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Changes in hepatic branched-chain α-keto acid dehydrogenase activity in response to isoleucine imbalance in growing chickens

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Three experiments were carried out to determine the influence of isoleucine imbalance on hepatic branched-chain α-keto acid dehydrogenase (BCKAD) activity in growing chickens. An isoleucine imbalance was induced by adding a 5% imbalancing amino acid mixture to a basal diet that contained adequate concentrations of all indispensable amino acids except isoleucine, which was marginally adequate (0.64–0.76% of the diet). The imbalancing mixture caused depressions ($P < 0.05$) in feed intake and growth rate. The depression in feed intake appeared to occur prior to the depression in growth rate. The isoleucine concentration in plasma decreased ($P < 0.05$), but not consistently, among experiments in response to the imbalancing mixture of amino acids. Basal and total activities of hepatic BCKAD were increased ($P < 0.05$) 21% and 28%, respectively, within 24 hours in one experiment and were elevated ($P < 0.05$) 19% and 14%, respectively, at the end of the 13 days of a second experiment. The moisture, protein, and fat contents of whole body and liver were not affected by the imbalancing mixture of amino acids. It appears likely that broiler chicks did not adapt to the imbalanced diets because the depressed feed intake and growth rate and alterations in plasma isoleucine and hepatic BCKAD activity persisted through 13 days of experiment. The isoleucine requirement, expressed as percent of diet, was increased by the imbalancing mixture of amino acids, and the efficiency of isoleucine utilization for growth (grams of weight gain per milligram of isoleucine intake) was decreased in two of three experiments. These results suggest that BCKAD may have a play in the increased isoleucine requirement of broiler chicks under conditions of isoleucine imbalance. (J. Nutr. Biochem. 9:687–696, 1998) © *Elsevier Science Inc. 1998*

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Introduction

Amino acid imbalances refer to the deleterious effects that occur when a second-limiting amino acid or a mixture of amino acids lacking a particular limiting amino acid is supplemented in diets marginal in one or more indispensable amino acids.[1,2] In spite of variations in the conditions that have been used to induce amino acid imbalances, such as protein level in the diet, the extent of difference in total nitrogen content between basal and imbalanced diets, and kinds of amino acids used as imbalancing agents, the conspicuous common features of amino acid imbalances have been a decreased concentration of the limiting amino acid in blood,[3–5] depression of feed intake and weight gain,[5–7] and increased dietary content of the limiting amino acid needed to correct the imbalances.[7–9]

There is strong evidence that the decrease of limiting amino acid in plasma or altered ratio of limiting amino acid to total amino acids under conditions of amino acid imbalance is detected in the anterior prepyriform cortex of brain.[10,11] A decrease in the concentration of a limiting amino acid in specific regions of the brain is followed by behavioral effects, especially a decrease in feed intake.[10,11] This might be due in part to the competition between the limiting amino acid and the amino acids in the imbalancing mixture for transport from blood into brain.[4,11] However,

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the metabolic fate of a limiting amino acid has yet to be determined: What causes the blood level of limiting amino acid to decrease? One hypothesis is that imbalancing amino acids stimulate protein synthesis in liver, thereby depleting blood of the amino acids.[12,13] This hypothesis is supported by evidence that protein synthesis in liver is increased.[14–16]

Salmon[17] proposed that the catabolism of a limiting amino acid increased simultaneously with enhanced catabolism of the surplus of other amino acids added to the diet to produce an amino acid imbalance. An increase in the activity of hepatic threonine dehydrogenase, a major enzyme of threonine catabolism, recently has been observed in chickens and rats under conditions of threonine imbalance.[7,18] Therefore, it appears possible that changes in protein synthesis and catabolism of the limiting amino acid contribute to the metabolic consequences of amino acid imbalance.

If amino acid catabolism has a role in amino acid imbalances, then an increase in activity of enzymes responsible for the degradation of the first limiting amino acid should be evident in imbalances other than that of threonine. Branched-chain α-keto acid dehydrogenase (BCKAD), which regulates the irreversible degradation of branched-chain keto acids is believed to be the primary regulated enzyme of branched-chain amino acid catabolism.[19] BCKAD is inactivated by a kinase that, in turn, is allosterically controlled by the concentrations of branched-chain α-keto acids and possibly certain other α-keto acids.[20,21] Because the precursor amino acids are usually present in imbalancing mixtures of amino acids, we hypothesized that isoleucine or valine imbalances might be due in part to increased BCKAD activity.

The objectives of the present study were to determine whether an isoleucine imbalance could be induced in chickens using a diet containing an adequate level of protein and metabolizable energy, and a marginally adequate concentration of isoleucine, and to determine whether the activity of hepatic BCKAD is altered under these conditions.

Methods and materials

Cockerels of a Peterson male (Peterson Farms, Inc., Decatur, AR USA) and Hubbard female (Hubbard Farms, Inc., NH USA) cross from a commercial supplier (Moyer's Chicks, Inc., Quakertown, PA USA) were used in all experiments. The chicks were received from the hatchery within 2 days of hatching, housed in environmentally-controlled Petersime (Petersime Incubation Co., Gettysburg, OH USA) battery brooders with raised wire floors, and allowed free access to water and feed. The temperature of the brooders was 35°C and the animal facility in which the cages were located was maintained at approximately 22°C throughout all the experiments. Fifteen hours of lighting were provided from 7:00 AM to 10:00 PM. At the beginning of each experiment, equal weights of chicks in individual pens were achieved by weighing, assigning chicks to pens according to weight class, and discarding those with extreme weights. Pens of chicks were randomly assigned to all treatments. All animal protocols were approved by the Institutional Animal Care and Use Committee of Cornell University.

Experimental design

Experiment 1 was designed to induce an isoleucine imbalance by the addition of a mixture of amino acids to a basal diet that was marginally limiting in isoleucine. The basal diet contained 23.2% dietary crude protein and 3,016 kcal/kg of dietary metabolizable energy (*Table 1*). The isoleucine content was 0.7% of the diet, below the level of 0.8% estimated by the National Research Council[22] as the requirement of the broiler chicken through 3 weeks of age. However, this level was similar to the dietary concentration that maximized weight gain, feed consumption, and efficiency of feed utilization in a preliminary study using the same strain of chicks, diet, and housing conditions as in the present experiments (Park and Austic, unpublished observations). The basal diet was supplemented with L-amino acids to supply 120% of the estimated requirements of all indispensable amino acids, except isoleucine, according to the estimated requirements of the National Research Council.[23] The imbalancing mixture of amino acids is shown in *Table 2*. It contained molar equivalent amounts of each of the amino acids listed.

In Experiment 1, five replicate pens of four chicks weighing an average 43 g per chick were used. The basal diet and the basal containing the imbalancing mixture of amino acids (*Table 2*) were either unsupplemented or supplemented with 0.3% or 0.6% L-isoleucine. All diets were made isonitrogenous and isocaloric by adjusting the concentrations of L-glutamate, corn starch, and corn oil, respectively. Chicks were fed the experimental diets for 13 days. To determine the temporal response to the diets, chicks were weighed and feed intake was determined at 2, 4, 6, 8, 11, and 13 days. Blood was collected from two chicks per pen on the 13th day. After feed was withheld overnight, the remaining two chicks per replicate from treatments 1, 4, and 5 were sampled on the 14th day for analysis of body composition.

Experiment 2 was performed to determine whether the activity and activity state of liver BCKAD are changed under the condition of an isoleucine imbalance. Experimental treatments were composed of two diets: a basal diet (*Table 1*) similar to the diet used in Experiment 1 and the basal diet supplemented with the imbalancing mixture of amino acids (*Table 2*). The two diets were isonitrogenous. Chicks were fed a practical starter diet during the first 5 or more days prior to experiment. At the end of the fifth day, four replicate pens of five chicks were assigned randomly by weight to each of the two diets on 3 consecutive days. Body weights and feed intake were measured, and the activity of BCKAD was determined after 13 days of experiment with the four replicates being assayed on 4 consecutive days. In 1 day, two replicates per treatment were used for the assay of basal activity of BCKAD, and the other two replicates per treatment were used for assay of total BCKAD activity. Two chicks from each replicate were randomly selected for the liver to be pooled for determination of hepatic BCKAD activity.

Experiment 3 was designed to verify the effect of an isoleucine imbalance on BCKAD activity and to determine whether the change in enzyme activity would occur during the first day of the imbalance. If BCKAD is an important mediator of isoleucine imbalance, a rapid change of BCKAD activity would have to be manifested. In addition, isonitrogenous and nonisonitrogenous diets were used to determine the effect of dietary nitrogen content on the change of BCKAD activity. Dietary treatments were composed of four diets: two basal diets (*Table 1*) and the two basal diets supplemented isonitrogenously (to basal diet 1) or nonisonitrogenously (to basal diet 2) with the imbalancing mixture (*Table 2*) of amino acids. Each basal diet was fed to a large group of chicks for the first 5 days. At the end of the fifth day, two replicate pens of five chicks were distributed to each of four diets on 4 consecutive days; one replicate of each treatment was used for basal determination of BCKAD and the other for total activity after 1 day of feeding. After the fifth day, the remaining chicks were continued on their basal diets. The concentration of isoleucine in the basal diets and blood plasma were measured as described below.

Table 1 Composition of basal diets

Ingredients	Experiment 1 (g/kg)	Experiment 2 (g/kg)	Experiment 3 (g/kg)	
			Diet 1	Diet 3
Wheat, soft	625.0	625.0	625.0	534.3
Peanut meal (48% crude protein)	147.0	147.0	147.0	238.9
Amino acid supplement	46.3[1]	46.3[1]	46.3[1]	30.3[2]
Corn starch[3]	—	3.0	2.8	48.8
Corn oil[3]	27.7	31.2	31.4	33.4
Mineral mixture	53.0[4]	53.3[5]	53.3[5]	48.7[6]
Vitamin mixture[7]	10.0	10.0	10.0	10.0
Choline chloride (60% choline)	2.4	2.4	2.4	2.4
L-glutamic acid[3]	88.6	81.8	81.8	52.0
Composition[8]				
By calculation:				
Metabolizable energy, kcal/kg	3016	3039	3040	3023
Crude protein, %	23.0	22.6	22.6	22.7
Calcium, %	1.15	1.15	1.15	1.15
Phosphorus, available, %	0.52	0.52	0.52	0.52
By analysis:				
Crude protein, %	23.2	22.4	22.8	22.7
Isoleucine, %	0.69	0.73	0.75	0.76

[1]Supplied the following as L-form to provide 20% above the amino acid requirements estimated by National Research Council (NRC) (1994) except for lysine, which provided 20% above the requirement estimated by the (NRC) (1984): arginine, 0.46; cystine, 0.24; histidine, 0.12; leucine, 0.64; lysine, 0.99; methionine, 0.44; phenylalanine, 0.23; threonine, 0.57; tryptophan, 0.09; tyrosine, 0.25; valine, 0.45; isoleucine, 0.15. Lysine was in the form of lysine · HCl.
[2]Supplied the following in g/kg of diet: L-cystine, 1.9; L-histidine, 0.4; L-leucine, 4.3; L-lysine, 8.7; L-methionine, 4.2; L-phenylalanine, 0.5; L-threonine, 4.8; L-tryptophan, 0.5; L-tyrosine, 1.2; L-valine, 2.8; L-isoleucine, 1.0. Lysine was in the form of lysine · HCl.
[3]All treatments were made isonitrogenous and isocaloric by adjusting the concentration of L-glutamate, corn starch, and corn oil.
[4]Supplied the following in g/kg of diet; $CaHPO_4 \cdot 2H_2O$, 23.9; $CaCO_3$, 13.2; $MgSO_4 \cdot 7H_2O$, 6.1; NaCl, 2.5; Na_2CO_3, 2.1; $KHCO_3$, 4.6; $FeSO_4 \cdot 7H_2O$, 0.28; $MnSO_4 \cdot H_2O$, 0.22; ZnO, 0.050; $CuSO_4 \cdot 5H_2O$, 0.021; $Na_2MoO_4 \cdot 2H_2O$, 0.00084; $CoCl_2 \cdot 6H_2O$, 0.0017; KI, 0.00069; Na_2SeO_4, 0.00032.
[5]Supplied the following in g/kg of diet: $CaHPO_4 \cdot 2H_2O$, 20.5; $CaCO_3$, 20.0; $MgSO_4$, 3.0; NaCl, 2.5; Na_2CO_3, 2.1; $KHCO_3$, 4.6; $FeSO_4 \cdot 7H_2O$, 0.28; $MnSO_4 \cdot H_2O$, 0.22; ZnO, 0.05; $CuSO_4 \cdot 5H_2O$, 0.021; $Na_2MoO_4 \cdot 2H_2O$, 0.00084; $CoCl_2 \cdot 6H_2O$, 0.0017; KI, 0.00069; Na_2SeO_4, 0.00032.
[6]Supplied the following in g/kg of diet: $CaHPO_4 \cdot 2H_2O$, 20.0; $CaCO_3$, 18.7; $MgSO_4 \cdot 7H_2O$, 3.0; NaCl, 4.1; Na_2CO_3, 2.3; $FeSO_4 \cdot 7H_2O$, 0.31; $MnSO_4 \cdot H_2O$, 0.22; ZnO, 0.050; $CuSO_4 \cdot 5H_2O$, 0.019; $Na_2MoO_4 \cdot 2H_2O$, 0.00084; $CoCl_2 \cdot 6H_2O$, 0.0017; KI, 0.00069; Na_2SeO_4, 0.00032.
[7]Supplied the following in mg/kg of diet: thiamin · HCl, 15.0; riboflavin, 15.0; nicotinic acid, 50.0; *d*-calcium pantothenate, 20.0; pyridoxine · HCl, 6.0; folic acid, 6.0; biotin, 0.6; cyanocobalamin, 0.02; menadione sodium bisulfite, 1.50; ethoxyquin, 100.0; retinyl acetate, 1.754; cholecalciferol, 0.0625; *dl*-α-tocopheryl acetate, 110; glucose monohydrate, 9,570 to make 10 g.
[8]As fed basis.

Analytical procedures

Diets. The nitrogen and dry matter contents of feed samples were determined in duplicate using Association of Official Analytical Chemists (AOAC)[24] procedures. Feed samples of basal diets were analyzed for amino acids. Duplicate ground samples of feeds were extracted of fat using acetone:chloroform (3:1 vol/vol) as suggested by Ashworth.[25] After the defatted samples were dried, subsamples containing 12 to 13 mg of nitrogen were hydrolyzed in 6 N HCl at 110°C for 21 hours using a modified method of Gehrke et al.[26] Subsamples for determination of branched-chain amino acids were hydrolyzed in duplicate in 6 N HCl for 21, 45, 69, and 93 hours. Isoleucine and valine concentrations of the basal diets are based on 69 hours of hydrolysis, the time at which the amount of these amino acids released by hydrolysis was maximal.

Table 2 Imbalancing mixture of amino acids

Amino acid	% of diet
L-arginine	0.48
L-lysine · HCl	0.40
L-leucine	0.36
L-valine	0.32
L-histidine	0.43
L-methionine	0.41
L-phenylalanine	0.46
L-tryptophan	0.57
L-tyrosine	0.50
L-alanine	0.25
L-glycine	0.21
L-serine	0.29
L-threonine	0.33
Total	5.01

The amino acid concentrations in the acid hydrolysates were determined by high performance liquid chromatography (HPLC; Beckman model 334 Gradient Liquid Chromatography System, Beckman Instruments, Inc., Altex Division, Palo Alto, CA USA) using a two-buffer gradient system consisting of lithium citrate buffer (pH 2.7) and lithium hydroxide buffer (pH 12.0) on a cation exchange column. Post-column derivatization with o-phthalaldehyde, fluorescence spectroscopy, and a peak integration system (Spectra-Physics, San Jose, CA USA) were used for the detection and calculation of amino acid concentrations in samples.

Plasma amino acids. Blood samples were taken by cardiac puncture. Feed and water were available ad libitum before blood was sampled. One milliliter of blood was collected from each of two randomly selected chicks per pen and was pooled for analysis. Chicks were euthanized immediately after collection. Pooled

Table 3 Influence of dietary treatments on growth, feed consumption, and efficiencies of feed and isoleucine utilization for body weight gain[1] (Experiment 1)

Diet		Cumulative			Daily	
		Weight gain[2]	Feed consumption[2]	Gain/feed[3]	Isoleucine intake	Weight gain/ isoleucine intake
					(mg/day)	(g/mg)
1	Basal	296	389	0.763	239	0.0961
2	+ 0.3% Ile	300	379	0.794	321†	0.0714†
3	+ 0.6% Ile	306	380	0.806*	409†	0.0588†
4	Basal + 5% ImbAA[4]	265*	343†	0.769	211†	0.0943
5	+ 0.3% Ile	294‡	356*	0.826†‡	301†§	0.0763†§
6	+ 0.6% Ile	290‡	355*	0.820†	382†§	0.0575†§
SEM[5]		8.2	10.4	0.0022	7.18	0.00137

[1]Means (per chick) of five replicates of four chicks. Average initial weight of day 2 posthatching was 43 g. Diets were fed from day 2 to day 15 posthatching.
[2]g/13 days/chick.
[3]g weight gain/g feed consumed.
[4]ImbAA (imbalancing amino acid mixture), *Table 2*.
[5]Pooled standard error of the mean.
*,†Means different from those of chicks fed diet 1 (†, $P < 0.01$; *, $P < 0.05$).
‡,§Means different from those of chicks fed diet 4 (§, $P < 0.01$; ‡, $P < 0.05$).

samples were centrifuged at 600 × g for 10 minutes. Protein was precipitated from diluted plasma samples with 1.6% sulfosalicylic acid. Samples were stored at 4°C overnight and centrifuged at maximum speed of Eppendorf centrifuge for 2 minutes. The supernatants obtained were filtered into sample vials and analyzed for free isoleucine by HPLC. Norleucine (150 nmol/mL) was used as an internal standard.

Body composition. The remaining two chicks per replicate of selected treatments in Experiment 1 were sampled for analysis of body composition after 1 day of feed withdrawal. They were weighed and euthanized by carbon dioxide gas. Livers were removed, dried at 65°C in a forced air oven until constant weights were obtained, and ground. Chicks used for carcass analyses were dried in a large air-forced oven until constant weights were obtained, weighed, and passed through a 1/8-in screen in a comminuting machine with powered dry ice. After the dry ice had dissipated, the dry ground carcasses were blended, re-dried in an oven, and blended again enough to get homogenous samples just before the analysis. Weights before and after drying were used for calculation of percent moisture. Samples were used for the determination of crude protein (N × 6.25) and lipid (Soxhlet apparatus) contents using standard assays of the AOAC.[24]

Liver BCKAD activity. The homogenization of liver, isolation of mitochondria, and preparation of mitochondrial extracts was carried out according to the procedures of Patston et al.,[27] with the exception that dichloroacetate was substituted for α-ketoisocaproic acid as inhibitor of BCKAD kinase, and the assay medium contained 30% higher concentrations of phosphate buffer, cofactors, and substrate (α-ketoisocaproic acid). The BCKAD assay is based on measurement of NADH production by mitochondrial extracts in a spectrophotometer equipped with a recorder and a temperature-controlled cuvette chamber at 37°C. The mitochondrial BCKAD multienzyme complex is subject to reversible covalent modification. Phosphorylation inactivates and dephosphorylation reactivates the complex.[28,29] Therefore, the basal BCKAD activity (active form only) was measured without activating the complex, whereas the total BCKAD activity (active plus inactive forms) was determined after an initial incubation for 30 minutes at 37°C to provide maximum activation. Units of enzyme activities were determined as micromoles of NADH produced per 10 minutes at 37°C after subtraction of the amount of NADH produced in the blanks using the extinction coefficient of NADH (6.22×10^3 M^{-1} cm^{-1} at 340 nm). BCKAD activities were expressed as milliunits per milligram of mitochondrial protein.

Statistical analyses

In Experiment 1, the general linear models (GLM) procedure was used to evaluate preplanned comparisons[30,31] among treatment means. In Experiments 2 and 3, the GLM procedure was performed using day, treatment, and day × treatment as the variables to evaluate comparisons[30,31] between the basal diet and the imbalanced diet. SAS[32] statistical software (SAS Proprietary Software Release 6.09, SAS Institute, Inc., Cary, NC USA) was used.

Results

Experiment 1

When the basal diet was supplemented with 0.3% or 0.6% isoleucine, growth rate and feed consumption were not improved, but the efficiency of feed utilization was increased at the highest level of isoleucine supplementation (*Table 3*). The addition of 5% imbalancing amino acids to the basal diet decreased weight gain and feed consumption, but did not affect the efficiency of feed utilization. Addition of 0.3% or 0.6% isoleucine to the diet containing the 5% imbalancing amino acids resulted in a similar weight gain, but lower feed consumption, compared with that which occurred in chicks fed the basal diet. Consequently, the efficiency of feed utilization by chicks fed these isoleucine-supplemented diets was greater than that of chicks fed the basal diet or the basal supplemented with the 5% imbalancing mixture of amino acids.

Changes in cumulative weight gain and feed intake and daily feed intake of chicks fed the basal diet and the basal supplemented with the 5% imbalancing amino acid mixture during the course of experiment are presented in *Figure 1*. The cumulative weight gain of chicks fed the diet contain-

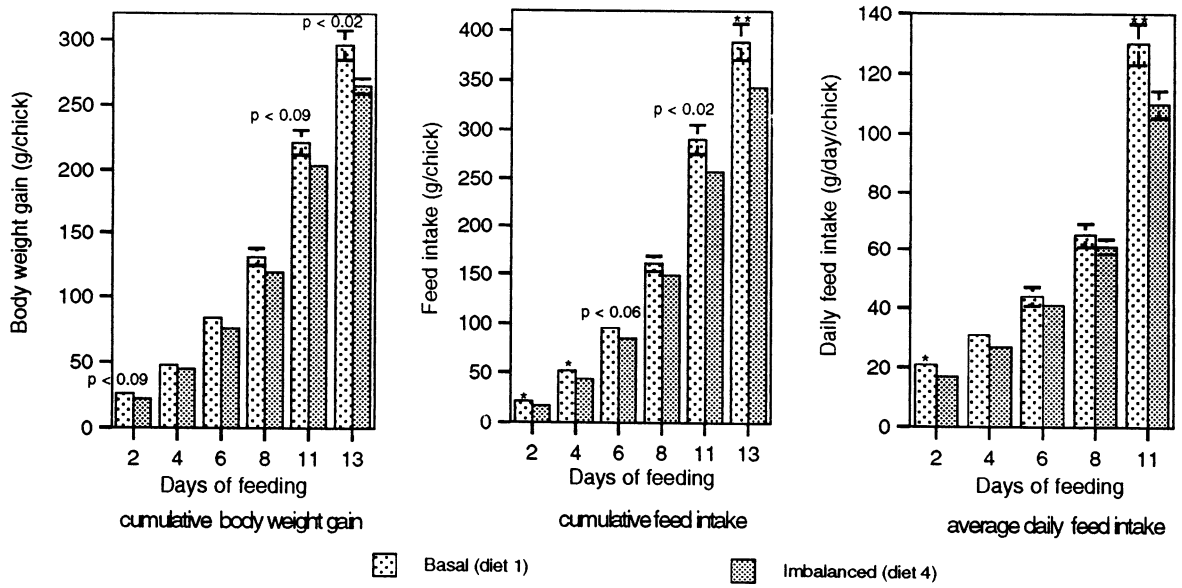

Figure 1 Changes in cumulative body weight gain (*left*), cumulative feed intake (*middle*), and average daily feed intake (*right*) at 2-day intervals (Experiment 1). Stippled bars, basal (diet 1); shaded bars, imbalanced (diet 4). Significant difference between the basal and imbalanced groups denoted by * ($P < 0.05$) and ** ($P < 0.01$). Other *P*-values are indicated in the figures for selected comparisons.

ing the imbalancing amino acids appeared to be slightly lower than that of chicks fed the basal diet, but the difference was not significant ($P > 0.05$) until day 13 of feeding. The cumulative feed intake and average daily feed intake of chicks fed the diet containing the imbalancing amino acids were significantly lower by the second day of the experiment than those of chicks fed the basal diet.

Daily isoleucine intake, feed intake, and weight gain were calculated based on cumulative feed intake, weight gain, and composition of diets (*Table 3*). The efficiency of utilization of isoleucine was indirectly determined using the ratio of daily weight gain to isoleucine intake. The addition of the imbalancing amino acid mixture to the basal diet did not alter the ratio. Dietary isoleucine increased and imbalancing amino acids decreased plasma isoleucine concentrations (*Table 4*) ($P < 0.01$).

There were no differences ($P > 0.05$) in the moisture, protein, and fat contents of chicks fed the basal diet or the diet containing the imbalancing mixture of amino acids. The composition of chicks fed either diet was 74%, 14%, and 9% of body weight, respectively, for these three components. The moisture, protein, and fat contents of livers from chicks fed the basal diet (72%, 18%, and 4% of weight, respectively) also did not differ from those of chicks fed the diet containing the imbalancing mixture of amino acids (68%, 20%, and 4%, respectively).

Table 4 Plasma isoleucine concentrations (Experiment 1)

Dietary isoleucine %	Imbalancing mixture of amino acids (μmoles/liter)		Pooled mean[1]
	0	+	
0.7	124[2]	102	113–a
1.0	255	205	230–b
1.3	287	263	275–c
Pooled mean[1]	222–a	190–b	

[1]Pooled means within a row or column having a different letter (a, b, c) are different ($P < 0.01$).
[2]Mean values of five replicates of two chicks per replicate (SEM = 11). Blood samples were drawn from fed chicks after 13 days of experiment.

Experiment 2

Chicks fed the diet containing the 5% imbalancing mixture of amino acids had lower weight gain and feed consumption and were less efficient in feed and isoleucine utilization for gain than chicks fed the basal diet (*Table 5*). The basal BCKAD activity of the chicks fed the diet containing the 5% imbalancing mixture was 19% higher than that of chicks fed the basal diet. Chicks fed the diet containing the imbalancing mixture had 14% higher total activity of BCKAD than did chicks fed the basal diet. The activity states of BCKAD (i.e., basal activity as percent of total activity) of chicks fed the basal diet and the diet containing the imbalancing mixture were 42.9% and 44.8%, respectively. The activity states were not different ($P > 0.05$).

Experiment 3. Chicks fed the diets supplemented with 5% imbalancing mixture of amino acids for 1 day had lower weight gains, feed consumptions, and efficiencies of feed and isoleucine utilization than did chicks fed basal diets (*Table 6*). The basal diets were isonitrogenous or nonisonitrogenous with the diets that contained the imbalancing mixture of amino acids. Therefore, the diet containing the imbalancing amino acid mixture (diet 4) that was not isonitrogenous with the basal diet (diet 3) contained approximately 27% dietary crude protein whereas both the isonitrogenous imbalanced diet (diet 2) and basal diet (diet 1) contained approximately 23% crude protein. There was no difference in efficiency of feed utilization between the basal diets even though the composition of two diets was slightly

Table 5 Influence of isoleucine imblance on weight gain, feed consumption, efficiencies of feed and isoleucine utilization for body weight gain, and branched-chain α-keto acid dehydrogenase, (BCKAD) activity[1] (Experiment 2)

	Cumulative			Daily				BCKAD activity (munit/mg mitochondrial protein)	
Diet	Weight gain[2]	Feed consumption[2]	Gain/feed[3]	Weight gain (g/day)	Feed intake (g/day)	Isoleucine intake (mg/day)	Weight gain/isoleucine intake (g/mg)	Total (active + inactive)	Basal (active only)
1 Basal	390	600	0.649	30	46	369	0.0813	8.4	3.6
2 + ImbAA[4]	348*	563*	0.617*	27*	43*	346*	0.0775*	9.6†	4.3†
SEM[5]	6.1	7.7	0.00597	0.48	0.61	4.85	0.000755	0.25	0.08

[1]Means (per chick) of 12 replicates of five chicks. Average initial weights were 77 g, 92 g, and 106 g (5, 6, and 7 days posthatching, respectively) for 1st, 2nd, and 3rd days of assignment to diets, respectively. Values for BCKAD activity (total and active) in milliunits per mg mitochondrial protein are means of six replicates of four livers pooled from two pens of chicks fed the same diet.
[2]g/13 days/chick.
[3]g weight gain/g feed consumed.
[4]ImbAA (imbalancing amino acid mixture); see *Table 2*.
[5]Pooled standard error of the mean.
,†Means with symbols are significantly different from those of chicks fed diet 1 (, $P < 0.01$; †, $P < 0.05$).

different (*Table 1*). Therefore, when two basal diets were compared as a group with the two diets containing the imbalancing mixture, highly significant differences were observed in growth rate, feed intake, and feed and efficiency of isoleucine utilization.

The basal activities of BCKAD were 23% and 19% higher, respectively, in chicks fed the isonitrogenous and nonisonitriogenous diets containing the imbalancing amino acids. The chicks fed the diets containing the imbalancing mixture also had 22% and 34% higher total activities of BCKAD, respectively, than did chicks fed the basal diets (*Table 6*). The basal activity ranged from 44.8 to 45.7% of total activity and did not differ between treatments ($P < 0.05$).

The concentration of isoleucine in plasma was decreased significantly ($P < 0.05$) by the imbalancing amino acid mixture when the diets were isonitrogenous, but did not decrease when the diets were not isonitrogenous (*Table 7*).

Discussion

Induction and degree of an isoleucine imbalance in broiler chickens

In most of the studies of amino acid imbalance using rats, imbalances were created by supplementing 6 to 7% imbalancing amino acid agents into basal diets that were low in dietary protein (5–7% dietary crude protein).[3,4,6,13–16,18,33] Depressed growth rate and even some pathologic conditions derived from toxicities, antagonism, and imbalances in animals fed a diet containing such a low level of dietary protein may not occur in animals that are fed a diet adequate in protein.[13] One of the reasons that isoleucine imbalance was selected for the present study is the possibility that increasing amounts of several synthetic amino acids such as methionine, lysine, tryptophan, and threonine in practical diets[34] might cause isoleucine to become a limiting amino

Table 6 Influence of isoleucine imblance on weight gain, feed consumption, efficiency of feed and isoleucine utilization for body weight gain, and branched-chain α-keto acid dehydrogenase, (BCKAD) activity of broiler chicks[1] (Experiment 3)

	Cumulative			Daily			BCKAD activity (munit/mg mitochondrial protein)	
Diet	Weight gain[2]	Feed consumption[2]	Gain/feed[3]	Weight gain (g/day)	Isoleucine intake (mg/day)	Weight gain/isoleucine intake (g/mg)	Total (active + inactive)	Basal (active only)
1 Basal 1	24.1	38.1	0.629	24	298	0.0794	6.7	3.0
2 + 5% ImbAA[4]	16.1†	34.1†	0.467†	16†	271†	0.0585†	8.2*	3.7†
3 Basal 2	22.7	36.5	0.617	23	296	0.0763	7.0	3.2
4 + 5% ImbAA[4]	16.6§	32.1§	0.510§	17§	260§	0.0625§	8.4‡	3.8§
SEM[5]	0.5	0.6	0.0140	0.54	11.0	0.00340	0.1	0.1

[1]Means (per chick) of eight replicates of five chicks. Average initial weights were 140 g, 163 g, 177 g, and 248 g (7, 8, 9, and 11 days posthatching, respectively) for 1st, 2nd, 3rd, and 4th days of assignment to experimental diets, respectively. Values for BCKAD activity (total and active) in milliunits per mg mitchohondrial protein are means for four replicates of livers pooled from two pens of chicks fed the same diet. Diets 1 and 2 were isonitrogenous. Diets 3 and 4 were non-isonitrogenous. Chicks were sampled for measurement of BCKAD activities after 24 hours of experiment.
[2]g/24 hours/chick.
[3]g weight gain/g feed consumed.
[4]See *Table 2*.
[5]Pooled standard error of the mean.
,†Means significantly different from those of chicks fed diet 1 (, $P < 0.05$; †, $P < 0.01$).
‡,§Means significantly different from those of chicks fed diet 3 (‡, $P < 0.05$; §, $P < 0.01$).

Table 7 Plasma isoleucine concentrations[1] (Experiment 3)

Diet	Ile
	nmole/ml
1 Basal 1	132
2 + 5% ImbAA[2]	96*
3 Basal 2	101
4 + 5% ImbAA	98
SEM[3]	10

[1]Mean value of four replicates of four chicks pooled by replicate from each treatment after 24 hours of experiment. Blood samples were taken after 24 hours of initial feeding of experimental diets. Chicks had access to each diet *ad libitum* before blood was collected.
[2]ImbAA (Imbalancing amino acids), see *Table 2*.
[3]Pooled standard error of the mean.
*Significant difference between chicks fed diets 1 and 2 ($P < 0.05$).

acid when feeds are formulated to lower levels of protein or with unconventional feedstuffs.

The amounts of individual amino acids needed to cause adverse effects of antagonisms and toxicities usually exceed twice the requirements of the amino acids, or 2 to 4% of the diet.[13,35,36] However, the concentrations of individual amino acids needed to induce imbalances are relatively low when compared with those needed to produce toxicities or antagonisms. In the present study, 5% of imbalancing amino acid mixture was used to induce an isoleucine imbalance in broilers fed an adequate level of dietary protein containing a marginally adequate level of isoleucine, the first limiting amino acid. None of the amino acids were present in the mixture at sufficient concentration to manifest the deleterious effects of antagonisms or toxicities.

It is clear from the first experiment that the addition of a mixture of L-amino acids lacking L-isoleucine to a diet containing an adequate level of crude protein but marginally limiting in isoleucine caused a growth depression in broiler chickens that could be prevented by supplementation of the diet with isoleucine. This finding in itself is not unexpected because the effect of dietary protein level on requirements of growing chickens for other amino acids in chickens have been described previously.[37–44] However, it establishes a model in the chicken that can be used to determine the effect of isoleucine imbalance on the activity of the primary regulated enzyme of isoleucine catabolism and to determine the effect of the imbalance on the efficiency of isoleucine utilization for growth. The effect of the imbalancing mixture on food intake was observed within 2 days of experiment, the earliest time at which the measurement of food intake was made. Rapid decreases in food intake (usually within a few hours) have been reported to occur under conditions of amino acid imbalance.[3,5,13] Evidence obtained in studies on the rat suggests that the depressed food intake is due in part to the detection of altered amino acid profiles in the prepyriform cortex of the central nervous system.[10,11]

An aspect of isoleucine balance that differs in this avian model and the conventional rat model is the phenomenon of adaptation.[3,11,13] When isoleucine imbalance or threonine imbalance are produced using the rat model, weight gain and food consumption are decreased within 1 day of feeding a basal diet limited in isoleucine or threonine to which an imbalancing mixture of amino acids is added. However, rats adapt to the diet over a period of several days, and food intake and body weight are improved. This phenomenon has not been noted in the published examples of amino acid imbalance of poultry,[7,18,38,39] and it did not occur during the 13-day experimental period used in the present study. This difference between the low-protein rat model and the adequate-protein chicken model has been observed during experiments within the same laboratory.[18] Therefore, we conclude that the two models differ in this regard, but because the models differ not only in species (chicken versus rat) dietary protein level (low versus adequate) and weight ratio of imbalancing mixture of amino acids to dietary crude protein (5% added to 21.3% crude protein for chicken, 6–10% added to 5–6% crude protein for rats), it is not clear what accounts for the lack of adaptation in one case but not in the other.

The present studies assessed the activity of hepatic BCKAD as an indicator of possible changes in isoleucine catabolism. BCKAD activity was increased in both experiments in which it was assayed. Feed intake and weight gain of chicks fed an isoleucine-imbalanced diet were inversely related to BCKAD activities. It is clear from these studies that the activity of hepatic BCKAD in broilers rapidly increases under conditions of an isoleucine imbalance.

Interestingly, the activity of threonine dehydrogenase in rats fed a threonine-imbalanced diet appeared to be increased for the first 3 days, but not to be different from that of rats fed a basal diet after 3 days, which coincided with the adaptive responses such as restored feed intake and weight gain and increased plasma threonine concentration.[18] Leghorn chicks, however, did not adapt in feed intake, weight gain, or enzyme activity during 9 days of experiment.[18] Chicks fed a threonine-imbalanced diet for 24 hours or 9 days showed consistently twice the hepatic threonine dehydrogenase activity of chicks fed the basal diet.[18] The results of the present studies are in good agreement with the previous studies of threonine imbalance in chickens in which no adaptation was observed.

It has been generally accepted that activities of enzymes of amino acid and nitrogen metabolism increase in response to intakes of amino acids beyond those levels required for protein metabolism in the body, and that this contributes to the maintenance of homeostasis. These enzymes include, among others, arginase,[45,46] serine-threonine dehydratase,[47,48] threonine dehydrogenase,[49] tryptophan pyrrolase,[50] glutamate dehydrogenase,[51] leucine-α-ketoglutarate amino transferase,[52] and BCKAD.[52–56]

The imbalancing amino acid mixture of amino acids might increase the substrate availability for BCKAD in liver because BCKAD exhibits broad specificity toward α-keto acids such as α-ketoisocaproate (the keto analog acid of leucine), α-ketoisovalerate (the keto analog of valine), α-keto-γ -[methylthio]butyrate (the keto analog of methionine), and α-ketobutyrate (the α-keto acid derived from catabolism of methionine and threonine).[57,58] Keto acids originating from catabolism of imbalancing amino acids in the present study might inhibit BCKAD kinase, because studies in mammals have shown that the kinase can be inhibited by various α-keto acids.[20,21]

Little is known about chicken BCKAD and its regulation. If the mechanism is similar to those in mammals, α-keto acids derived from the amino acids in the imbalancing mixture could cause hepatic BCKAD to be less phosphorylated (i.e., more active), possibly increasing the catabolism of branched-chain amino acids such as isoleucine. The activity state of hepatic BCKAD, however, was not significantly increased by imbalancing amino acids. It appears that the main effect of the imbalancing mixture of amino acids was to increase the BCKAD content of liver. Based on studies in mammals, BCKAD is widely distributed in other tissues, where it often has a lower activity state than in liver.[27,28,55,58] It is possible that these tissues may contribute to changes in branched-chain amino acid metabolism in response to the imbalancing mixture of amino acids. Other investigators have noted that the activity state of BCKAD in liver often is not as responsive to dietary or hormonal treatments as the activity state of BCKAD in other tissues.[53]

Plasma isoleucine

The concentration of limiting amino acid characteristically decreases when an imbalancing mixture of amino acids is included in the diet.[3–5,14–18] The concentration of isoleucine in plasma decreased in both experiments in which it was measured, although not consistently. In the third experiment, for example, isoleucine concentration decreased significantly only when isoleucine imbalance was produced by use of isonitrogenous diets. This apparent difference between isonitrogenous and nonisonitrogenous diets may not be biologically significant, because others[3–5,14–18] have observed decreases of first limiting amino acids when imbalancing amino acids were added to the diet without maintaining isonitrogenous conditions.

The decrease in plasma isoleucine concentration may be a consequence of increased catabolism of isoleucine via hepatic BCKAD activity. In addition, it is possible that the α-keto acid derivatives of several amino acids in the imbalancing mixture activated BCKAD in peripheral tissues, resulting in greater capacity for increased isoleucine catabolism than was evident in the approximately 20% increase in activity of the hepatic enzyme. Reduced food intake also may have contributed to reduced plasma isoleucine concentrations. However, in rat models of amino acid imbalance the concentration of limiting amino acid in plasma decreased even when food intake was controlled (i.e., equal) in basal and imbalanced groups.[3,5,33] Other investigators[12,13] have provided evidence that hepatic protein synthesis is increased in amino acid imbalance and have suggested that this contributes to the decrease in the concentration of first-limiting amino acid in plasma. The physiologic basis for the change in concentration of first-limiting amino acid in plasma may be multifactorial in nature, involving BCKAD among other factors.

Efficiency of isoleucine utilization

The efficiency of utilization of isoleucine was indirectly measured using the ratio of daily weight gain to isoleucine intake. The efficiency of isoleucine utilization after 13 days of feeding in the first experiment was not different for chicks fed the basal diet and the diet containing the imbalancing amino acids. However, the efficiency was lower for chicks fed the diets containing the imbalancing amino acid mixture in the two subsequent short-term experiments and it appeared to be much lower in Experiment 3 than in Experiment 2. This reduced efficiency may be a consequence of the increased BCKAD activity, leading to catabolism of isoleucine. However, studies on the flux of isoleucine through the metabolic pathway in vivo would be required to determine whether the increased BCKAD activity is indicative of an actual increase in isoleucine catabolism. If an increase in isoleucine catabolism occurs, but it is not reflected in altered feed efficiency, it may be small enough or sufficiently transient that it is not easily detected by carcass analysis or nutritional balance studies. For example, in Cieslak and Benevenga's[60,61] studies on the effect of imbalancing mixtures of amino acids on lysine and threonine utilization in growing rats, they did not detect alterations in feed efficiency or the amount of carcass protein gained per unit of lysine or threonine intake when the diet contained the imbalancing mixture. The chick, however, may respond to imbalance somewhat differently from the rat. Based on strong responses to 5% imbalancing mixture of amino acids added to a 23% protein diet in the present studies and others[7,18,39] involving threonine imbalance, in contrast to small responses in rats to 4.5 to 5% imbalancing mixtures of amino acids added to crystalline amino acid diets containing 11 to 13% amino acids, it appears that the chick may be more susceptible than the rat to amino acid imbalance. Perhaps the more transient nature of amino acid imbalances in the rat is another manifestation of this difference.

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