Nutritional Equivalence of β-Lactoglobulin and Its Corresponding Amino Acids. (26817)

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One of the most important problems in protein metabolism is the comparison of the nutritional behavior of a protein with that of its constituent amino acids. Many growth studies utilizing amino acids have been reported, but to the authors' knowledge in no case has a comparison been made with a crystalline protein, free from carbohydrate, phosphorus, prosthetic groups and possible unidentified contaminants which might possess growth stimulating properties. A frequent comparison has been with casein, which obviously does not fill the above requisites and in such studies the amino acid mixture does not provide growth equal to that obtained with the intact protein(1,2,3). During the period 1945 to 1948 studies with crystalline β-lactoglobulin were conducted with mice. The difficulties in obtaining sufficient quantities of the crystalline protein necessitated small groups of animals and these early studies were not reported except for an ACS meeting abstract (Sept. 1948). It has been hoped that an extension of this work would provide valuable additional data, but the importance of the original observations is so great that results of these experiments are being presented even though expanded observations have not been possible.

Materials and methods. Crystalline β-lactoglobulin was made from fresh raw skim milk by modification of the procedure of Palmer(4) adapted for large scale isolation. The purity of the β-lactoglobulin was established by electrophoretic homogeneity at pH 4.8 and 6.5(5). The crystalline protein was maintained wet and under toluene until all measurements of purity were complete and was then dried by lyophilization before incorporating into diets. The amino acid composition described by Brand *et al.*(6) was used in preparing the amino acid counterpart shown in Table I. Amino acids were purchased from commercial suppliers and purity was established by checking optical rotation and melting points. Since basic amino acids were to be fed as their hydrochlorides, a calculated equivalence of $NaHCO_3$ was added to the mixture (Table I).

Diet composition is given in Table II. In addition to β-lactoglobulin and its amino acid counterpart, 2 crude protein sources, a vitamin test casein and a commercial lactalbumin, were used as additional controls. All proteins were incorporated in the diets at equal nitrogen levels and amounts used were selected to give growth rates that would be somewhat less than the maximum achievable by the mouse. As closely as possible, by calculation, the diets were isocaloric. Sodium chloride was added to the protein-containing diets to balance the sodium of the $NaHCO_3$ in the amino acid diet.

Since mice, as well as other animals, must adjust to diets containing amino acids, the amino acid diet was fed for 3 days to 30 male weanling mice selected for uniformity of weight at 18 days of age (Swiss-Webster Strain, Sharp and Dohme Colony). During the adjustment period 1.5% of 1:20 liver powder was incorporated in the diet. These experiments were conducted prior to crystallization of Vit. B_{12}, but it was recognized that a source of an unidentified factor was required by the mouse. In the light of subsequent studies it is probable that during the preweanling period and with the liver powder fed during the 3 day adjustment period, sufficient stores of Vit. B_{12} were accumulated so that the absence of this vitamin during the subsequent 4 week test was not limiting for the animals. Sixteen mice that showed uniform growth responses during the 3 day adjustment period were divided into 4 groups of 4 mice each with equalized weight distribution. The mice were caged individually and

* Deceased.

TABLE I. Amino Acid Composition of β-lactoglobulin and Amino Acid Mixture.

	β-lactoglobulin		Amino acid mixture		
	Amino acid yield/100 g protein, g	N/100 g protein, g		Amino acid, g	N, g
Glycine	1.4	.26		1.4	.26
Alanine	(7.8)*	(1.23)*	L	7.8	1.23
Leucine	15.6	1.67	L	15.6	1.67
Proline	4.1	.50	L	4.1	.50
Cysteine	1.11	.13	—	—	—
Cystine	2.29	.27	L	3.4	.40
Tryptophan	1.94	.27	L	2.0	.28
Tyrosine	3.8	.29	L	3.8	.29
	38.04	4.62		38.10	4.63
Arginine	2.88	.93	L-HCl	3.5	.93
Histidine	1.58	.43	L-HCl-H_2O	2.1	.43
Lysine	11.40	2.18	L-HCl-H_2O	15.6	2.18
	15.86	3.54		21.2	3.54
Aspartic acid	11.4	1.20	L	3.3	.35
Asparagine	—	—	L	7.9	1.67
Glutamic acid	19.5	1.86	L	17.1	1.63
Glutamine	—	—	L	2.2	.42
Methionine	3.22	.30	L	3.2	.30
Phenylalanine	3.54	.30	L	3.5	.30
Valine	5.8	.70	L	5.8	.70
Isoleucine	(6.0)*	(.64)*	L	6.0	.64
Serine	5.0	.67	L	5.0	.67
Threonine	5.85	.69	L	5.85	.69
	60.31	6.36		59.85	7.37
Amide NH_3	1.3	1.08			
Na HCO_3				8.7	
Total	115.51	15.60		127.85	15.54
	15.60/115.51 = 13.50%			15.54/127.85 = 12.16%	

* Values revised from those given in original references.

TABLE II. Composition of Diets.

	Holding	A	B	C	D
	g				
Casein[1]	—	17.0	—	—	—
Lactalbumin[2]	—	—	19.5	—	—
β-lactoglobulin	—	—	—	15.3	—
Amino acid mixture	19.0	—	—	—	19.2
Hydrogenated cottonseed oil[3]	25	25	25	25	25
Corn oil[4]	2	2	2	2	2
Glucose[5]	20	20	20	20	20
Salt mixture[6]	4	4	4	4	4
Cellulose[7]	2	2	2	2	2
1:20 liver conc. powder	1.5	—	—	—	—
Choline chloride	.2	.2	.2	.2	.2
Sodium chloride	—	.9	.9	.9	—
White dextrin	26.3	28.9	26.4	30.6	27.6
Total	100.0	100.0	100.0	100.0	100.0
% nitrogen in diet	2.33	2.34	2.34	2.34	2.53

Each diet supplemented to contain per 100 g: 4 mg of α-tocopherol, 900 U.S.P. units of vit. A, 180 U.S.P. units of vit. D, 1 mg of 2-methyl-1,4-naphthoquinone diacetate, 0.8 mg of thiamine HCl, 1.6 mg of riboflavin, 0.8 mg of pyridoxine HCl, 4.0 mg of niacin, 4.4 mg of calcium pantothenate, 4.0 mg of para-aminobenzoic acid, and 21.6 mg of inositol.

Footnotes: [1] Bordens Labco, [2] Bordens 15-42, [3] Primex, [4] Mazola, [5] Cerelose, [6] Jones, J. H., and Foster, C., *J. Nut.*, 1942, v24, 245; [7] Cellu Flour.

fed *ad libitum* in a temperature controlled room. Daily weights and food consumption were measured.

Results and discussion. Performance data at 14 and 28 days are given in Table III. Growth rate and efficiency of nitrogen utilization were the same for the crystalline β-lactoglobulin and its corresponding amino acid mixture. On the other hand, these 2 groups appear to have had lower growth rates and ratios of gain to nitrogen intake than was obtained with the 2 crude proteins, casein

TABLE III. Growth and Nitrogen Utilization of Mice Fed Diets Containing Intact Proteins and Amino Acid Mixture.

		14 days			28 days		
	Initial wt, g	Wt gain, g	N intake, g	Wt gain / N intake	Wt gain, g	N intake, g	Wt gain / N intake
Casein	9.9	11.3	1.00	11.3	12.6	2.03	6.2
	9.7	10.8	1.08	10.0	12.8	2.15	6.0
	9.5	9.4	1.00	9.4	11.8	2.03	5.8
	8.9	12.1	.98	12.3	15.5	2.15	7.2
Avg	9.5	10.9	1.02	10.7	13.2	2.10	6.3
Lactalbumin	12.1	7.1	.91	7.8	9.3	1.96	4.7
	8.5	13.3	1.08	12.3	16.2	2.32	7.0
	8.6	11.9	1.03	11.6	13.1	2.11	6.2
	9.1	9.4	.90	10.4	13.3	1.94	6.9
Avg	9.6	10.4	.98	10.5	13.0	2.08	6.2
β-lactoglobulin	8.2	7.9	.75	10.5	10.7	1.79	6.0
	9.9	10.6	.99	10.7	12.0	2.10	5.7
	9.4	9.0	1.00	9.0	11.4	2.08	5.5
	10.6	8.6	.99	8.7	11.0	1.94	5.7
Avg	9.5	9.1	.93	9.7	11.3	1.98	5.7
Amino acid mix	9.4	7.0	.81	8.6	11.3	1.84	6.1
	9.4	8.5	.97	8.8	12.6	2.15	5.9
	9.1	9.7	1.02	9.5	11.8	2.31	5.1
	9.9	8.8	.99	8.9	11.2	2.08	5.4
Avg	9.4	8.5	.95	9.0	11.7	2.09	5.6

and lactalbumin. Since small groups had to be used, calculations of significance of differences does not seem warranted and therefore individual results have been given. It will be very important to ascertain whether the apparent difference between β-lactoglobulin and the crude proteins is a reflection of amino acid pattern or some other factor.

In numerous comparisons of growth responses to crude proteins and amino acid mixtures, the proteins have been superior. In the present studies a crystalline protein, free from carbohydrate, phosphorus, prosthetic groups and unidentified constituents gave equivalent growth response to an amino acid mixture corresponding exactly to the native protein. This is probably the only time a comparison has been made in which the complete amino acid composition of a protein has been accurately reproduced. In spite of the extremely high cost of this type of investigation, this model experiment must be extended to establish a complete understanding of the nutritional contributions of proteins and amino acids.

Summary. In mouse growth studies crystalline β-lactoglobulin has been compared with a mixture of L amino acids duplicating its composition and these materials were compared with casein and lactalbumin. The pure protein and its amino acid counterpart gave equal growth and efficiency of dietary nitrogen utilization. Casein and lactalbumin provided superior growth rates to both the pure protein and its amino acid counterpart.

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