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Journal of Nutritional Biochemistry 12 (2001) 274–284

THE JOURNAL OF Nutritional Biochemistry

Dietary supplements of mixtures of indispensable amino acids lacking threonine, phenylalanine or histidine increase the activity of hepatic threonine dehydrogenase, phenylalanine hydroxylase or histidase, respectively, and prevent growth depressions in chicks caused by dietary excesses of threonine, phenylalanine, or histidine☆

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Received 18 July 2000; received in revised form 4 January 2001; accepted 9 January 2001

Abstract

Experiments were carried out to determine whether the addition of a mixture of indispensable amino acids (IAA) lacking in threonine, phenylalanine or histidine, respectively, to a nutritionally complete diet would increase the hepatic activities of the rate-limiting enzymes for catabolism of threonine, phenylalanine or histidine and prevent the adverse effects of the amino acid on growth when the dietary level of the amino acid is excessive. Week old Leghorn chicks were fed semi-purified diets containing 19% crude protein to which were added no IAA supplement or 10% crude protein from an IAA mix and 5 graded levels of either L-threonine, L-phenylalanine or L-histidine in a 2×5 factorial arrangement of treatments. Each amino acid was investigated in a separate experiment involving four replicate pens (seven chicks each) per diet. Weight gains and feed consumptions were determined on the fourteenth day of each experiment. The groups receiving no excess, and 1.0% or 2.0% excesses of amino acids were sampled on the fifteenth day for enzyme activities and plasma amino acid concentrations. Weight gain and/or feed consumption were lower, and plasma concentrations of threonine, phenylalanine and histidine were higher, in chicks receiving 1.5 to 2.0% dietary additions of threonine, phenylalanine, and histidine, respectively, than in chicks that did not receive these amino acids. Chicks that received the amino acids in diets that also contained the IAA supplement had better growth and feed consumption, lower plasma concentrations of threonine, phenylalanine or histidine, higher plasma concentrations of other indispensable amino acids, and higher activities of threonine dehydrogenase, phenylalanine hydroxylase, and histidase than chicks receiving excess amino acids in the absence of IAA supplements. We conclude that the dietary level of protein, not the dietary level of individual amino acids, is the primary determinant of the activity of amino acid degrading enzymes in liver. The increased activity of these enzymes may be the mechanism by which dietary protein alleviates the adverse effects of excessive levels of individual amino acids. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Chicks; Dietary protein; Amino acid excess; Amino acid catabolism

1. Introduction

Dietary concentrations of individual amino acids that greatly exceed the requirement can have a negative impact on growth and feed intake of an animal [1]. Raising the protein content of the diet has been reported to ameliorate this impact in rats [2–6]. Positive relationships between dietary protein concentration and the hepatic activities of enzymes of catabolism of tyrosine (Tyr) [5,7], phenylalanine (Phe) [8], histidine (His) [9,10], branched-chain amino acids [11,12], threonine (Thr) [13,14], serine (Ser) [7], cysteine (Cys) [15], glycine (Gly) [16,17], and ornithine (Orn) [18] have been reported.

☆This research project was supported in part through Hatch project number 157405.

Presented in part at Experimental Biology 2000, April 15–18, San Diego, CA [Keene, J.C. and Austic, R.E. Dietary additions of mixtures of indispensable amino acids (IAA) increase the activities of hepatic threonine (Thr) dehydrogenase, phenylalanine (Phe) hydroxylase, and histidase, and prevent the depressions of growth and feed intake of chickens receiving excess dietary Thr, Phe, or histidine (His), respectively. FASEB J. 14(4):A93].

Abbreviations used: IAA, indispensable amino acids; CP, crude protein.

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PII: S0955-2863(01)00139-5

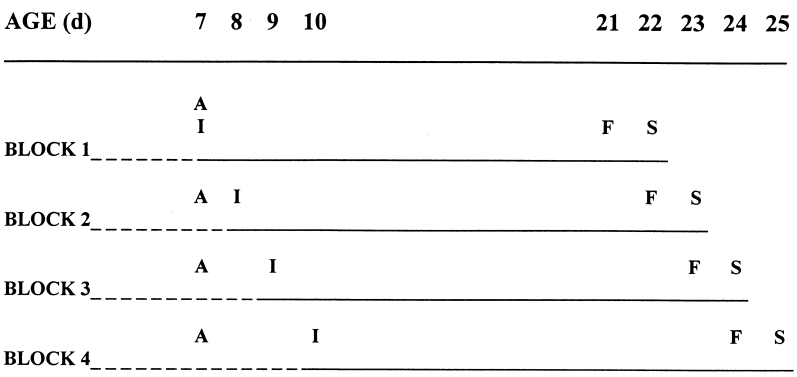

Fig. 1. Experimental design. In each experiment chicks were fed a practical diet until 7 days of age at which time all chicks were assigned (A) by weight, 7 chicks per cage, to brooder cages. Each experiment involved a 2 × 5 factorial arrangement of treatments in four blocks (by day of age). The ten experimental diets were randomly assigned to one cage each on days 7, 8, 9 and 10. Chicks and the initial supply of feed for each cage in blocks 1 through 4 were weighed (I) on days 7 through 10, respectively. Chicks and the feed remaining for each cage were weighed (F) on days 21 through 24, respectively (14 days of experiment) and blood and liver samples (S) were taken on days 22 through 25 (15 days of experiment) in blocks 1 through 4, respectively. Blood and liver samples were obtained from 3 chicks per cage only for chicks that received the 0, 1.0 and 2.0% dietary additions of the amino acid under investigation. ——, practical diet; -------- , experimental diets.

In a model of amino acid imbalance in which the imbalance is produced by the addition of a mixture of indispensable amino acids (IAA) lacking one indispensable amino acid [1], the imbalancing mixture was reported to increase the activity of threonine dehydrogenase in chicks and rats [19,20], branched-chain keto acid dehydrogenase in chicks [21], and histidase in rats [22] that received diets containing IAA lacking Thr, isoleucine (Ile), and His, respectively. These findings suggest that the effect of protein on the activity of first-limiting enzyme of the catabolism of an amino acid may be independent of the dietary concentration of the amino acid.

The present study was carried out to determine the effects of dietary excesses of individual amino acids and dietary supplements of mixtures of IAA lacking the amino acids on growth, concentrations of amino acids in plasma, and hepatic activities of enzymes of amino acid metabolism. The amino acids and respective enzymes of interest were Thr and threonine dehydrogenase (EC 1.1.1.103), Phe and phenylalanine hydroxylase (EC 1.14.16.1), and His and histidase (L-histidine ammonia lyase, EC 4.3.1.3).

2. Materials and methods

Male White Leghorn chicks were raised on a practical diet for one week after hatching. Chicks were housed at 25 chicks per pen in thermostatically-controlled battery brooder cages (26" × 39") with free access to water and feed. Room lights were timer-controlled for 16 hr of light per day. At the start of each experiment, the chicks were sorted by weight and individuals with exceptionally high or low body weights were excluded from the study. The remaining chicks were randomly allocated to 13" × 39" brooder cages with 7 chicks per pen in the same room and with the same light cycle. Experimental diets were randomly allocated to one pen per diet in each of four blocks (Fig. 1). Blocking was necessary to facilitate timely sample collection and analysis. Free access to food and water was continued for 15 days. All animal procedures were approved by the Institutional Animal Care and Use Committee of Cornell University.

2.1. Diet preparation and analysis

A nutritionally-adequate semipurified basal diet (Table 1) containing 19% crude protein (CP) was used in each experiment. Soy protein isolate provided the major portion of the CP, but L-cystine, L-methionine, L-threonine and L-tryptophan were added to the basal diet to satisfy the minimum requirement of chicks for these nutrients [23]. A factorial arrangement of treatments, 2 levels of IAA mixture and 5 levels of amino acids, was used. Indispensable amino acids (IAA), not including Thr in Experiment 1, Phe in Experiment 2 or His in Experiment 3, were added at the same relative levels as contained in soy protein isolate to increase the CP content of the diet by 10% over that of the basal diet. Threonine (in Experiment 1), Phe (in Experiment 2), or His (in Experiment 3) was then added at 0.5%, 1.0%, 1.5%, and 2.0% of the diets to diets unsupplemented and supplemented with IAA. L-Glutamic acid was used to balance the nitrogen levels across the diets within the IAA unsupplemented and supplemented groups and glucose monohydrate was used to equalize the final weights. All amino acids except lysine HCl, threonine and tryptophan were pharmaceutical grade. Feed grade L-lysine HCl, L-threonine, and L-tryptophan were of 98.5%, 98.5% and 98% purity, respectively. The basal diets of Experiments 1, 2 and 3 contained (by analysis) 0.68% Thr, 0.76% Phe, and 0.38% His, respectively.

Samples of all diets were analyzed in duplicate for crude protein content by the Kjeldahl method for a Labconco hood

Table 1
Composition of diets[1]

Ingredients	Basal diet (Expt. 1, 2, 3)	Basal + IAA (Expt. 1)	Basal + IAA (Expt. 2)	Basal + IAA (Expt. 3)
	g/100 g diet			
Soy protein isolate[2]	18.20	18.20	18.20	18.20
Glucose · H_2O[3]	59.82	47.66	47.99	46.85
Cellulose[4]	3.00	3.00	3.00	3.00
Corn oil[5]	4.00	4.00	4.00	4.00
Vitamin premix[6]	1.20	1.20	1.20	1.20
Mineral premix[7]	6.59	6.59	6.59	6.59
Amino acid supplement[8]	0.66	0.66	0.66	0.66
L-Arginine	–	1.74	1.74	1.74
L-Cystine	–	0.13	0.13	0.13
L-Histidine	–[9]	0.55	0.55	–[9]
L-Isoleucine	–	1.20	1.20	1.20
L-Leucine	–	1.72	1.72	1.72
L-Lysine · HCl	–	1.43	1.43	1.43
L-Methionine	–	0.21	0.21	0.21
L-Phenylalanine	–[10]	1.12	–[10]	1.12
L-Threonine	–[11]	–[11]	0.86	0.86
L-Tryptophan	–	0.21	0.21	0.21
L-Tyrosine	–	0.81	0.81	0.81
L-Valine	–	1.15	1.15	1.15
L-Glutamic acid[12]	6.53	7.39	7.32	7.89
$NaHCO_3$[13]	–	1.03	1.03	1.03

[1] The variable levels of threonine, phenylalanine, and histidine are indicated in Footnotes 9, 10, and 11.
[2] Approximately 90% protein. USB, Cleveland, OH 44128.
[3] Best Foods, formerly CPC International, Amman, Jordan.
[4] Solka Floc 200, F S & D Corp., Urbana, OH 43078.
[5] SYSCO Corp., Houston, TX 77077.
[6] Provided the following in mg/100 g diet: thiamin-HCl, 1.5; riboflavin, 1.5; nicotinic acid, 5.0; folic acid, 0.6; pyridoxine-HCl, 0.6; biotin, 0.06; d-Ca-pantothenate, 2.0; menadione sodium bisulfite, 0.15; choline chloride, 285; vitamin B_{12}, 0.002; butylated hydroxytoluene, 10; dl-α-tocophery acetate, 10; retinyl palmitate, 0.7; cholecalciferol, 0.7; glucose H_2O, 881.
[7] Provided the following in g/100 g diet: $CaHPO_4 2H_2O$, 2.07; $CaCO_3$, 1.48; KH_2PO_4, 1.0; $NaHCO_3$, 0.3; KCl, 0.1; $KHCO_3$, 0.64; NaCl, 0.6; $MnSO_4H_2O$, 0.035; $FeSO_4 7H_2O$, 0.05; $MgSO_4$, 0.3; KIO_3, 0.0002; $CuSO_4 5H_2O$, 0.003; ZnO, 0.01; $CoCl_2 6H_2O$, 0.00017; $NaMoO_4 2H_2O$, 0.00083; Na_2SeO_3, 0.00002.
[8] Contained, in mg/100 g diet: L-cystine, 0.27, L-methionine, 0.19, L-threonine, 0.15, and L-tryptophan, 0.05. This supplement was used to ensure the amino acid adequacy of the basal diet.
[9] In experiment 3, the diet contained no added histidine or 0.5, 1.0, 1.5 or 2.0% L-histidine added at the expense of glucose monohydrate and glutamic acid to make diets isonitrogenous.
[10] In experiment 2, the diet contained no added phenylalanine or 0.5, 1.0, 1.5 or 2.0% L-phenylalanine added at the expense of glucose monohydrate and glutamic acid to make diets isonitrogenous.
[11] In experiment 1, the diet contained no added threonine (except the amount in the amino acid supplement[8]) or 0.5, 1.0, 1.5 and 2.0% L-threonine added at the expense of glucose monohydrate and glutamic acid to make diets isonitrogenous.
[12] The differing amounts of glutamic acid for Experiments 1, 2 and 3 were used to maintain equal nitrogen contents of the three basal + 10% IAA diets.
[13] Added to neutralize the acid from L-lysine · HCl.

system [24] and for dry matter content by drying to constant weight in forced-air convection oven at 90°C. Samples of IAA unsupplemented diets containing no added amino acid or 1.0% or 2.0% amino acid were prepared for amino acid analysis in duplicate by subjecting them to acid hydrolysis using the method of Krick et al. [25] with the following modification; lipids were extracted from the diets prior to the evaporation step by rinsing the aliquot three times with 2 ml hexane. Norleucine was added prior to hydrolysis as an internal standard. Basal diets were analyzed for amino acids by ion exchange HPLC using a Beckman System Gold (Beckman Coulter, Inc., Fullerton, CA 92834) with three-step lithium buffer gradient, post-column ninhydrin reaction, and with detection at 560 nm.

2.2. Tissue preparation and enzyme assays

Fifteen days after the start of the feeding trial, three chicks from each pen were selected at random for blood and tissue sampling from the basal groups and the groups fed the basal diets supplemented with either 1.0% or 2.0% of the experimental amino acid. The remaining chicks were euthanized by CO_2 gas. The selected chicks were weighed as a group. One-half ml samples of blood were drawn by cardiac

puncture with a 1 ml heparinized syringe and 22 × 1.5" needle. The blood samples were pooled per pen and maintained on ice. The chicks were subdued with CO_2 gas and euthanized by cervical dislocation. The livers were collected and covered with 0.25 M sucrose (pH 7.4) in beakers on ice, one breaker per pen.

Blood samples from all three experiments were prepared for plasma amino acid analysis as described by Davis and Austic [20]. Plasma amino acid concentrations were obtained by HPLC as previously described.

Liver samples collected for Experiment 1 were prepared as described by Davis and Austic [20]. Liver mitochondria were isolated using the method of Schneider and Hogeboom [26]. The threonine dehydrogenase assay, based on the measurement of two alternative products, glycine and aminoacetone, followed the method of Bird et al. [27] with modifications by Davis and Austic [20]. Glycine was determined by HPLC as previously described [20], using taurine as the internal standard. Liver samples collected for Experiment 2 were prepared as described by Powell et al. [28]. The supernatant was collected and assayed for phenylalanine hydroxylase activity by the method of McGee et al. [29] with modifications by Powell et al. [28]. Tyrosine was determined by the method of Udenfriend and Cooper [30] with modifications by Nielsen [31] using a wavelength of 450 nm.

Liver samples collected for Experiment 3 were prepared for centrifugation prior to measurement of histidase activity as described by Powell et al. [28] with the following modification; samples were homogenized with four parts 0.25 M sucrose at pH 7.4. Samples were centrifuged at 37,000 rpm (105,000 × g) for one hour at 4°C in a Beckman L-60 ultracentrifuge with 70Ti rotor (Beckman Instruments, Inc., Palo Alto, CA 94304). Urocanic acid production over 10 min was determined by the method of Schirmer and Harper [10] using a wavelength of 277 nm. Urocanase activity was measured separately by replacing the L-histidine in the preparation with purified water (Milli-Q System, Millipore Corp., Bedford, MA 01730), and adding enough of a 240 μM solution of urocanic acid to bring the starting concentration close to that of the ending concentration obtained during histidase analysis of the individual sample. The urocanic acid production during the histidase assay was corrected for the loss of urocanic acid due to urocanase activity.

The protein contents of the enzyme preparations in all experiments were determined by the colorimetric method of Lowry et al. [32] and measured with a Hitachi U-2000 spectrophotometer (Hitachi Instruments, Inc., Danbury, CT 06810) at 660 nm.

2.3. Statistical analysis

Data were analyzed using the general linear model procedure of MINITAB 11 for Windows (Minitab, Inc., State College, PA 16801). Natural log transformations were used prior to statistical analysis when unequal variances were detected. The model included amino acid (Thr, Phe or His)

Table 2
Influence of dietary threonine (Thr) and IAA on chick growth, feed consumption, feed utilization, threonine dehydrogenase activity and plasma threonine concentration

Dietary supplement		Weight gained[2]	Feed consumed[2]	F/G[1]	Threonine dehydrogenase activity	Plasma threonine[3]
Threonine, %	IAA	g/chick day			nmol/(15 min · mg mitochondrial protein)	μmol/L
−	−	11.1	21.1	1.90	6.90	381
0.5	−	11.0	19.8	1.80	−	−
1.0	−	10.8	20.6	1.89	8.60	5286[†]
1.5	−	10.2	18.4[†]	1.81	−	−
2.0	−	9.1[†]	17.3[†]	1.91	11.51[†]	12428[†]
−	+	10.7	19.4	1.82	30.77[§]	98
0.5	+	11.3	19.8	1.75	−	−
1.0	+	11.5	21.5	1.86	29.68[§]	1617[§]
1.5	+	11.5[§]	19.1	1.67	−	−
2.0	+	11.7[§]	20.7[§]	1.77	26.75[§]	1695[§]
Pooled SEM		0.23	0.55	0.05	0.10[4]	608
Source of variation[5]						
Thr		*	**	*	ns	***
IAA		***	ns	**	***	***
Thr x IAA		***	***	ns	*	***

[1] Grams feed consumed/g weight gained.
[2] Mean of four pens of seven chicks each, one pen from each block.
[3] Mean of four pens, three chicks pooled from each pen, one pen from each block.
[4] Pooled SEM of data after natural log transformation,
[†] Significantly ($P < 0.05$) different from chicks fed the diet with no added threonine within the same protein level.
[§] Significantly ($P < 0.05$) different from chicks fed the diet with the same added threonine level at the lower level of protein.
[5] *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

Table 3
Plasma concentrations of amino acids other than threonine (Thr) in chicks in Experiment 1

IAA	−			+			SEM	Source of variation		
Threonine, (% added)	0	1.0	2.0	0	1.0	2.0		Thr	IAA	Thr x IAA
	μmol amino acid/L plasma									
Aspartate	85	77	86	54	52	41	9.1	ns	***	ns
Serine	513	709	1000†	372	445	440§	111.9	**	***	*
Asparagine	238	303	317	134	151§	122§	19.0	ns	***	ns
Glutamate	350	281	233	204	188	174	20.0	ns	**	ns
Glutamine	576	561	601	443	471	455	40.2	ns	**	ns
Proline	157	251	309†	176	175	156§	23.2	*	**	**
Glycine	305	520†	782†	188	299§	328§	39.2	***	***	**
Alanine	1051	1065	1100	494	481	464	0.09[1]	ns	***	ns
Valine	103	130	146	306	333	248	24.4	ns	***	ns
Cystine	30	40	49	32	29§	28§	3.0	ns	**	**
Methionine	44	47	52	55	56	38	2.7	ns	ns	−
Isoleucine	85	97	105	155	172	127	16.4	ns	**	ns
Leucine	104	113	119	214	227	169	18.9	ns	***	ns
Tyrosine	100	114	109	196	205	174	12.8	ns	***	ns
Phenylalanine	66	77	79	123	126	97	11.0	ns	***	ns
Lysine	177	167	172	538	491	405	58.6	ns	***	ns
Histidine	99	97	101	140	144	104	13.9	ns	*	ns
Arginine	272	312	312	346	369	282	35.8	ns	ns	−

† Significantly ($P < 0.05$) different from chicks fed the diet wiht no added threonine within the same protein level.
§ Significantly ($P < 0.05$) different from chicks fed the diet with the same added threonine level at the lower level of protein.
[1] Pooled SEM obtained from data after natural log transformation.

level, IAA level, block, and interaction between amino acid level and IAA level. The model included interactions between amino acid level and block and between IAA level and block only when these terms were significant at $P < 0.05$. When significant ($P < 0.05$) interactions of amino acid and IAA occurred, pairwise comparisons were made using the Bonferroni procedure with a family confidence interval of 95%.

3. Results

3.1. Experiment 1

Chicks fed the largest excess of Thr in the diets unsupplemented with IAA had lower weight gain, feed consumption and over 30-fold higher plasma Thr concentrations than chicks fed the diets that did not contain excess Thr (Table 2). Those receiving excess Thr in diets containing IAA had weight gains, feed consumptions, feed conversion ratios, and threonine dehydrogenase activities that were not significantly different, and plasma Thr concentrations that were higher, than those of chicks fed the diet containing IAA but no supplemental Thr. Chicks fed the diets containing excess Thr plus the IAA supplement had weight gains and feed consumptions that were not different from those of chicks fed the diet without added Thr or IAA. Threonine dehydrogenase activity tended to be higher in chicks fed the diets lacking the IAA supplement and containing excess Thr than in chicks fed the diet lacking the IAA supplement and not containing excess Thr. Plasma Thr concentrations were markedly lower in chicks that received excess Thr plus IAA than in chicks that received excess Thr but no IAA supplement.

The concentrations of serine (Ser), proline (Pro) and glycine (Gly) in plasma were higher in chicks that received excess Thr without IAA than in chicks that did not receive excess Thr or the IAA supplement (Table 3). Chicks that received the IAA supplement had lower plasma concentrations of aspartate (Asp), Ser, asparagine (Asn), glutamate (Glu), glutamine (Gln), Pro, Gly, alanine (Ala) and Cys and higher concentrations of Ile, leucine (Leu), tyrosine (Tyr), Phe, lysine (Lys) and His than chicks that did not receive the dietary supplement of IAA. There were significant interactions: chicks that received excess Thr had higher Ser, Pro, and Gly concentrations than chicks not receiving excess Thr only when the diet did not contain the IAA supplement. Cys tended to be higher in chicks fed excess Thr, but only when the diets were not supplemented with IAA. There were no significant effects of dietary treatment on plasma arginine (Arg) and methionine (Met) concentrations.

3.2. Experiment 2

Chicks fed diets containing excess Phe (1.5 and 2.0%) but without IAA had lower weight gains and feed consumptions and higher feed conversion ratios than chicks fed the diet containing no added Phe without IAA (Table 4). Weight gains, feed consumptions and feed conversion ratios of chicks fed diets containing excess Phe with IAA supplement were not significantly different from those of chicks

Table 4
Influence of dietary phenylalanine (Phe) and IAA on chick growth, feed consumption, feed utilization, phenylalanine hydroxylase activity and plasma phenylalanine concentration

Dietary supplement		Weight gained[2]	Feed consumed[2]	F/G[1]	Phenylalanine hydrolylase activity[3]	Plasma phenylalanine[3]
Phenylalanine, %	IAA	g/chick day			nmol Tyr/(20 min. · mg cytosolic protein)	μmol/L
−	−	11.7	22.1	1.89	6.89	87
0.5	−	11.4	22.6	1.98	−	−
1.0	−	10.9	21.5	1.96	6.93	210†
1.5	−	9.1†	19.7†	2.15†	−	−
2.0	−	6.7†	17.3†	2.59†	4.69	778†
−	+	12.2	20.8§	1.71	10.81	58
0.5	+	11.9	21.3§	1.78	−	−
1.0	+	11.6	21.0	1.80	9.66	1.31†
1.5	+	11.8§	21.5§	1.82§	−	−
2.0	+	11.5§	21.5§	1.86§	8.30	217§,†
Pooled SEM		0.22	0.25	0.05	1.13	0.15[4]
Source of variation[5]						
Phe		***	***	***	ns	***
IAA		***	**	***	**	***
Phe x IAA		***	**	***	ns	*

[1] Grams feed consumed/g weight gained.
[2] Mean of four pens of seven chicks each, one pen from each block.
[3] Mean of four pens, three chicks pooled from each pen, one pen from each block.
[4] Pooled SEM of data after natural log transformation.
† Significntly ($P < 0.05$) different from chicks fed the diet with no added phenylalanine within the same protein level.
§ Significantly ($P < 0.05$) different from chicks fed the diet with the same added phenylalanine level at the lower protein level.
[5] *, $P < 0.05$; ** , $P < 0.01$; ***, $P < 0.001$; ns not significant.

receiving the diet with IAA containing no added Phe or the diet containing no added Phe or IAA. Phenylalanine hydroxylase activity was higher in chicks that received the IAA supplement than in those that did not receive the supplement, irrespective of dietary Phe level. Plasma Phe concentrations were lower in chicks that received diets containing IAA than in those fed diets without the IAA supplement.

The plasma concentrations of Ser were higher and Glu were lower in chicks that received diets containing excess Phe without IAA supplement than in chicks that received the diet without excess Phe and without IAA supplement (Table 5). Tyr concentrations were higher in chicks that received excess dietary Phe. Chicks that received the IAA supplement had lower Asp, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Cys and Tyr, and higher concentrations of Thr, Val, Ile, Leu and Lys, than chicks that did not receive the IAA supplement. There were significant interactions of Phe and IAA for Tyr and Cys: the effect of dietary Phe on plasma Tyr was less when the diet contained the IAA supplement, and chicks receiving excess Phe without IAA tended to have higher Cys, and those receiving excess Phe with IAA tended to have lower plasma Cys concentrations, than chicks that received the diets containing no added Phe. There were no significant effects of treatments on plasma Met, His and Arg concentrations.

3.3. Experiment 3

Chicks fed diets having the highest levels of His had lower body weight gains and higher feed conversion ratios than chicks receiving the diets that did not contain added His (Table 6). Chicks receiving IAA had lower feed consumption and feed conversion ratios than chicks that did not receive IAA. Chicks that received diets containing excess His plus the IAA supplement had weight gains and feed conversion ratios that did not differ from those of chicks fed the diet without added His or IAA. Chicks receiving excess His or supplemental IAA had higher histidase activity than chicks that did not receive excess His or IAA supplement. Chicks that received diets containing excess His had higher plasma concentrations of His than chicks that did not receive excess His, but the effect of dietary His on plasma His concentration was markedly less when the diets contained the IAA supplement.

The concentration of plasma Ser was higher, and concentrations of Ala, Tyr and Lys were lower, in chicks that received diets containing excess His (Table 7). Chicks that received IAA had lower plasma concentrations of Ser, Asn, Glu, Gln, Pro, Gly, and Ala, and had higher concentrations of Thr, Val, Met, Ile, Leu, Tyr, Phe, Lys, and Arg than chicks that did not receive IAA. Two interactions were detected: the effects of dietary His on plasma Ser were

Table 5
Plasma concentrations of amino acids other than phenylalanine (phe) in chicks in Experiment 2

IAA	0			+			SEM	Source of variation		
Phenylalanine, (% added)	0	1.0	2.0	0	1.0	2.0		Phe	IAA	Phe x IAA
	μmol amino acid/L plasma									
Aspartate	154	118	209	119	80	85	30.2	ns	**	ns
Threonine	593	450	465	1071	937	813	79.4	ns	***	ns
Serine	596	670	724	439	486	478	29.3	*	***	ns
Asparagine	234	236	253	112	149	122	25.0	ns	***	ns
Glutamate	466	362	317	298	261	253	32.3	*	**	ns
Glutamine	703	649	677	445	470	514	46.8	ns	***	ns
Proline	241	249	306	205	234	203	19.7	ns	**	ns
Glycine	409	398	428	305	310	313	22.7	ns	***	ns
Alanine	982	951	893	548	620	454	0.08[1]	ns	***	ns
Valine	119	130	173	399	393	365	34.9	ns	***	ns
Cystine	42	44	55	43	37	36[§]	2.9	ns	**	**
Methionine	57	54	59	69	66	63	9.0	ns	ns	ns
Isoleucine	102	106	117	205	203	184	0.13[1]	ns	***	ns
Leucine	130	134	154	277	273	251	0.11[1]	ns	***	ns
Tyrosine	115	350[†]	651[†]	100	183	295[†,§]	26.0	***	***	***
Lysine	244	261	311	610	584	466	66.1	ns	***	ns
Histidine	138	133	170	176	157	147	23.4	ns	ns	ns
Arginine	403	307	300	406	382	376	55.0	ns	ns	ns

[†] Significantly ($P < 0.05$) different from chicks fed the diet with no added phenylalanine within the same protein level.
[§] Significantly ($P < 0.05$) different from chicks fed the diet with the same added phenylalanine level at the lower level of protein.
[1] Pooled SEM obtained from data after natural log transformation.

significantly smaller, and those on Tyr were greater, when the diet contained supplemental IAA. No significant effects of treatment on plasma Asp, Pro, and Cys were detected.

4. Discussion

The addition of 2.0% Thr, Phe or His to a nutritionally complete diet significantly reduced growth in chicks over unsupplemented controls by 18%, 43% and 16%, respectively. When added to the amount of each amino acid already present in the basal diet, the 2.0% addition raised the Thr content of the diet by a factor of 4, the Phe content by a factor of 5, and the His content by a factor of 9 over the requirements as listed by the NRC [23]. Of these three amino acids, Harper et al. [1] ranked His as the most potent growth inhibitor of weanling rats followed closely by Phe, and Thr was listed as the least likely to inhibit growth of all indispensable amino acids. Edmonds and Baker [33] fed 4.0% excesses of individual amino acids to chicks starting with a 23% protein basal diet, and found that Thr depressed growth by 29%, His by 50% and Phe by 79%. Given the more substantial amino acid excess in their study, it is not surprising that they demonstrated more severe growth depressions than found in this study. However, the fact that His fed at 9 times the requirement had essentially the same effect on the chicks as Thr fed at 4 times the requirement was unexpected. While the exact order of severity of effects among the amino acids varies with the study design, it is generally accepted that His is one of the most toxic amino acids based on growth depression when fed in excess [34–37].

Increasing the dietary content of either Thr or His significantly increased the plasma concentration of that amino acid when chicks were fed the protein-adequate basal diet, but not when Thr or His were added to the diets supplemented with the IAA mixture lacking the amino acid. The plasma concentration of Phe increased when Phe was added to the IAA-supplemented diet, but not to the same degree as it did when Phe was added to the basal diet. Supplementing the diet with IAA significantly reduced the plasma concentration when 2.0% of any of the three amino acids was added. Glycine is a product of Thr metabolism, and its concentration pattern followed that of Thr when Thr was added to the diets. Similarly, the concentration of Tyr, a product of Phe metabolism, closely followed that of Phe in the experiment involving Phe.

The effect on other plasma amino acids was variable, but a few trends can be noted. The plasma concentration of the amino acids contained in the IAA mixtures tended to be higher in chicks fed the mixtures. Conversely, the plasma concentration of dispensable amino acids tended to decrease with the addition of the IAA mixture. Although Glu was used to balance the added nitrogen from the investigated amino acids, and thus dietary content decreased with increasing Thr, Phe or His, there was no overall trend in plasma concentration of either Glu or Gln. Interestingly, plasma Ser concentration increased with increasing dietary Thr, Phe, and His in chicks fed the IAA unsupplemented diets. Working with rats, Moundras et al. [38] observed a

Table 6
Influence of dietary histidine (His) and IAA on chick growth, feed consumption, feed utilization, histidase activity and plasma histidine concentration

Dietary supplement Histidine %	IAA	Weight gained[2] g/chick day	Feed consumed[2]	F/G[1]	Histidase activity[3] nmol Urocanic acid/(15 min · mg mitochondrial protein)	Plasma histidine[3] μmol/L
−	−	11.6	24.1	2.08	0.82	119
0.5	−	12.3	24.4	1.98	—	—
1.0	−	11.0	23.3	2.12	0.93	398
1.5	−	10.9	23.8	2.18	—	—
2.0	−	9.7	21.6[†]	2.24	1.16	1806[†]
−	+	12.4	22.0[§]	1.77	1.46	58
0.5	+	12.4	22.2[§]	1.79	—	—
1.0	+	11.9	22.1	1.85	1.79	244
1.5	+	11.1	22.0[§]	2.00	—	—
2.0	+	11.7	22.7	1.94	1.81	461[§]
Pooled SEM		0.04[4]	0.02[4]	0.05	0.06	147
Source of variation[5]						
His		**	ns	***	**	***
IAA		**	***	***	***	**
His x IAA		ns	**	ns	ns	**

[1] Grams feed consumed/g weight gained.
[2] Mean of four pens of seven chicks each, one pen from each block.
[3] Mean of four pens, three chicks pooled from each pen, one pen from each block.
[4] Pooled SEM of data after natural log transformation.
[†] Significantly ($P < 0.05$) different from chicks fed the diet with no added histidine within the same protein level.
[§] Significantly ($P < 0.05$) different from chicks fed the diet with the same added histidine level at the lower level of protein.
[5]*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant.

significant increase in plasma Glu and Gln concentration with a 7.2% supplement of Glu or Gln added to an adequate protein diet. They also reported increased fractional extraction of Thr, Ser, Gly and Ala by liver and an approximate two-fold increase of hepatic serine (threonine) dehydratase activity. The amount of Glu added to their diets was large compared to the amount used to balance nitrogen in the present study.

In all three experiments, supplementing the diet with a mixture of IAA lacking the experimental amino acid (thereby raising the crude protein content of the diet from 19% to 29%) alleviated the negative effect of feeding the amino acid in excess. Weight gain, feed consumption and feed conversion of chicks fed the IAA-supplemented diet plus 2.0% of the experimental amino acid were equal to or better than those of the chicks fed the basal diet. This mitigating effect of increasing the IAA supplement is similar to effect observed in rodents when the dietary levels of actual proteins were increased [2–6,8]. It is not known whether the response to protein is a response only to the indispensable amino acids in the protein. If it is, the responses to 10% CP from IAA may be equivalent to that of 20% protein, assuming that proteins contain approximately 50% indispensable amino acids.

The mixture of IAA significantly increased the specific activity of the rate-limiting enzyme of catabolism in liver for each amino acid investigated, whereas the increase in dietary level of the amino acid had no detectable effect (Thr, Phe) or less (His) of an effect. The lack of effect of Thr on threonine dehydrogenase activity is consistent with an earlier observation in this laboratory [13] and with the observation of Watanabe et al. [39] that hepatic Thr catabolizing enzymes did not increase in response to increasing dietary Thr in mature female chickens. Sarwar et al. [40] found no effect of increasing dietary Thr on threonine dehydrogenase activity in rats. Watanabe et al. [41] subsequently reported that increasing dietary Thr did not affect liver enzyme activities in rats except for threonine aldolase which first decreased and then increased, and serine-hydroxymethyltransferase which decreased.

The IAA supplement increased histidase activity at all levels of added His. Similarly in rats, high dietary protein has been shown to increase hepatic histidase activity [42–44], and protein and an imbalancing mixture of amino acids has increased hepatic histidase mRNA concentrations [22, 44,45]. Unlike Thr and Phe, there was also an effect of His on histidase activity. This is in contrast with earlier research on rats [42–44], but consistent with the results of previous studies with chickens [46] and rainbow trout [47]. It is possible that His has a regulatory mechanism in addition to one(s) triggered by increasing dietary protein. Histidine, for example, may be involved in the initiation of the response by regulating preproglucagon mRNA levels [48].

The effects of protein may not be limited to indispensable amino acids. The decrease in plasma concentration of dispensable amino acids suggests a generalized effect of IAA on the metabolism of amino acids that were not included in the IAA mixture.

Studies of threonine dehydrogenase have indicated similar responses to protein and IAA supplements.[1] These ob-

Table 7
Plasma concentrations of amino acids other than histidine (his) in chicks in Experiment 3

IAA	−			+			SEM	Source of variation		
Threonine, (% added)	0	1.0	2.0	0	1.0	2.0		His	IAA	His x IAA
	μmol amino acid/L plasma									
Aspartate	113	85	67	79	71	76	0.26[1]	ns	ns	ns
Threonine	461	382	506	804	766	722	68.1	ns	***	ns
Serine	635	657	834[†]	461[§]	449[§]	492[§]	0.03[1]	**	***	*
Asparagine	182	218	277	132	144	145	49.8	ns	*	ns
Glutamate	465	542	581	433	425	410	54.1	ns	*	ns
Glutamine	342	455	610	334	372	336	53.2	ns	*	ns
Proline	280	224	259	176	235	173	39.8	ns	ns	ns
Glycine	403	317	346	298	286	271	25.0	ns	**	ns
Alanine	881	717	467	570	482	355	112.6	*	*	ns
Valine	133	99	105	374	346	302	0.15[1]	ns	***	ns
Cystine	30	40	39	40	36	36	3.9	ns	ns	ns
Methionine	51	40	46	72	70	59	9.5	ns	*	ns
Isoleucine	116	86	86	209	197	160	0.16[1]	ns	***	ns
Leucine	145	106	98	242	229	181	0.16[1]	ns	**	ns
Tyrosine	110	102	93	228[§]	189[†,§]	173[†,§]	5.7	**	***	*
Phenylalanine	80	79	102	130	127	116	16.1	ns	**	ns
Lysine	388	171	172	574	510	424	44.0	*	***	ns
Arginine	274	199	148	419	396	293	60.0	ns	**	ns

[†] Significantly ($P < 0.05$) different from chicks fed the diet with no added histidine within the same protein level.
[§] Significantly ($P < 0.05$) different from chicks fed the diet with the same added histidine level at the lower level of protein.
[1] Pooled SEM obtained from data after natural log transformation.

servations, the results of the present study, and those of Torres et al. [44], and Tovar et al. [45], suggest that the IAA supplement is similar to raising the protein level of the diet. Although at first it would seem logical that the dietary content and thus the plasma concentration of an amino acid would regulate the activity of its own pathway of catabolism, when natural selection is taken into account it is appropriate that total dietary protein would be the primary control mechanism. A feral animal rarely encounters a diet containing a single amino acid at a much higher concentration than all others, and when it does research has shown that intake is depressed or the diet is avoided [1,33,49]. Much more common would be the consumption of a diet that is either low or high in total protein content relative to the animal's needs. Likely, mechanisms would have evolved to allow the animal's metabolism to respond accordingly, efficiently utilizing protein if the content is low, or degrading excess amino acids if the content is high.

Understanding how this occurs is much more complicated. Evidence is growing that glucagon is one of the major regulatory factors in the response to a high-protein diet. In a study with human volunteers, Charlton et al. [50] separated the effects of insulin, glucagon and growth hormone in response to a dietary protein load, and showed that glucagon was responsible for clearance of excess amino acids and inhibition of protein synthesis. Kita et al. [51] showed that feeding chicks high-protein diets significantly decreased protein synthesis in breast muscle at the pretranslational level. Ip and Harper [3] observed that glucagon administration increased the activity of hepatic tyrosine aminotransferase, decreased plasma Tyr concentration, but had no effect on the decrease in weight gain caused by feeding rats excessive Tyr. Fisher et al. [52] reported that insulin antagonized glucagon stimulation of phenylalanine hydroxylase activity in *in vitro* studies with rat liver cells. Lee and Harper [53] and Morris et al. [54] reported that the administration of glucagon to rats increased the activities of histidase, urocanase and histidine-pyruvate aminotransferase. Alemán et al. [55] reported that glucagon administration to rats increased histidase activity at the pretranslational level.

Returning to the question of individual amino acids influencing enzymes involved in their catabolism, it should be noted that there are several well known examples of enzymes affected by their substrates. Increases in hepatic tryptophan pyrrolase activity in response to tryptophan [56], hepatic tyrosine transaminase in response to tyrosine [57], and hepatic and muscle branched-chain keto acid dehydrogenase in response to branched-chain keto acids [58] in the rat have been reported. The mechanisms differ: tryptophan reducing the rate of degradation of tryptophan pyrrolase [59], for example, and branched-chain keto acids allosterically inhibiting branched-chain keto acid dehydrogenase kinase activity [58]. In the chicken the effect of dietary histidine on hepatic histidase activity [46] as well as the effects of lysine on hepatic lysine-α-ketoglutarate reductase activity [60] and dietary arginine on renal arginase activity [61,62] have been reported. Individual amino acids also have been known to increase the activities of enzymes involved in the catabolism of other amino acids. The effect of tryptophan on hepatic threonine dehydratase, ornithine

transaminase and tyrosine transaminase in the rat [63,64] and the effects of lysine, tyrosine, histidine and phenylalanine on renal arginase activity in chickens [61,62] are well documented examples. The mechanisms by which these changes in enzyme activity occur are not known. Therefore, it is possible that the effects of dietary excesses of protein, mixtures of indispensable amino acids, and individual amino acids have some mechanisms in common.

At this time we can conclude only that dietary protein level appears to be a major determinant of the activities of the regulatory enzymes of amino acid catabolism in the chick when dietary levels of individual amino acids are limiting [19–22], adequate [5,7–18], or excessive. The increase in enzyme activity may account for the alleviation by protein of the growth depressions caused by excesses of individual amino acids.

Note

1. Austic, R.E., Keene, J.C., and Yuan, J.-H. (2000). Effect of dietary protein level on amino acid imbalance and toxicity. Proceedings of the Cornell Nutrition Conference for Feed Manufacturers (Rochester, NY), pp. 65–71.

Acknowledgments

The authors gratefully acknowledge the gifts of amino acids from BioKyowa, Inc., Chesterfield, MO and chickens from ISA Babcock, Inc. (Ithaca, NY) that were provided in support of this project. We also thank Barbara Smagner for her word processing and editorial help in the preparation of this report.

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