Research Notes

The Effect of Dietary Protein Level on Threonine Dehydrogenase Activity in Chickens

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ABSTRACT An experiment was carried out to determine the effect of dietary protein level on the specific activity of hepatic L-threonine dehydrogenase in young growing chicks. Six replicate pens of seven Leghorn chicks were fed semipurified diets containing 23, 27, or 32% CP with identical relative proportions of amino acids in each protein group. Body weights and feed consumption were measured for 3 d, and hepatic mitochondria were isolated for assay of threonine dehydrogenase (TDH) activity. Weight gains and feed efficiency increased at each level of protein supplementation, but feed consumption was not affected by protein level. The specific activity of threonine dehydrogenase in isolated liver mitochondria was significantly ($P < 0.05$) higher in the 32% CP group than in the 23% CP group, and the activity in the 27% CP group was intermediate. We conclude that moderate increases in dietary protein level result in elevated hepatic threonine dehydrogenase activity in growing chicks.

(*Key words*: threonine dehydrogenase, chicken, dietary protein, aminoacetone, glycine)

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INTRODUCTION

Threonine dehydrogenase (TDH) is considered to be the major enzyme of L-threonine catabolism (Bird and Nunn, 1983). The enzyme is closely associated in mitochondria with 2-amino-3-oxobutyrate coenzyme A ligase that catalyzes the conversion of 2-amino-3-oxobutyrate to glycine and acetyl coenzyme A (Dale, 1978). An alternative product derived from 2-amino-3-oxobutyrate is aminoacetone, which is formed nonenzymatically by rapid spontaneous decarboxylation (Laver et al., 1959; Tressel et al., 1986). The specific activity of TDH in isolated chicken hepatic mitochondria is reported to be significantly increased by dietary threonine imbalance caused when 5 to 10% of a mixture of indispensable amino acids lacking threonine is added to a 23% CP diet marginally limiting in threonine (Davis and Austic, 1994; Yuan et al., 2000). TDH activity has been reported to decrease to 10% or increase by 400% within 3 d when chicks are fed a protein-free diet or a diet containing 51% protein, respectively, compared with the activity in chicks fed a 23% CP basal diet (Davis and Austic, 1997). The increases in TDH activity observed under conditions of threonine imbalance may reflect the higher amino acid content of the diet. The present experiment was carried out to determine whether dietary supplements of a nutritionally balanced mixture of protein and amino acids equivalent to 5 and 10% of the diet would increase hepatic TDH activity.

MATERIALS AND METHODS

Birds

Day-old male Single Comb White Leghorn chicks, obtained from a local breeding company,[2] were housed in battery brooder cages with raised wire floors. Brooders were set at 35 C for the first week and 32 C in the second week and were housed in a room maintained at approximately 21 C. The room and cages were lighted from 0800 to 2200 h daily. Chicks had free access to feed and water throughout the pre-experimental and experimental periods, except for the overnight hours when feed was withheld prior to 0800 h on the first day of experiment. All procedures were approved by the Institutional Animal Care and Use Committee of Cornell University.

Diets

Chicks were fed a practical chick starter diet for 5 to 7 d and then were fed a 23% CP basal diet for 5 d. The basal diet (Table 1) (Davis and Austic, 1994) was formulated to contain all nutrients at levels sufficient to satisfy the nutrient requirements of Leghorn chicks according to the

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Abbreviation Key: TDH = threonine dehydrogenase.

TABLE 1. Composition of diets

Ingredients	Dietary group (g/100 g of diet) 23% CP	27% CP	32% CP
Soybean protein (91.5% CP)	18.20	21.65	25.66
L-Aspartic acid	0.67	0.82	0.90
L-Serine	0.13	0.15	0.18
L-Glutamic acid	0.81	0.96	1.14
L-Proline	0.27	0.32	0.38
Glycine	0.62	0.74	0.87
L-Alanine	0.19	0.23	0.27
L-Valine	0.23	0.27	0.32
L-Methionine	0.09	0.11	0.13
L-Isoleucine	0.26	0.31	0.37
L-Leucine	0.35	0.42	0.49
L-Tyrosine	0.16	0.19	0.23
L-Phenylalanine	0.27	0.32	0.38
L-Lysine·HCl	0.47	0.56	0.66
L-Histidine	0.16	0.19	0.23
L-Arginine·HCl	0.70	0.83	0.99
L-Tryptophan	0.07	0.08	0.10
DL-Methionine	0.60	0.71	0.85
Cellulose	3.00	3.00	3.00
Corn oil	4.00	4.00	4.00
Minerals[1]	1.20	1.20	1.20
Vitamins[2]	6.59	6.59	6.59
Glucose monohydrate	60.69	56.37	51.02

[1]Provided the following (g/100 g of diet): $CaCO_3$, 1.48; $CaHPO_4 \cdot 2H_2O$, 2.07; KH_2PO_4, 1.00; $NaHCO_3$, 0.3; KCl, 0.10; $KHCO_3$, 0.64; NaCl, 0.60; $MnSO_4 \cdot H_2O$, 0.035; $FeSO_4 \cdot 7H_2O$, 0.05; $MgSO_4$, 0.3; KI, 0.00016; $CuSO_4 \cdot 7H_2O$, 0.03; ZnO, 0.01; $CoCl_2 \cdot 6H_2O$, 0.00017; $NaMoO_4 \cdot 2H_2O$, 0.00083; Na_2SeO_3, 0.00002.

[2]Provided the following (mg or IU/100 g diet): thiamin-HCl, 1.5; riboflavin, 1.5; nicotinic acid, 5.0; folic acid, 0.6; pyridoxine, 0.6; biotin, 0.06; vitamin B_{12}, 0.002; choline Cl, 285; d-calcium pantothenate, 2.0; menadione sodium bisulfite, 0.15; vitamin E, 5 (IU); vitamin D_3, 225 (IU); vitamin A, 425 (IU); butylated hydroxytoluene, 10.0.

National Research Council (1994) except for the threonine level (0.67% of the diet), which was marginally inadequate (Davis and Austic, 1982). Dietary CP was raised by increasing the isolated soybean protein and crystalline amino acids of the basal diet proportionately to achieve 27 and 32% CP. Chicks were sorted by weight before assignment to experimental groups in such a manner as to achieve equal weight distributions among all pens. Two replicate pens of seven chicks were started on experimental diets on 3 consecutive d, beginning at 10 d of age. Chicks were provided with the experimental diets for 3 d, at which time, liver samples were obtained and the specific activity of TDH in liver mitochondria was determined. Feed consumption and body weight gains were measured during the 3-d experiment.

Liver mitochondria were prepared and assayed for TDH activity as previously described by Davis and Austic (1994). The assay is based on the accumulation of glycine and aminoacetone, representing the two alternative pathways of 2-amino-3-oxobutyrate disappearance.

Data from each experiment were subjected to one-way ANOVA. All statistical procedures were performed using Minitab statistical software,[3] and differences were considered significant when $P < 0.05$. Fisher's pairwise comparison procedure was used to detect differences among treatment means.

[3]Version 11.12, Minitab, Inc., State College, PA 16801-3008.

RESULTS

Weight gain and the efficiency of feed utilization were significantly higher in chicks fed diets containing 27 and 32% CP compared with chicks fed the 23% CP diet and were higher in the 32% group than in the 27% group (Table 2). Feed consumption was equal in all three groups. The specific activity of TDH was twice as high in the 32% CP group as in the 23% CP group. The specific activity of the 27% CP group was intermediate between the chicks given the 23% CP diet and those given 32% CP. Glycine accumulations were three- to fourfold higher than aminoacetone accumulations in all three groups, but the proportion of product that was recovered as glycine did not differ among groups.

DISCUSSION

Birds fed high-protein diets have been reported to have increased activities of many rate-limiting enzymes of amino acid catabolism as compared with those fed lower dietary levels of protein (Ashida and Harper, 1961; Freedland and Avery, 1964; Wergedal and Harper, 1964; Horwitz and Waisman, 1966; Reynolds et al., 1971; Featherston and Horn, 1973; Muramatsu et al., 1975; Kang-Lee and Harper, 1979; Gillim et al., 1983; Mueckler et al., 1982; Dixon and Harper, 1984; Miller et al., 1988; Ewart et al., 1992; Le Floc'h et al., 1994; Bella et al., 1996; Davis and Austic, 1997; Torres et al., 1998). The increases in enzyme

TABLE 2. Effects of dietary protein level on growth, feed consumption, feed utilization, and threonine dehydrogenase activity of liver mitochondria

Dietary group	Weight gain	Feed consumed	Feed utilization	Threonine dehydrogenase activity: Aminoacetone[1]	Glycine[1]	Total activity[2]	Glycine, % of total[3]
	(g/day)	(g/day)	(gain/feed)	nmol/(15 min·mg mitochondrial protein)			
23% CP	6.3[c4]	13.1	0.48[c]	3.8[b]	14.4[b]	18.3[b]	78.7
27% CP	7.5[b]	13.2	0.57[b]	5.8[ab]	16.8[ab]	22.7[ab]	74.0
32% CP	8.4[a]	13.4	0.63[a]	7.7[a]	27.7[a]	35.4[a]	78.2
Pooled SEM	0.25	0.38	.010	1.02	3.8	4.4	0.033

[a–c]Means within the same column lacking a common letter are significantly different ($P < 0.05$).

[1]Amounts of aminoacetone and glycine that had accumulated in reaction mixtures during 15 min incubation.

[2]Aminoacetone plus glycine accumulation.

[3]100[glycine/(glycine + aminoacetone)].

[4]Means of six replicates of seven chicks for each diet.

activity are not surprising because animals must be able to metabolize amino acids when the dietary intake exceeds requirements for the synthesis of protein and other essential nitrogenous compounds.

In the present studies, chicks fed the 27 and 32% CP diets had better growth responses and higher specific activities of TDH than chicks fed the basal diet. This result agrees with the report of Davis and Austic (1997) in which chicks fed a very high-protein diet (51% CP) gained more weight and the specific activity of hepatic TDH was four to five times as high as in the chicks fed a 23% CP diet. In the latter studies, it was also observed that the specific activity of TDH was not affected addition of 1.7% L-threonine to the 23% CP diet. This amount of threonine approximated the threonine increment of the 51% protein diet. The increase in TDH activity in response to protein is consistent with results in other species (Le Floc'h et al., 1994; Hammer et al., 1996).

Five to 10% dietary additions of amino acid mixtures lacking threonine have been used to cause threonine imbalance and have been reported to increase the specific activity of TDH in mitochondria of chickens (Davis and Austic, 1994; 1997) and rats (Davis and Austic, 1994). The present results indicate that relatively small increments of a threonine-containing mixture of protein plus crystalline amino acids in the range of CP additions that have been used to cause amino acid imbalances result in increases in TDH activity that are similar to those reported to occur in threonine imbalance (Yuan et al., 2000). These increases occur despite the fact that growth and feed intake improves with increased levels of a well-balanced dietary protein (present study) but decreases under conditions of imbalance. The effects of threonine imbalance on the specific activity of hepatic mitochondrial TDH, therefore, appears to be due primarily to dietary amino acid load per se rather than a unique condition attributable to the lack of threonine in the unbalanced mixture of amino acids.

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