rats fed poly-l-lysine gained equally as well as those fed the diet supplemented with l-lysine·HCl. Growth performance during the initial nine day adjustment and collection period are illustrated in Table IV and Figure I. Statistical inferences cannot be made because of low sample size, however, the effect of poly-l-lysine was striking, compared to the diet without lysine. Feed to gain ratio appeared to favor the l-lysine·HCl diet although PER were comparable between the two sources of lysine. Biological value of the poly-l-lysine diet

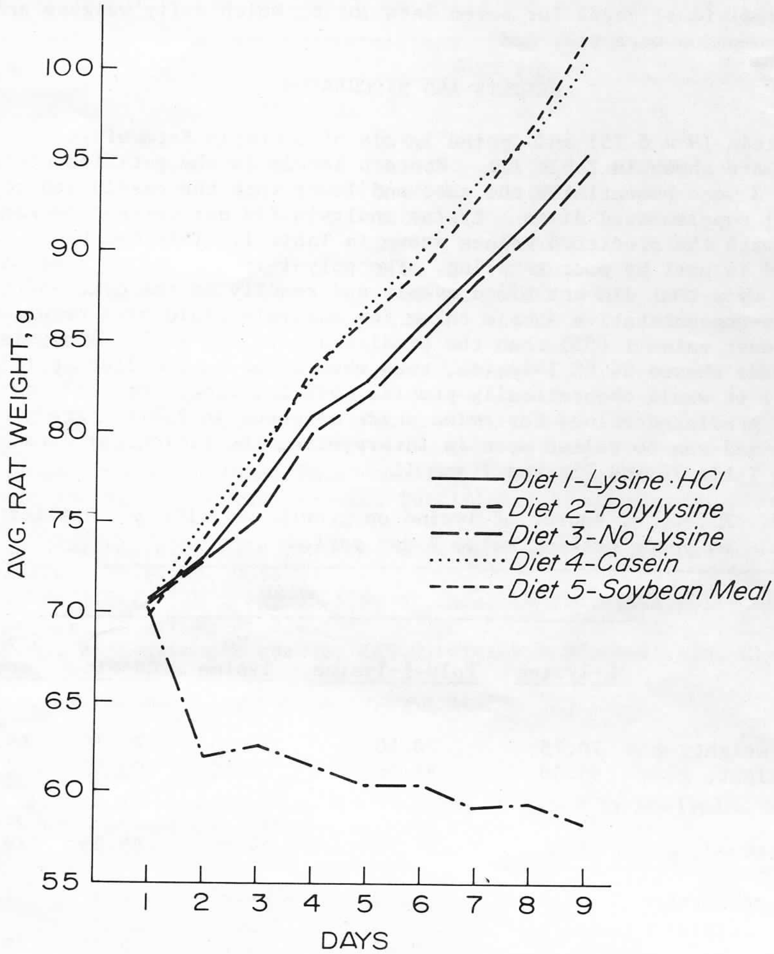

Figure I. Cumulative rat growth curves on purified and semi-purified diets.

appeared somewhat lower although TPD was about equal compared to the l-lysine·HCl diet. The higher BV gave the l-lysine·HCl the highest NPU. Feed consumption was not different between the l-lysine·HCl and poly-l-lysine diets (Table IV) and rats fed these diets consumed the total 10 grams presented daily from day four through day nine.

The absence of lysine in Diet 3 produced the expected poor performance. Feed intake was reduced by 50% on days one and two compared to the lysine supplemented rats and continued to be 30 to 40% less from that point through day nine. These rats immediately lost about 10% of the body weight, and then continued to lose at a slow rate from day three through day nine (Figure I). Although TPD was relatively good on

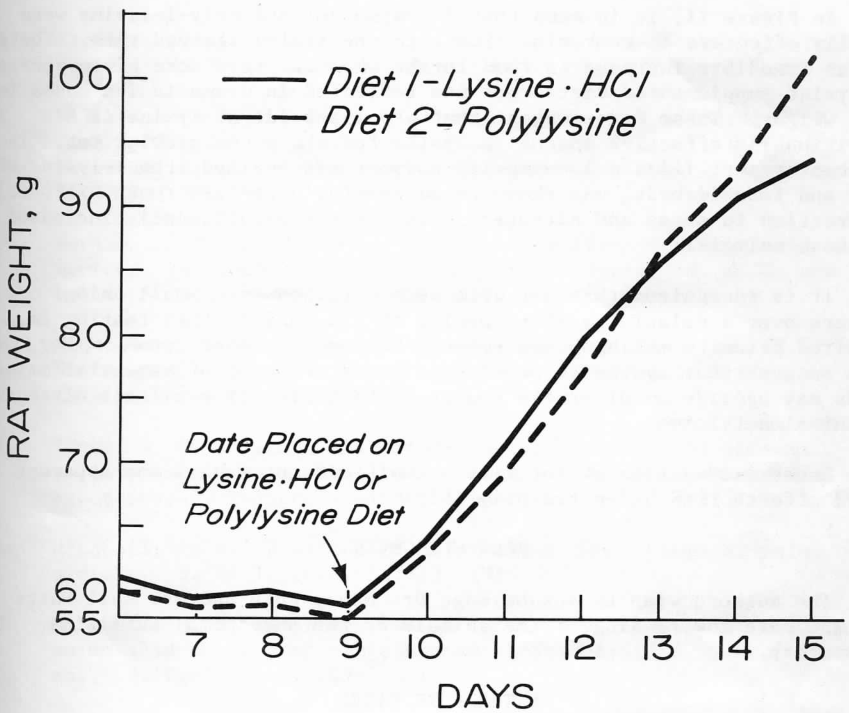

Figure II. Lysine and polylysine reversal of lysine starvation.

the lysine-free diet, NPU was very poor due to the low BV (Table IV). The BV of the lysine-free diet is quite near to that predicted by Mitchell and Block (13) for a protein having a 100% deficit of a single indispensable amino acid. Comparing rat performance on both l-lysine·HCl and poly-l-lysine diets to that of those fed the lysine-free diet (Table IV and Figure I), it is readily apparent that both sources of lysine were effective in promoting growth and nitrogen utilization in young growing rats.

The higher protein level in the casein and soybean meal supplemented diets was reflected in faster growth and an improvement in feed to gain ratio. However, PER, BV, and NPU were not as high for these diets compared to the lysine supplemented purified diets (Table IV). Variation in total protein (Table III) and in certain dispensable amino acids (Table I) make the data from the semipurified and purified diets difficult to compare. Evidently lysine was not the limiting nutritional factor in promoting maximum growth since all diets were supplemented with equal levels of this amino acid. However, it is noteworthy that the poly-l-lysine supplemented diet compared favorably in promoting rat growth, to both casein and soybean meal and nitrogen utilization was significantly greater on this diet, especially in comparison with soybean meal.

In Figure II, it is seen that l-lysine·HCl and poly-l-lysine were equally effective in restoring growth to the lysine starved rats. There was an immediate increase in food intake when the rats were given access to lysine supplemented diets that was reflected in dramatic increases in body weight. These data suggest that a polypeptide of lysine is a nutritionally effective source of lysine for the young growing rat. In a recent report (14), a non-peptide polymer synthesized from l-lysine·HCl, urea and formaldehyde, was shown to successfully by-pass rumen microbial destruction in sheep and nitrogen retention was significantly increased in these animals.

It is recognized that our data were obtained with small animal numbers over a relatively short period of time and further testing is required prior to making broad general recommendations. However, our findings suggest that synthetic peptide polymers composed of essential amino acids may provide an alternate source of biologically available nitrogen in animal nutrition.

Gross examination of the live animals did not reveal any apparent toxic effects from being fed poly-l-lysine.

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