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Sulfur Amino Acid Activity of Glutathione, DL-α-Hydroxy-Methionine, and α-Keto-Methionine in Chicks (39906)

JEANNINE M. HARTER AND DAVID H. BAKER[1]

Department of Animal Science, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

The total sulfur amino acids (TSAA) requirement of an animal can be met by dietary methionine alone or by a proper combination of methionine and cysteine (1). Therefore, any compound that can furnish methionine or cysteine activity could spare a portion of the TSAA requirement. Glutathione, α-OH-methionine (α-OH-M), and α-keto-methionine (α-keto-M) are compounds that may spare some or all of the TSAA requirement of an animal.

Glutathione (L-γ-glutamyl-L-cysteinylglycine), a naturally occuring tripeptide, is the most abundant sulfhydryl compound present in tissues (2), and its wide occurrence in feeds and foods could conceivably provide usable sulfur as cysteine for protein synthesis.

The hydroxy and keto analogs of methionine are both natural metabolites of methionine, as shown in Fig. 1. Both the hydroxy and keto analogs are available as chemical additives and have been shown capable of providing usable sulfur for methionine and cysteine biosynthesis (3-7). However, the quantitative efficacy of each analog is a point of contention (5).

The purpose of the assays reported herein was to establish quantitative efficacy of glutathione, and α-keto-M using chick growth as the principal criterion of evaluation.

Materials and methods. Male chicks resulting from the cross of New Hampshire males and Columbian females were used in all assays. Care of chicks prior to treatment and the experimental allotment procedure are described elsewhere (7). All assays were of 8-days duration. The basal diet (Table I) for all assays was devoid of sulfur amino acids, and all additions to the diet were made at the expense of cornstarch. Feed and water were offered *ad libitum*. Data were subjected to analysis of variance, and pooled standard errors were calculated for each response parameter.

Assay 1 was designed to evaluate exogenous glutathione[2] as a source of cysteine. L-Methionine was present in all diets at its physiological requirement of 0.30% (1), and isosulfurous levels of cysteine and glutathione were fed separately or in combination to determine efficacy of exogenous glutathione as a source of cysteine.

Assays 2 and 3 were designed to evaluate α-keto-M[3] and DL-α-OH-M[4] as sources of TSAA. Isosulfurous levels of the two analogs and L-methionine were fed in assay 2. In assay 3, 1% glutamine was fed in addition to DL-α-OH-M to determine if any additional response would occur in the presence of excess glutamine. Glutamine has been shown to be the principal amino donor in rats (8) in the transamination reaction that yields L-methionine from α-keto-M (Fig. 1).

Results and discussion. Chicks in assay 1 (Table II) gained as rapidly and as efficiently when given glutathione as a source of cysteine as when given an isosulfurous level of cystine. Weight gains of chicks fed glutathione in combination with L-cystine were similar to those of chicks fed an isosulfurous concentration of L-cystine alone. These results indicate that chicks have sufficient proteolytic activity to cleave the γ linkage of the tripeptide and to hydrolyze the resulting dipeptide into its constituent amino acids, thereby liberating cysteine for protein synthesis. Similar results were obtained with rats by Dyer and du Vigneaud

[1] To whom reprint requests should be sent.

[2] Glutathione purchased from Nutritional Biochemicals Corp.

[3] α-Keto-methionine supplied by De Gussa, West Germany. α-Keto-M provided as its calcium salt, analyzed to contain 11.9% Ca and 14.3% moisture.

[4] DL-α-OH-methionine (Ca) supplied by Monsanto Corp., St. Louis, Missouri.

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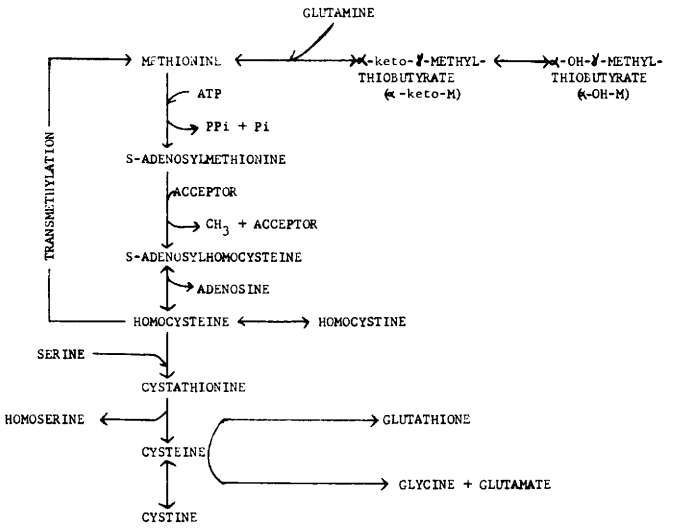

FIG. 1. Interconversion of sulfur compounds.

TABLE I. COMPOSITION OF BASAL DIET.

Ingredient	%	Amino acid mix	g/19.78 g
Cornstarch	to 100	L-Arginine HCl	1.15
Amino acid mix	19.78	L-Histidine HCl H_2O	0.45
Corn oil	15.00	L-Lysine HCl	1.14
Salt mixture[a]	5.37	L-Tyrosine	0.45
Cellulose[b]	3.00	L-Tryptophan	0.15
$NaHCO_3$	1.00	L-Phenylalanine	0.50
Choline chloride	0.20	L-Methionine	–
Vitamins[a]	0.20	L-Cystine	–
α-Tocopheryl acetate (20 mg/kg)	+	L-Threonine	0.65
Ethoxyquin (125 mg/kg)	+	L-Leucine	1.00
		L-Isoleucine	0.60
		Glycine	0.60
		L-Valine	0.69
		L-Proline	0.40
		L-Glutamic acid	12.00
			19.78

[a] Katz and Baker (7).
[b] Solka Floc, Brown Company, Chicago, Illinois.

(9). However, our results with chicks eliminate the confounding factor of coprophagy always present in rat feeding trials. Thus, the increased growth of rats fed glutathione may have been due to breakdown of glutathione to its constituent amino acids by intestinal bacteria, followed by reingestion of the liberated L-cysteine.

Results in assay 2 (Table III) clearly indicate that α-keto-M has greater ($P < 0.01$) methionine activity than DL-α-OH-M. However, both methionine analogs were inferior ($P < 0.01$) to L-methionine as a source of total sulfur amino acids. Compared with L-methionine, the TSAA-sparing value of α-keto-M was 83%, and only 53% for DL-α-OH-M. This suggests that the major loss of activity occurs in converting DL-α-OH-M to α-keto-M. This may be due to inefficient conversion of the D-OH component of the DL racemic mixture to the keto compound. Thus, it has been shown that D-methionine, which must also go through a keto derivative to be converted to L-methionine, is less efficacious than L-methionine, not only for chicks (10) but for man (11) as well.

It has been suggested by Langer (8) that glutamine is the principal amino donor in the transamination reaction which converts

TABLE II. PERFORMANCE OF CHICKS FED GLUTATHIONE AS A SOURCE OF CYSTEINE (ASSAY 1).[a]

Diet	Gain (g)	Gain/feed[b]
(1) Basal + 0.30% L-methionine	41.7	0.38
(2) As (1) + 0.10% L-cystine	84.1	0.55
(3) As (1) + 0.256% glutathione[c]	95.5	0.58
(4) As (1) + 0.20% L-cystine	110.4	0.64
(5) As (2) + 0.256% glutathione	110.8	0.67
Pooled SE	4.8	0.02

[a] Average of triplicate groups of seven male chicks for the period 8 to 16 days posthatching; average initial weight was 77 g.
[b] Weight gain (g) ÷ feed intake (g).
[c] Isosulfurous to 0.10% L-cystine.

TABLE III. PERFORMANCE OF CHICKS FED L-METHIONINE OR METHIONINE ANALOGS AS A SOURCE OF TOTAL SULFUR AMINO ACIDS (ASSAY 2).[a]

Diets[b]	Gain (g)	Relative gain (%)	Gain/feed[c]
(1) Basal + 0.400% L-methionine	64.3	100	0.55
(2) Basal + 0.530% α-keto-M (Ca)	53.2	83	0.50
(3) Basal + 0.476% DL-α-OH-M (Ca)	34.1	53	0.39
Pooled SE	4.7	7	0.02

[a] Average of triplicate groups of seven male chicks for the period 8 to 16 days posthatching; average initial weight was 59 g.
[b] All diets are isosulfurous; corrections were made for calcium and moisture content of the compounds.
[c] Weight gain (g) ÷ feed intake (g).

α-keto-M to L-methionine. Results in assay 3 (Table IV) show no additional response to DL-α-OH-M when 1% glutamine was added to the diet, and this indicates that glutamine was not a limiting factor in our diet.

The relative efficacy of all the sulfur-containing compounds used in these assays is largely dependent upon the amount of L-methionine or L-cysteine activity that they can furnish. However, the relative timing with which these compounds are made available for protein synthesis is also of consequence. Thus, both L- and DL-methionine are absorbed from the gut much more

TABLE IV. PERFORMANCE OF CHICKS FED DL-α-OH-METHIONINE WITH AND WITHOUT ADDED GLUTAMINE (ASSAY 3).[a]

Diet[b]	Gain (g)	Relative gain (%)	Gain/feed[c]
(1) Basal + 0.50% L-methionine	89.3	100	0.64
(2) As (1) + 1.0% L-glutamine	92.2	103	0.66
(3) Basal + 0.595% DL-α-OH-M (Ca)	51.3	57	0.49
(4) As (3) + 1.0% L-glutamine	53.3	60	0.49
Pooled SE	3.9	4	0.01

[a] Average of triplicate groups of five male chicks for the period 8 to 16 days posthatching; average initial weight was 66 g.
[b] All diets are isosulfurous; corrections were made for the calcium content of the compounds.
[c] Weight gain (g) ÷ feed intake (g).

rapidly then DL-α-OH-M (12), and this may partially explain the marked superiority of L- or DL-methionine over DL-α-OH-M when purified L-amino acid diets are fed (5). To our knowledge, the absorption velocity of α-keto-M is not known. In a recent study from our laboratory (unpublished data), individual chicks fasted for 16 hr were fed exactly 4 g of a diet containing either 1.075% α-keto-M or 0.952% DL-α-OH-M. Blood samples taken 0, 20, and 40 min postprandial revealed plasma free methionine concentrations of 5, 18, and 27 μg/ml, respectively, for chicks fed α-keto-M, and 5, 11, and 13 μg/ml, respectively, for chicks fed DL-α-OH-M. This suggests, therefore, either that α-keto-M is absorbed more rapidly than DL-α-OH-M or that its conversion to methionine after absorption is more rapid.

Summary. Male crossbred chicks were fed crystalline amino acid diets to evaluate the sulfur amino acid activity of glutathione, DL-α-OH-methionine (DL-α-OH-M), and α-keto-methionine (α-keto-M). Relative to L-cystine at isosulfurous levels, glutathione had a cystine-sparing value of 100%. Chicks fed α-keto-M gained 83% as fast as those fed an equal sulfur contribution from L-methionine, and those fed DL-α-OH-M gained 53% as fast. Addition of 1% glutamine to the purified diet failed to enhance

the efficacy of DL-α-OH-M, indicating that glutamine is not a limiting dietary factor in the conversion of DL-α-OH-M to L-methionine.

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