

# Effect of Lysine and Tryptophan Supplementation on Nicotinic Acid Metabolism in Pigs Given Raw or Lime-Treated Corn Diets

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Three experiments with baby Duroc-Jersey pigs were done. The animals received either a corn or a lime-treated corn diet supplemented with all the needed vitamins except nicotinic acid and with lysine and tryptophan. When lysine was withdrawn stepwise from the diet, there was an increase in urinary N-methyl nicotinamide excretion values regardless of whether the raw or the lime-treated corn diet was fed. When

tryptophan was withdrawn from the diet, there was an increase in N-methyl nicotinamide excretion values followed by a decrease to normal levels. Nitrogen balance was less affected by the withdrawal of the amino acids in the lime-treated corn than in the raw corn diet, suggesting that the former may contain a better balanced protein than the latter. (Metabolism 15: No. 6, June, 548-556, 1966)

**T**REATMENT OF CORN with lime as done by the rural population of Central America and Mexico has been known to improve the weight gain of rats as compared with that of rats fed on raw corn<sup>1,2</sup> and to increase the availability of nicotinic acid to the rat<sup>3</sup> and pig.<sup>2</sup> This effect has been ascribed to the fact that nicotinic acid in raw corn is present mainly in a bound form<sup>4,5</sup> which is hydrolyzed by alkali treatment releasing free nicotinic acid during the process. On the other hand, the apparent difference between lime-treated and raw corn in amino acid balance<sup>1</sup> and relative availability of the amino acids<sup>6</sup> has been suggested as an important factor in influencing the relative amounts of nicotinic acid available from both products. Necessarily, this suggestion applies more to the nicotinic acid resulting from tryptophan conversion than to that already present in corn or lime-treated corn.

Working with casein diets, Koeppe and Henderson<sup>7</sup> and Henderson et al.<sup>8</sup> demonstrated in rats given marginal levels of nicotinic acid and tryptophan that the addition of other amino acids resulted in growth suppression and nicotinic acid deficiency. This finding suggests that the relative amounts of other amino acids influence the utilization of tryptophan for either protein synthesis or conversion to nicotinic acid. Furthermore, Cherkes and Filchagin<sup>9</sup> reported that the addition of the sulphur-containing amino acids to a casein diet resulted in a decreased urinary excretion of nicotinic acid metabolites in

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rats. These results suggest that the overall amino acid composition of a diet is an important factor in determining the amount of nicotinic acid available to a growing organism. The experiments now described were designed to study further the relationship between amino acids and nicotinic acid in corn and lime-treated corn.

### MATERIAL AND METHODS

Pure-bred Duroc Jersey pigs were placed in individual metabolism cages and fed ad libitum on a basal diet of the percentage composition: raw or lime-treated corn, 96.7; minerals (containing 33 per cent  $\text{CaCO}_3$ , 33 per cent bone meal, 33 per cent sodium chloride and 1 per cent minor elements\*), 3.0; cod liver oil, 0.3, and an adequate amount of all B-complex vitamins except nicotinic acid.<sup>10</sup> Water was offered to the animals 3 times a day. After an adaptation period of 1 day, urine and feces were collected for a period of 3 days. The urine was collected in dark bottles containing 1 ml. of concentrated acetic acid. During every day of each balance period a portion of the urine from each animal was taken in a 15 ml. stoppered test tube and kept under refrigeration until analyzed for N-methyl nicotinamide by the method of Huff and Perlzweig.<sup>11</sup> Urine, feces and food were analyzed for nitrogen by the Kjeldahl procedure with selenious acid as the catalyst. All the animals were weighed at the beginning and at the end of every balance period. The basal diet given during the first balance period of every experiment had no amino acid supplement added but the experimental diets were supplemented with 0.48 per cent L-lysine hydrochloride and 0.09 per cent DL-tryptophan. These quantities of amino acids are about twice the amount usually found in corn.

Three experiments were done. The first was divided into 2 parts. In the first part 3 baby Duroc-Jersey pigs weighing 22, 16 and 14 pounds were fed on the raw corn diet supplemented with 0.48 per cent lysine and 0.09 per cent tryptophan, except for the first balance period during which the corn diet was fed unsupplemented. The food was offered in adequate amounts which were increased according to the appetite of the animals. The level of tryptophan was kept constant and lysine was withdrawn in amounts of 0.2 Gm. per cent in successive balance periods. In the second part of the experiment, the level of lysine was kept constant and tryptophan was withdrawn in amounts of 0.02 Gm. per cent of diet for each balance period. In experiment 2 the same procedure was repeated with a different set of pigs, using the lime-treated corn diets.

In experiment 3, four 6-week old Duroc-Jersey pigs weighing between 17 and 19 pounds were used. Two were fed on the raw corn and the other 2 the lime-treated corn diet. Both diets were supplemented with the same levels of lysine and tryptophan as at the beginning of the previous experiment. The level of lysine was kept constant and tryptophan was withdrawn stepwise in amounts of 0.01 Gm. per cent from 0.1 to 0 Gm. per cent of the diet.

### RESULTS

Table 1 shows the initial and final weights and the food consumption for the individual pigs in each of the experiments. Figure 1 shows the results for N-methyl nicotinamide excretion for the raw and lime-treated corn from experiments 1 and 2 when lysine was gradually withdrawn from the diet and tryptophan was kept constant. The first point in the curve corresponds to the basal diet without amino acid supplementation. As the lysine level of the ration decreased, excretion of N-methyl nicotinamide/100 Gm. of ration was increased when both the raw and the lime-treated corn diets were fed. The

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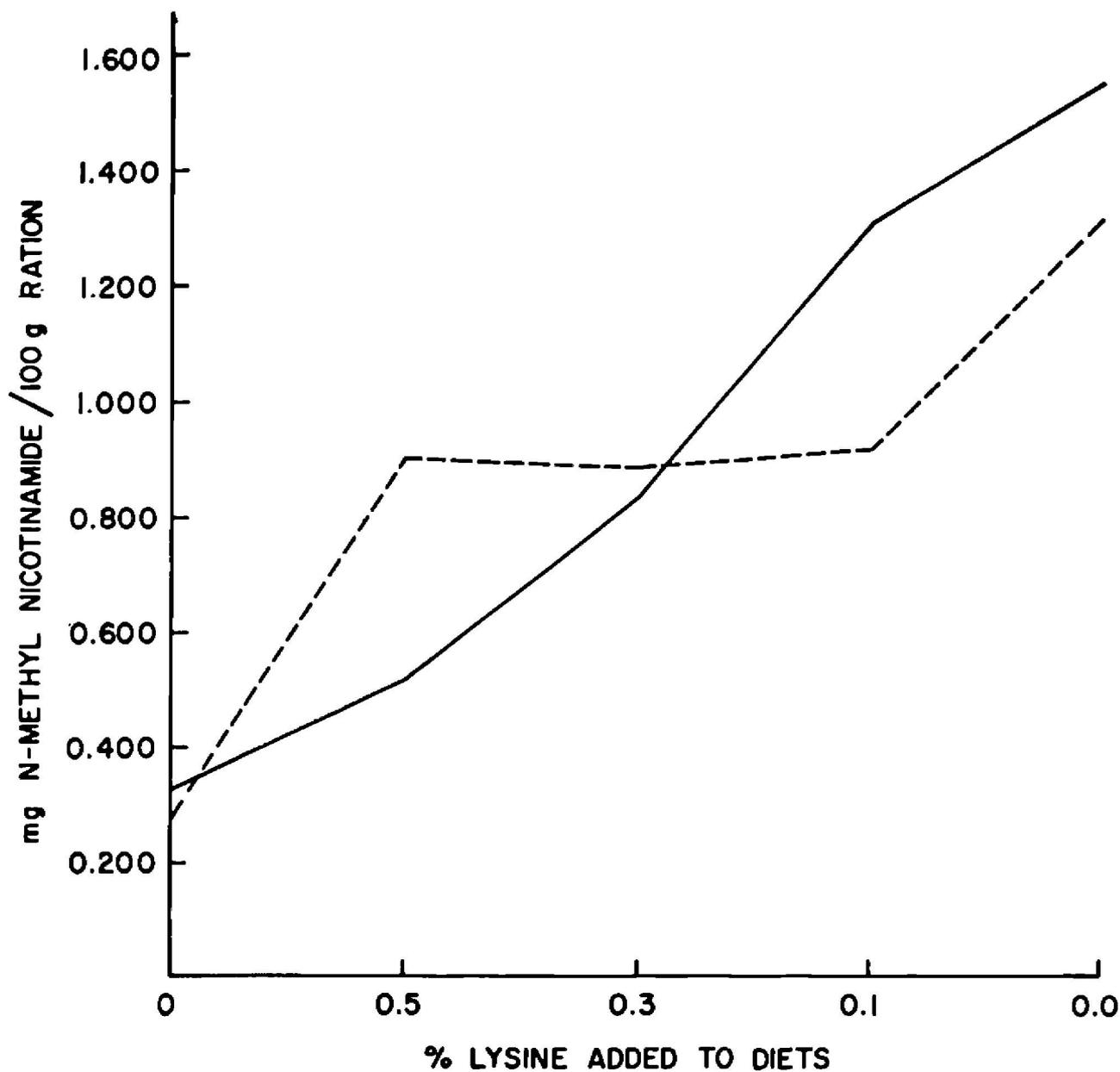
Table 1.—*Initial and Final Weight and Food Consumption of Pigs Fed Raw or Lime-treated Corn Diets*

Pig No.	Ration	Initial Weight lb.	Final Weight lb.	Food Consumption lb.
<i>Experiment 1</i>				
1	Raw corn	22	24	24.5
2		16	25	18.2
3		14	26	20.0
1		35	46	25.3
2		27	34	18.8
3		28	40	24.3
<i>Experiment 2</i>				
1	Lime-treated corn	32	42	31.1
2		29	35	21.4
3		20	25	18.0
1		42	56	16.1
2		35	48	14.2
3		25	36	12.4
<i>Experiment 3</i>				
1	Raw corn	18	41	21.0
2		17	44	21.3
3	Lime-treated corn	17	21	15.9
4		19	44	20.6

nature of the response was, however, different since lysine withdrawal in the raw corn diet produced a linear increase in N-methyl nicotinamide excretion with no evidence of curvature while the lime-treated corn indicated significant curvature in the response to lysine withdrawal. The significance of the difference in trends was established by analysis of variance technique using as estimate of experimental error a within experimental pool of the pig by diet, pig by period and pig by period by diet interactions.

Figure 2 shows the nitrogen retention of these animals. As the lysine level of the ration decreased, there was, after an initial rise, a significant decrease in nitrogen retention in the animals receiving the raw corn diet, resulting in a highly significant quadratic component of the response after the linear fit. The animals given the lime-treated corn diet retained, after an initial rise, significantly less nitrogen as the lysine was withdrawn from the diet. In this case, the linear fit did not significantly reduce the sum of square and practically all the variation was due to curvature of essentially quadratic nature.

Figure 3 shows the urinary N-methyl nicotinamide excretion values when tryptophan was withdrawn stepwise from a raw corn or a lime-treated corn diet at a constant lysine intake. When the raw corn diet was fed, the linear and quadratic components of the response were highly significant, while when tryptophan was withdrawn from the lime-treated corn diet, the response was essentially of the quadratic nature. Figure 4 shows the nitrogen retention of these animals. There was a significant linear response when tryptophan was

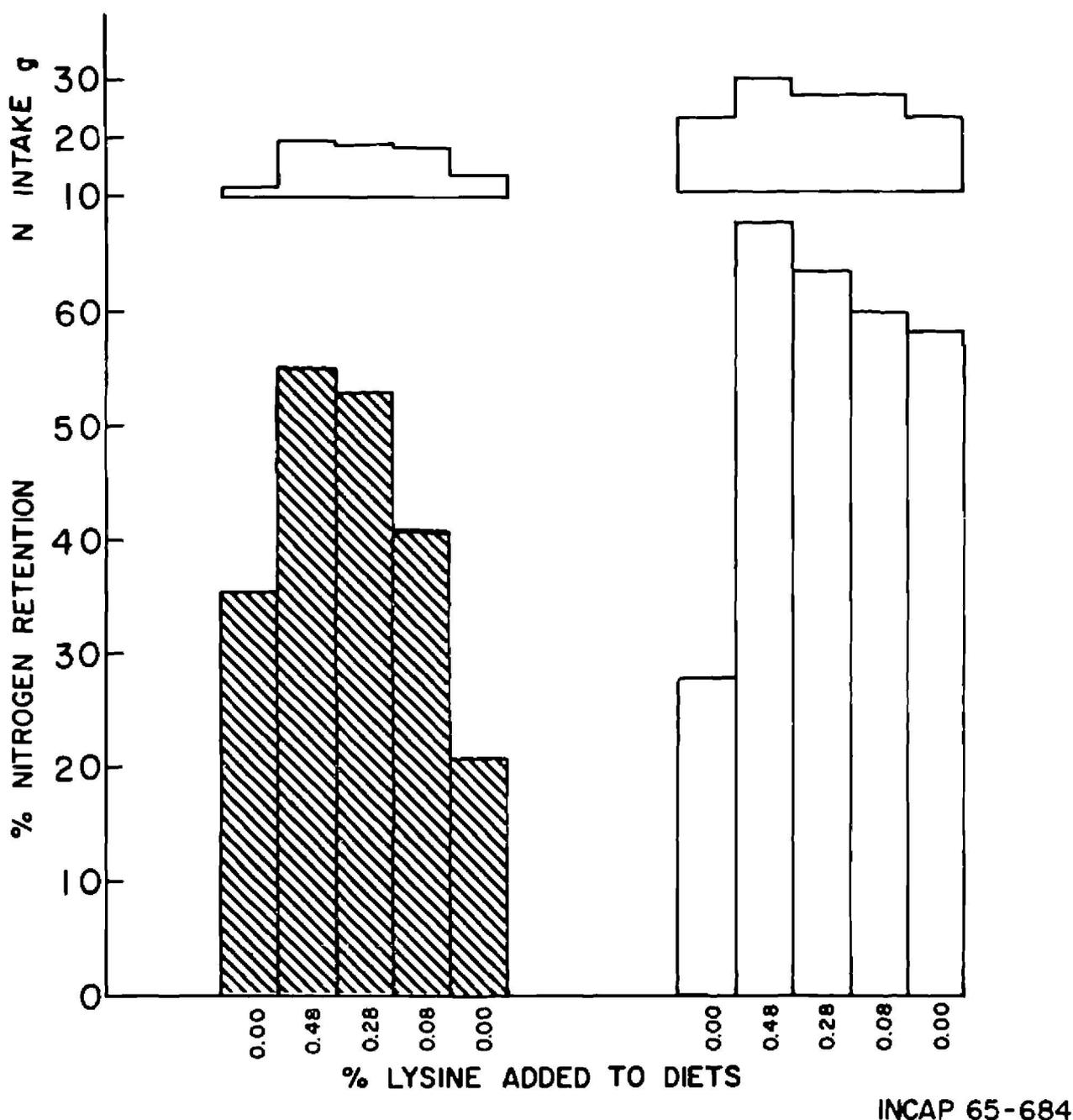


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Fig. 1.—Urinary N-methyl nicotinamide excretion in pigs fed raw or lime-treated corn diets. — raw corn - - - lime-treated corn. 0.09 per cent tryptophan added to diets, except for the first point in the curve where no amino acid was supplemented.

withdrawn from the raw corn diet, but no significant effect was observed with the lime-treated corn diet. In the analysis of these results, the data for the raw and the lime-treated corn were not combined because the error estimate was twice as large for the lime-treated corn than for the raw corn diet; furthermore, there was evidence of a significant diet-by-experiment interaction and accordingly pools of the between-pig variance in diets for each experiment were used as an estimate of experimental error.

Figure 5 shows the N-methyl nicotinamide excretion values, and Figure 6 the nitrogen retention with a raw or lime-treated corn diet supplemented with decreasing levels of tryptophan at a constant lysine intake. Again, the first point in the curve corresponds to the basal diet without amino acid supplementation. The results follow the same trend as those of experiments 1 and 2 when tryptophan was withdrawn from the diet and there was a significant effect due to the level of tryptophan in the diet.



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Fig. 2.—Nitrogen retention in pigs fed raw or lime-treated corn diets. Stripe box, raw corn; blank box, lime-treated corn. 0.09 per cent tryptophan added to diets, except for the first point in the graph where no amino acid was supplemented.

#### DISCUSSION

These results suggest that since lysine is the first limiting amino acid in corn, the relative quantities of this amino acid available to a growing organism, whose sole source of protein is corn, would be the limiting factor in regulating protein synthesis; as the lysine is added to the corn diet, more and more of the other amino acids present will be utilized for protein synthesis and less for other metabolic functions. When the tryptophan intake is fixed, the more lysine present in the diet the more tryptophan was used for protein synthesis and less for nicotinic acid conversion. The opposite was also true, as the lysine level of the ration decreased, N-methyl nicotinamide excretion was increased. Apparently under these circumstances less tryptophan is being used for protein synthesis and more for conversion to nicotinic acid.

The initial rise in N-methyl nicotinamide excretion observed when tryptophan was withdrawn at a constant lysine intake, suggests that with the level of lysine present, the amount of tryptophan that was being added to the diet was

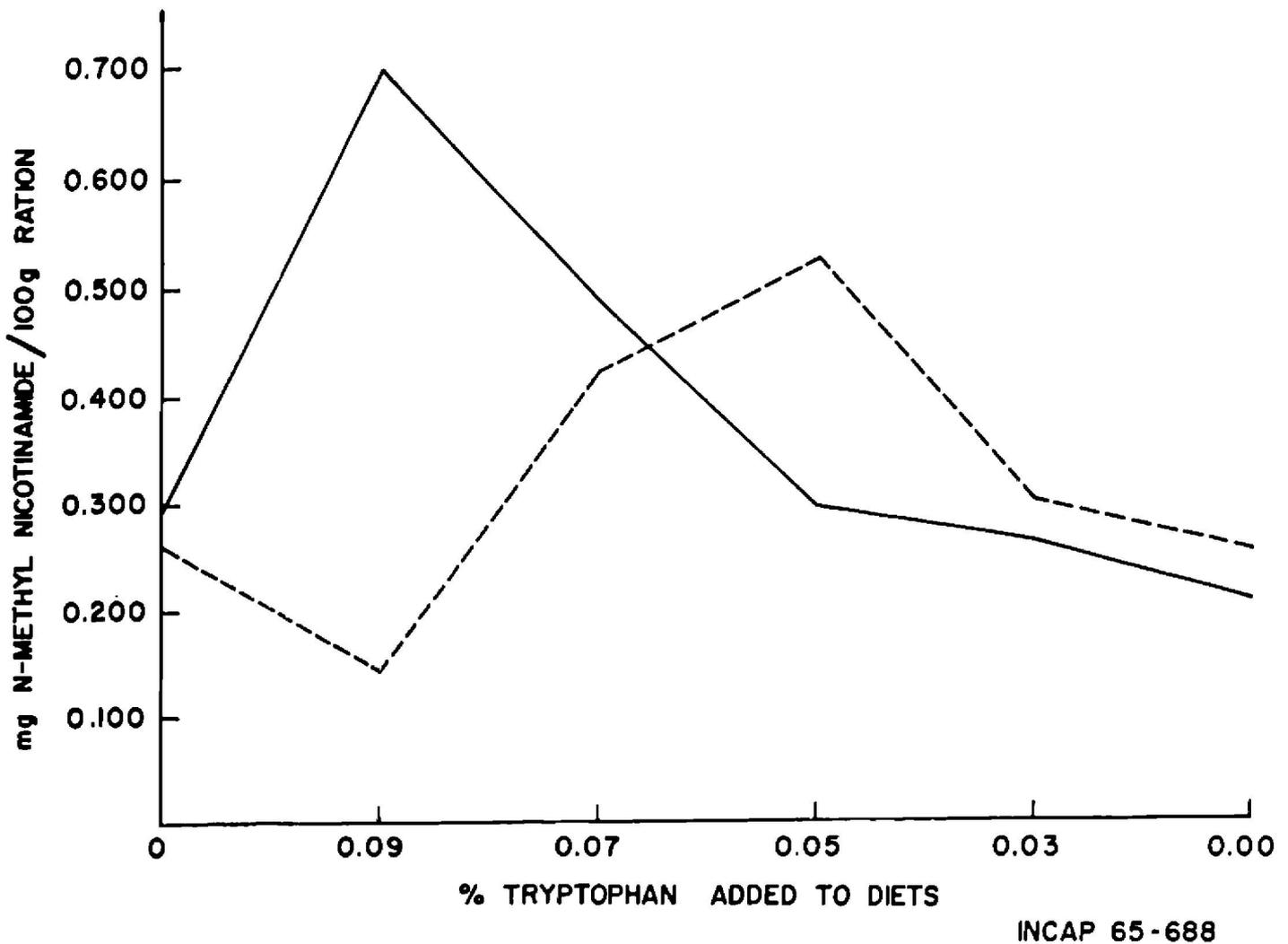


Fig. 3.—Urinary N-methyl nicotinamide excretion in pigs fed raw or lime-treated corn diets. — raw corn - - - - lime-treated corn. 0.48 per cent lysine added to diets, except for the first point in the curve where no amino acid was supplemented.

in excess of that required for protein synthesis, hence, the excess tryptophan was converted into nicotinic acid. When the tryptophan level of the ration was lowered, the relative ratio of both amino acids was such that all the tryptophan present was used for protein synthesis and none for nicotinic acid conversion. The results of measurements of N-methyl nicotinamide excretion shown on the right hand of the curve (Fig. 3) are probably a reflection of the amount of nicotinic acid present in the diet and not of the nicotinic acid resulting from tryptophan conversion. Since the difference within periods for a given diet was not significant, the length of the experimental period sufficiently balanced out any carry-over effect.

Although using different measurements, these results confirm the validity of the mechanism proposed by Koeppe and Henderson<sup>7</sup> and Henderson et al.<sup>8</sup> who suggested that when one of the amino acids becomes limiting for growth (i.e., protein synthesis), the latter would occur as long as the supply of the amino acid could allow it, then growth would cease making more amino acids available for other metabolic functions such as tryptophan conversion to nicotinic acid. The amino acid composition of a given diet is then a very important factor in determining whether tryptophan is to be used for protein synthesis or to be converted into nicotinic acid.

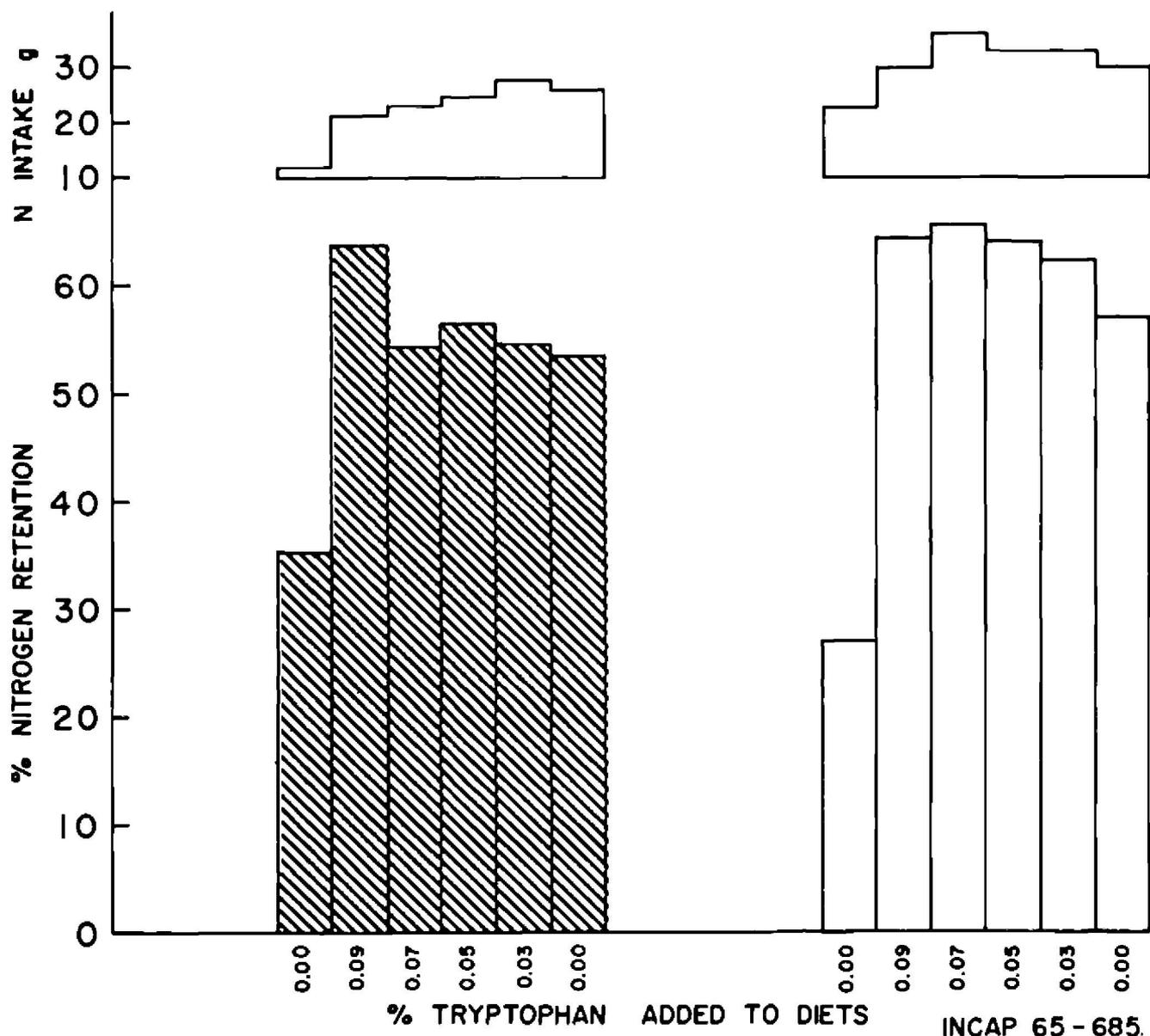


Fig. 4.—Nitrogen retention in pigs fed raw or lime-treated corn diets. Stripe box, raw corn; blank box, lime-treated corn. 0.48 per cent lysine added to diets, except for the first point in the graph where no amino acid was supplemented.

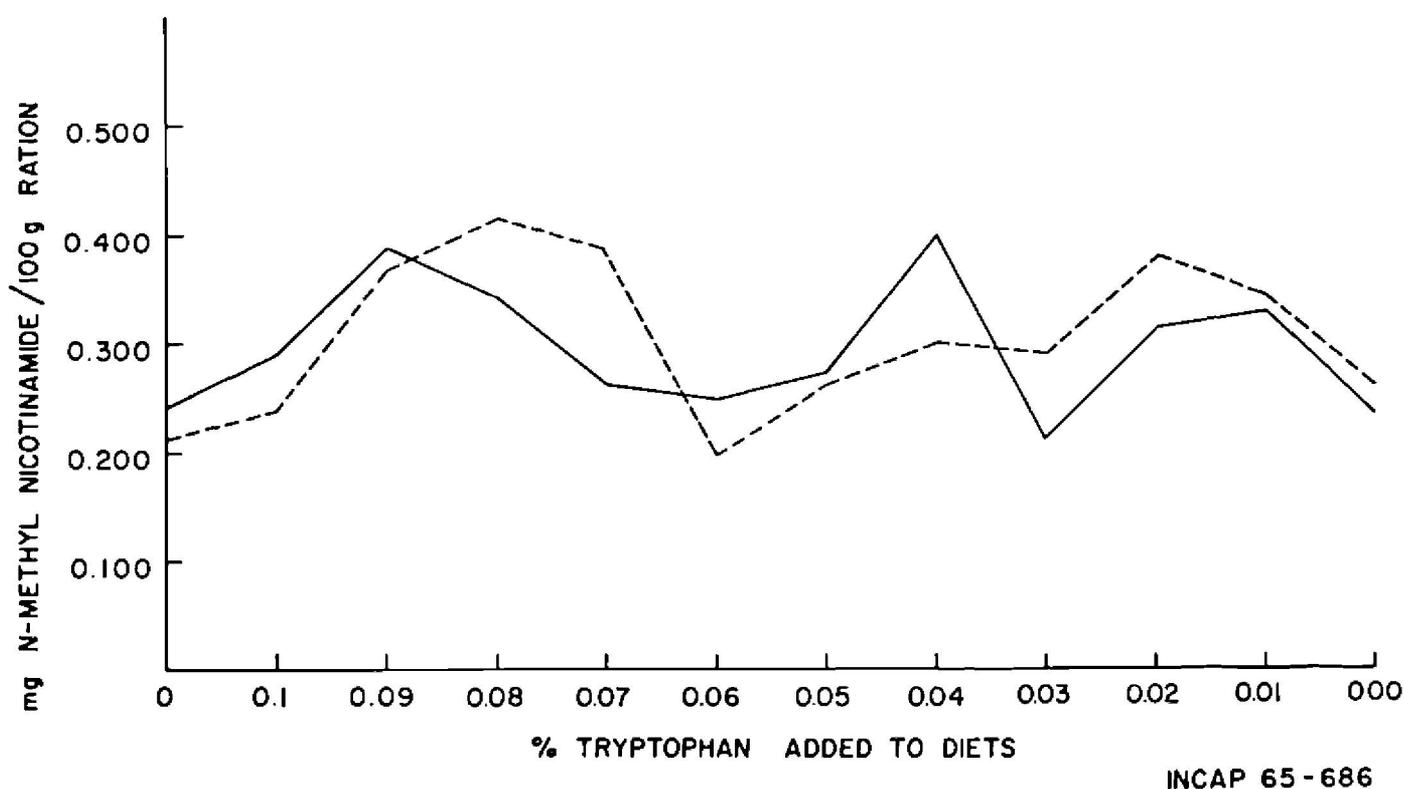


Fig. 5.—N-methyl nicotinamide excretion in pigs fed raw or lime-treated corn diets. — raw corn - - - lime-treated corn. 0.48 per cent lysine added to diets, except for the first point in the curve where no amino acid was supplemented.

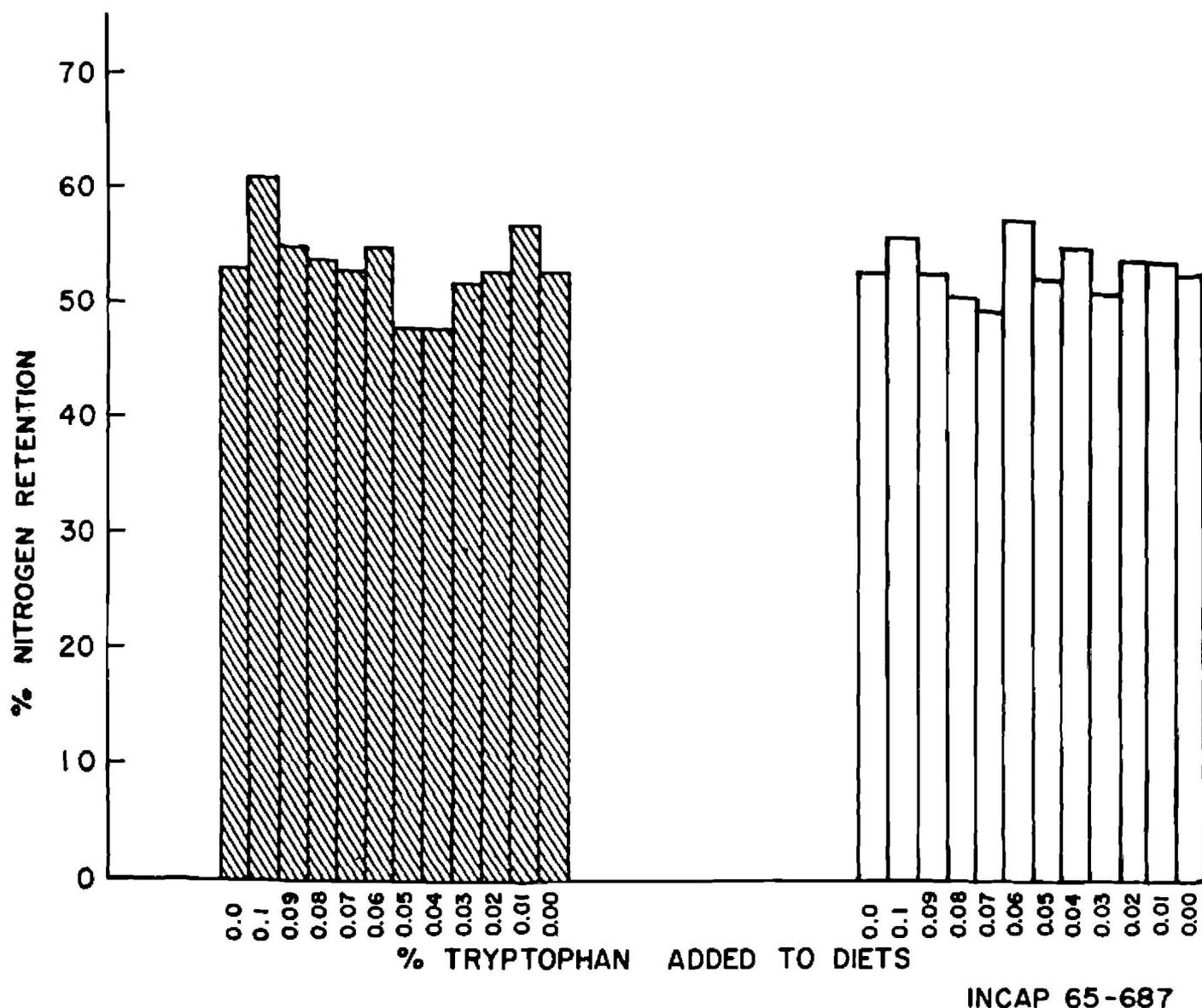


Fig. 6.—Nitrogen retention in pigs fed raw or lime-treated corn diets. Stripe box, raw corn; blank box lime-treated corn. 0.48 per cent lysine added to diets, except for the first point in the graph where no amino acid was supplemented.

The fact that the lime-treated corn diet gave a higher nitrogen retention than did the raw corn diet and that the former was less affected by the withdrawal of the amino acids from the diet, suggests that the lime treatment of corn results in a better balanced protein.<sup>6</sup>

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ARTERIOSCLEROTIC HEART DISEASE IN ROSETO, PENNSYLVANIA. A. *Keys*. *J.A.M.A.* 195:137, 1966.

Data from a recent electrocardiographic survey provide no support for the claim that people in the town of Roseto, Pennsylvania, are relatively protected from myocardial infarction. The evidence offered from death data for 1955 to 1961 does not prove that in those years the people of Roseto differed significantly from average U. S. white residents in the rate of death from arteriosclerotic heart disease. Consideration of the diet suggests that in the past Rosetans should have enjoyed considerable protection from arteriosclerotic heart disease. Failure to establish that fact could be expected from the inadequate size of the population concerned. There is no basis to propose a protective effect of the emotional climate of Roseto.