Dilution of proteins with nonessential amino acids and inorganic nitrogen[1]

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SUMMARY

The effect of adding one fourth, one half and the total amount of non-essential amino acids of different proteins at two different levels of protein in the diet was studied in rats. Also the effect of replacing the nonessential amino acid mixture with a nonspecific source of nitrogen (diammonium citrate) was studied. Results indicate that nonessential amino acid addition to a diet containing a good quality protein such as egg protein, has no significant effect on weight gain although nitrogen from this source was less efficiently utilized. The same results were obtained with nonprotein nitrogen in the diet. When proteins deficient in one of the essential amino acids, such as casein, soybean or cottonseed protein were fed at a 10 percent level, addition of nonessential amino acids decreased weight slightly and nitrogen retention significantly. Increasing the protein level to 15 percent counteracted the depressive effect of growth of nonessential amino acid addition. Nonprotein nitrogen added to the diet did result in low weight gains and nitrogen utilization.

The results suggest that response to nonessential amino acid addition is dependent on the amino acid make-up of the protein, while nonprotein addition in relatively large quantities depresses weight gain and nitrogen utilization, irrespective of protein make-up or level in the diet.

INTRODUCTION

Although it is recognized that ten of the commonly occurring amino acids are indispensable for the rat, during the last

1 Supported by National Institutes of Health, Grant No. AM-3811.
2 From the Division of Agricultural and Food Science.
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Recibido: 14-7-1969
INCAP Publication I-507

years attention has been given to the role played by the nonessential or dispensable amino acids, especially when they are fed in a nonprotein diet containing synthetic essential amino acids. Thus, Womack and Rose (1) showed that glutamic acid, proline and arginine stimulated the growth of weanling rats. Of these three amino acids, arginine is now considered an indispensable amino acid for the rat but proline and glutamic acid are considered dispensable. Rosenberg (2) suggested that approximately equal quantities of nitrogen should be supplied from dispensable and indispensable amino acids and Frost and Sandy (3) estimated, in protein-repletion studies with rats, that 20 to 30 percent of the nitrogen supplied in amino acid diets should be "other than essential amino acid nitrogen for maximum repletion".

The studies of Lardy and Feldott (4) showed that eight of the nonessential amino acids could be replaced on an isonitrogenous basis, with diammonium citrate when the diet contained all the essential amino acids in adequate proportions. Other sources of nonprotein nitrogen, such as glycine, urea and ammonium acetate, have also been studied.

The present study was carried out to determine the effect on the weight gain and nitrogen utilization of rats, of adding one fourth, one half and the total amount of nonessential amino acids of different proteins, at two different levels of protein intake. The effect of replacing the nonessential amino acid mixture by a nonspecific source of nitrogen such as diammonium citrate was also studied.

MATERIALS AND METHODS

Weanling rats of the Wistar strain from the INCAP colony were fed the basal diet shown in Table No. 1. To this diet the protein sources under study, casein and egg, soybean and cottonseed flours, were added at a level to provide 10 and 15 percent of protein in the diet. The essential and nonessential amino acid content of the proteins, under test, was tabulated and the amount contained in the percentage of protein for a given diet calculated. Mixtures of nonessential amino acids tó supply 25, 50 and 100 percent of the original concentration in the protein tested were added to the diets. The nonessential amino acids used were Glycine, L-Serine, L-Glutamic acid,

TABLE No. 1

COMPOSITION OF THE BASAL DIET

Ingredient	%
Mineral mixture[1]	4.0
Cottonseed oil	5.0
Cod liver oil	1.0
Cornstarch	90.0
Vitamin mixture[2], ml	5

[1] Hegsted, D. M. *et al.* *J. Biol. Chem.*, 138:459-466, 1941.

[2] Manna, L. & S. M. Hauge. *J. Biol. Chem.*, 202:91-96, 1953.

L-Aspartic acid, L-Proline, L-Alanine and L-Tyrosine. Table No. 2 shows the calculations for the 10 percent casein diet as an example. The first column of figures shows the percentage of the amino acids in casein, the second column the amount of these amino acids in 11.2 grams of casein which supplied 10 percent of protein to the diet, and the next three columns show the calculations for the amounts of amino acids to be added in order to supply 25, 50 and 100 percent of the concentration in the original protein. This porcedure was followed for all proteins tested at the two selected protein levels.

The rations were analyzed for nitrogen by the macro-Kjeldahl procedure (5). The essential and nonessential amino acid nitrogen was calculated as was also their ratio (I/D ratio), the latter according to Stucki and Harper (6). Table 3 shows the I/D ratios for the different diets at the 10 percent protein level. Since the proportions at the 15 percent level

TABLE No. 2

AMOUNTS OF NONESSENTIAL AMINO ACIDS ADDED TO EXPERIMENTAL DIETS

Amino acid	% in casein	in 11.2g casein g	25% concentration g	50% concentration g	100% concentration g
Glycine	1.987	0.222	0.056	0.111	0.222
L-Serine	6.645	0.744	0.186	0.372	0.744
L-Glutamic acid	23.052	2.582	0.646	1.291	2.582
L-Aspartic acid	7.393	0.828	0.207	0.414	0.828
L-Proline	11.749	1.313	0.328	0.656	1.313
L-Alanine	3.354	0.376	0.094	0.188	0.376
L-Tyrosine	5.819	0.652	0.163	0.326	0.652
TOTAL		6.717	1.680	3.358	6.717

Taken from: Orr, M. L. & B. K. Watt. Amino acid content of foods. Washington, D. C., U. S. Department of Agriculture, 1957. Home Economics Research Report No. 4. 41 p.

TABLE No. 3

I/D RATIOS OF EXPERIMENTAL DIETS AT A 10% PROTEIN LEVEL

Diet	% of nonessential amino acids added			
	0	25	50	100
Casein	1.15	0.92	0.77	0.58
Egg protein	1.44	1.15	0.96	0.72
Soybean protein	1.14	0.92	0.76	0.57
Cottonseed protein	1.39	1.11	0.93	0.69

were the same, the I/D ratios would also be the same as for the 10 percent level. The ratios in the control diet were close to one showing a 50-50 concentration of essential and nonessential amino acid nitrogen. As increasing amounts of nonessential amino acids were added, the ratio decreased to near half the original value.

The nitrogen supplied by the nonessential amino acid mixtures was calculated in terms of diammonium citrate; then added to diets at the two levels of protein tested and at the three levels of nonessential amino acid addition. These diets were also analyzed for nitrogen by the Kjeldahl method (5). All diets were fed to groups of 22 day old rats of the Wistar strain of the INCAP colony, 4 male and 4 female for a period of four weeks. The rats were placed in individual all wire screen cages with raised screen bottoms. Weight and feed consumption were recorded weekly and, at the end of the experiment, nitrogen efficiency ratio was calculated. Statistical significance of the data was determined by the methods outlined by Snedecor (7).

RESULTS

Table No. 4 shows the results for weight gain and nitrogen efficiency ratio when the casein diet was fed at 10 and 15 percent with nonessential amino acids and diammonium citrate. Addition of nonessential amino acids decreased weight gain significantly ($P<0.01$) at the 10 percent protein level. This effect was not detected at the 15 percent protein level. In both cases, nitrogen utilization decreased with increasing levels of either of the nitrogen sources added at both levels of protein intake. Addition of diammonium citrate decreased significantly ($P<0.01$) both weight gain and nitrogen efficiency ratio. Table No. 5 shows the results with egg protein. Addition of either nonessential amino acids or diammonium citrate did not affect significantly weight gain at any of the three levels of the protein tested. The tendency of the nitrogen efficiency ratio was to decrease with increasing levels of either source of nitrogen.

Table No. 6 shows the results when soybean meal was fed at levels to provide 10 and 15 percent of protein. Addition of nonessential amino acids resulted in slight increase in weight

TABLE No. 4

EFFECT OF DILUTING THE PROTEIN IN CASEIN WITH NONESSENTIAL AMINO ACIDS OR INORGANIC NITROGEN ON ITS NUTRITIVE VALUE

Protein in diet %	Parameter	ADDITION 0 %	25 %	50 %	100 %
		Nitrogen of nonessential amino acids			
10	Increase in weight, g	130	128	114	106
	NER	19.6	17.7	15.3	12.6
	Nitrogen in diet, %	1.65	1.83	2.01	2.39
15	Increase in weight, g	145	147	142	151
	NER	16.2	14.2	13.5	11.4
	Nitrogen in diet, %	2.26	2.54	2.75	3.36
		Inorganic Nitrogen			
10	Increase in weight, g	130	116	92	77
	NER	19.6	18.2	13.5	10.4
	Nitrogen in diet, %	1.65	1.71	2.02	2.24
15	Increase in weight, g	145	141	133	98
	NER	16.2	15.2	14.4	10.3
	Nitrogen in diet, %	2.26	2.38	2.69	3.30

Average initial weight: 46 g

TABLE No. 5

CHANGES IN WEIGHT AND NER OF RATS FED DIETS CONTAINING WHOLE EGG PROTEIN AND DILUTED WITH NONESSENTIAL AMINO ACIDS OR INORGANIC NITROGEN

Parameter	0 %	25 %	50 %	100 %
	Nitrogen of nonessential amino acids			
	10% Protein			
Increase in weight, g	123	123	121	124
NER	22.5	20.5	18.8	16.4
Nitrogen in diet, %	1.70	1.85	2.00	2.29
	12.5% Protein			
Increase in weight, g	132	126	130	131
NER	19.4	17.3	17.2	15.1
Nitrogen in diet, %	2.10	2.28	2.45	2.84
	15% Protein			
Increase in weight, g	133	136	128	127
NER	17.2	16.5	14.3	11.8
Nitrogen in diet, %	2.50	2.69	2.94	3.39
	Inorganic Nitrogen			
	10% Protein			
Increase in weight, g	123	114	109	117
NER	22.5	18.5	18.0	18.3
Nitrogen in diet, %	1.70	1.94	2.10	2.15
	12.5% Protein			
Increase in weight, g	132	137	124	122
NER	19.4	16.9	16.4	14.5
Nitrogen in diet, %	2.10	2.37	2.46	2.72
	15% Protein			
Increase in weight, g	133	133	123	125
NER	17.2	15.7	14.4	13.5
Nitrogen in diet, %	2.50	2.69	2.94	3.10

TABLE No. 6

EFFECT OF SOYBEAN PROTEIN DILUTION WITH NONESSENTIAL AMINO ACIDS AND NITROGEN ON ITS PROTEIN VALUE

Protein in diet %	Parameter	DILUTION 0 %	25 %	50 %	100 %
		Nitrogen of nonessential amino acids			
10	Increase in weight, g	91.	106	97	99
	NER	16.6	15.7	13.9	12.0
	Nitrogen in diet, %	1.49	1.64	1.78	2.07
15	Increase in weight, g	138	147	134	143
	NER	11.4	11.8	10.1	9.4
	Nitrogen in diet, %	2.62	2.77	3.08	3.47
		Inorganic Nitrogen			
10	Increase in weight, g	91	75	68	60
	NER	16.6	13.2	11.6	9.4
	Nitrogen in diet, %	1.49	1.66	1.89	2.16
15	Increase in weight, g	138	131	124	07
	NER	11.4	10.9	10.0	7.8
	Nitrogen in diet, %	2.62	2.84	3.05	3.49

Average initial weight: 47 g

gain at the 10 percent protein level but no significant effect could be detected at the 15 percent level. Addition of diammonium citrate resulted in significant ($P<0.01$) decrease in weight gain. Nitrogen efficiency ratio was decreased by adding nonessential amino acids at the 10 percent protein level but was not affected when the protein level was raised to 15 percent. Addition of diammonium citrate resulted in a significant ($P<0.01$) decrease of nitrogen utilization at either level of protein tested.

Table No. 7 shows the results obtained when cottonseed meal was fed to provide 10 and 15 percent of protein in the diet. Addition of nonessential amino acids resulted in lower weight gains only when the 10 percent level was fed; at 15 percent of protein the addition of nonessential amino acids did not have any effect on weight gain. At either level of

TABLE No. 7

EFFECT OF COTTONSEED PROTEIN DILUTION WITH NONESSENTIAL AMINO ACIDS OR INORGANIC NITROGEN ON THE PROTEIN VALUE

Protein in diet %	Parameter	DILUTION 0 %	25 %	50 %	100 %
		Nitrogen of nonessencial amino acids			
10	Increase in weight, g	97	100	88	101
	NER	13.5	12.6	10.9	11.1
	Nitrogen in diet, %	1.78	1.91	2.04	2.30
15	Increase in weight, g	144	144	136	138
	NER	14.0	13.0	11.6	10.3
	Nitrogen in diet, %	2.25	2.45	2.65	3.04
		Inorganic Nitrogen			
10	Increase in weight g	97	80	70	63
	NER	13.5	11.4	10.1	8.9
	Nitrogen in diet, %	1.78	1.91	2.04	2.30
15	Increase in weight, g	144	133	120	121
	NER	14.0	12.2	10.9	10.0
	Nitrogen in diet, %	2.25	2.45	2.64	3.03

Average initial weight: 47 g

protein the nitrogen efficiency ratio decreased. When diammonium citrate was fed there was a significant ($P<0.01$) decrease in weight gain and nitrogen utilization at either level of protein.

DISCUSSION

The results indicate that addition of nonessential amino acids to a diet containing a good quality protein, such as egg protein, has no significant effect on weight gain although nitrogen from this source is apparently less efficiently utilized by a growing organism. The same results were obtained when nonprotein nitrogen was added to the diet.

When proteins deficient in one of the essential amino acids, such as casein, soybean or cottonseed protein were fed at a ten percent level, addition of nonessential amino acids decreased weight slightly and nitrogen utilization significantly, particularly at the higher levels of addition. Increasing the protein level to 15 percent counteracted the depressive effect on growth caused by the addition of nonessential amino acids. This may be due to the fact that addition of nonessential amino acids could increase the requirement for some of the essential amino acids. Hepburn *et al.* (8) found that the requirement for arginine was increased to unusually high levels by the addition of nonessential amino acids to the diet.

If the requirement for an essential amino acid or amino acids is increased, this increase would result in lower weight gains and nitrogen utilization in those proteins which have marginal levels for one or more of the essential amino acids. In this case, the deficiency of a particular amino acid would be increased by the addition of nonessential amino acids and this in turn, would result in lower weight gains and nitrogen utilization as was the case in the present study. The increase of protein level to 15 percent would counteract the deficiency, but the efficiency of protein utilization will remain low.

Young and Villarreal (9), using 15 percent protein milk diets, reported a decreased utilization of the nitrogen of diets in which the milk protein was partially replaced by nonspecific nitrogen. Supplementation of the diets containing 10.5 percent protein and nonspecific nitrogen with sulfur amino acids, did not restore growth to the maximum rate obtained with the 15 percent milk protein diets.

The effects of the nonessential amino acid nitrogen and of the inorganic nitrogen of the present investigation are, however, confounded since the addition of either source of nitrogen increased total nitrogen of the diet. These increases in nitrogen content would tend to reduce nitrogen efficiency ratio (NER), which is the ratio of weight gained over total nitrogen consumed. It is well established that as nitrogen or protein content of the diet increases, NER or protein efficiency ratio (PER) decreases (10). The conclusions presented are, however, valid particularly with respect to the experiments in which inorganic nitrogen was used, not only because weight gained decreased with higher additions of this source but when comparisons of NER are made at similar levels of nitrogen in the diet in those groups fed nonessential amino acid nitrogen, lower NER values are found the higher the addition of inorganic nitorgen. This is also true for nonessential amino acid nitrogen additions, particularly for the proteins with a deficiency of an essential amino acid and at the lower levels of protein in the diet.

The highest depressing effects were observed with the vegetable proteins with either nonessential amino acid nitrogen or inorganic nitrogen and, furthermore, the effect seems to be independent of the I/D amino acid ratio of the protein.

Recently, Daniel *et al.* (11) indicated that the addition of glutamic acid to egg or milk protein did not alter weight gain from that obtained by feeding egg or milk alone. The addition, however, decreased PER in both proteins and in this case the decrease was not confounded with proteins levels since the diets were isonitrogenous.

This data indicate, therefore, that the attempts to dilute milk protein with nonessential nitrogen as a means of extending milk supplies should be discouraged particularly if such modified milks are intended for distribution in developing countries, where most, if not all protein ingested, is derived from vegetable sources (12). This is even more important in the case of vegetable protein concentrates which have deficiencies of essential amino acids and are of lower digestibility than animal proteins.

RESUMEN

Dilución de proteínas con aminoácidos no esenciales y nitrógeno inorgánico

Se llevó a cabo un estudio, en ratas, del efecto que tiene el agregado de un cuarto, un medio y una cantidad total de aminoácidos no esenciales de diferentes proteínas a dos niveles diferentes de contenido de proteína en la dieta. También se estudió el efecto que tiene el reemplazar mezclas de aminoácidos no esenciales con una fuente no específica de nitrógeno (citrato de diamonio). Los resultados indicaron que el agregado de los aminoácidos no esenciales a una dieta que contiene una proteína de buena calidad, tal como la proteína del huevo, no tiene ningún efecto significativo en el aumento de peso, aunque el nitrógeno de esta fuente fue el que se utilizó con menos eficiencia. Los mismos resultados se obtuvieron con el nitrógeno no proteínico en la dieta. Cuando las proteínas deficientes en uno de los aminoácidos esenciales, como es la caseína, frijol de soya y pro-

teína de semilla de algodón, fueron suministrados a un nivel de 10%, el agregado de aminoácidos no esenciales disminuyó el peso levemente y la utilización de nitrógeno significativamente. Al incrementarse la proteína a un nivel del 15% esto contrarrestó el efecto depresivo del agregado de aminoácidos no esenciales. El agregado de nitrógeno no proteínico a la dieta resultó en baja de peso y baja utilización de nitrógeno.

Los resultados obtenidos sugieren que la respuesta del agregado de aminoácidos no esenciales depende del patrón de aminoácidos de la proteína, mientras que el agregado no proteínico en cantidades relativamente grandes deprime la ganancia de peso y la utilización del nitrógeno sin importar el contenido o nivel de proteína en la dieta.

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