

Growth, Feed Intake, and Plasma Thyroid Hormone Levels in Chicks Fed Dietary Excesses of Essential Amino Acids

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ABSTRACT The consequences of dietary excesses of 10 essential amino acids, His, Ile, Phe, Trp, Val, Arg, Leu, Lys, Met, Thr, on growth, feed intake and plasma levels of triiodothyronine (T₃) and thyroxine (T₄) in growing chicks were investigated. Each amino acid was added to a starter ration to bring it to a level 2.84× above the National Research Council (1984) requirement. Excesses of all amino acids except His and Leu caused significant reductions in weight gain. Of the amino acid excesses that reduced growth, only Trp and Val did not also reduce feed intake. Gain:feed decreased significantly only in chicks consuming excess Arg, Lys, Phe,

and Trp. Chicks fed excesses of Ile and Val had plasma T₃ levels that were statistically higher than control levels; none of the other amino acid excesses significantly altered blood concentrations of this hormone. Compared to the control, plasma T₄ levels were not significantly altered by the amino acid excesses, but there was a significant difference between Trp and Val, the latter being lower. This study shows that high dietary levels of essential amino acids cause depressions in weight gain and feed intake, and, with Ile and Val, these depressions are accompanied by elevations in plasma T₃ levels. Otherwise, the amino acid excesses had little effect on plasma levels of thyroid hormones.

(*Key words:* essential amino acids, triiodothyronine, thyroxine, toxicity, growth)

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INTRODUCTION

Dietary deficiencies of individual essential amino acids cause decreases in weight gain, feed intake, and energy retention in growing chicks (Ousterhout, 1960; Kino and Okumura, 1986). Less is known about the effects of high dietary levels of essential amino acids on chick performance, although such excesses are known to affect growth and behavior, and to increase mortality (Snetsinger and Scott, 1961; Griminger and Fisher, 1968; Edmonds and Baker 1987; Han and Baker, 1993).

The thyroid gland and its hormones comprise an endocrine system in birds and mammals that has important regulatory effects on growth, energy utilization, and other physiological responses. Although the effects of dietary deficiencies of single essential amino acids on thyroid function have been studied in chicks (Pastro *et al.*, 1969; May, 1979; Elkin *et al.*, 1980; Carew *et al.*, 1983, 1997), nothing is known about the effects of amino acid excesses.

Because of increasing interest in adding relatively pure, single amino acids to poultry diets, knowledge of the metabolic effects of excesses that may occur with this practice is important. Monitoring changes in the endocrine system, in particular plasma thyroid hormone levels, may provide insight into such metabolic changes. The present experiments were done to evaluate growth, feed intake, and thyroid responses to diets containing excessive amounts of 10 essential amino acids (His, Ile, Phe, Trp, Val, Arg, Leu, Lys, Met, Thr).

MATERIALS AND METHODS

The experimental protocol was approved by the University of Vermont Institutional Animal Care and Use Committee.

Animals

These studies included two experiments with similar experimental designs. In each experiment, 175 day-old male broiler chicks³ were housed in electrically heated, battery brooders with raised wire floors. They were exposed to a 16:8 light:dark cycle and had free access to feed and water.

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Abbreviation Key: T₃ = triiodothyronine; T₄ = thyroxine.

Experimental Design and Diets

From 1 to 9 d of age, all chicks were fed a nutritionally complete broiler starter diet (Table 1). On Day 10, the midrange of chicks was distributed into 18 pens of eight chicks each. From Days 10 to 24, triplicate pens of chicks were fed one of the following diets: broiler starter (Table 1) alone or a diet with 1 of the 10 essential amino acids added at a level 284% above the NRC requirement (1984). The final levels of the essential amino acids in each diet were as follows (as a percentage of diet): His, 1.34; Ile, 3.07; Phe, 2.76; Trp, 0.88; Val, 3.15 (Experiment 1); or Arg, 5.53; Leu, 5.18; Lys, 4.61; Met, 1.92; Thr, 3.07 (Experiment 2). With the added amino acids, each diet contained a total of 3.84× the NRC requirement. All test diets were diluted to the same extent as the amino acid added in the largest amount by adding glucose to the diets to equal that concentration. Although a wide range of excessive levels of amino acids has been used by others in the research referred to above, we chose to relate the excesses to the required amounts needed in the diets as a more reasonable relationship between dietary need and possible toxicity rather than feeding all amino acids at fixed excesses as some have done, and also to avoid harsh effects on growth that have previously been reported with higher levels.

After 2 wk (24 d of age) on experiment, body weights were taken and blood was drawn by side heart puncture from each chick, placed into heparinized tubes, centrifuged at $1,800 \times g$ and the plasma frozen for later analysis.

Hormone Assays

Plasma total T_3 ⁴ and T_4 ⁵ levels were analyzed by radioimmunoassay validated for avian blood. Intra- and interassay coefficients of variation were 6.3 and 5.2% for T_3 and 10.4 and 2.9% for T_4 , respectively, in the two experiments.

Statistical Analysis

Data were analyzed by ANOVA with $P \leq 0.05$ considered significant. Means were compared using Duncan's multiple range test (Duncan, 1955).

RESULTS

Feed Intake, Weight Gain, and Gain:Feed Ratio

In Experiment 1 (Table 2) chicks fed excessive levels of four of the five amino acids (Ile, Phe, Trp, and Val) grew at a significantly slower rate than the control, whereas Phe caused the greatest growth depression. Only His had no effect on growth. However, only with Ile and Phe were the

TABLE 1. Composition of broiler starter diet¹

Ingredients	Percentage in diet (%)
Yellow corn meal	51.1
Soybean meal (dehulled)	36.0
Soybean oil	4.0
Meat scraps	3.0
Alfalfa meal	1.0
Distiller's dried grains	2.0
Dicalcium phosphate	1.0
Limestone	0.8
Salt	0.4
Trace mineral mix ²	0.1
Met hydroxy analog	0.1
Vitamin premix ³	0.5

¹Contains (by calculation) 23.7% protein, 3,148 kcal/ME kg, and, in percentages, Arg, 1.60; Cys, 0.47; Gly + Ser, 3.02; His, 0.63; Ile, 1.00; Lys, 1.31; Met, 0.51; Phe, 1.15; Thr, 0.90; Trp, 0.28; Tyr, 0.94; Val, 1.13.

²Mineral mix supplies in milligrams per 100 g of diet: $FeSO_4 \cdot 7H_2O$, 20; $MnSO_4 \cdot H_2O$, 23.1; ZnO, 7.4; $CuSO_4 \cdot 5H_2O$, 1.6; KIO_3 , 0.12; Na_2SeO_3 , 0.01; $CaCO_3$, 47.8.

³Vitamin premix supplies per kilogram of diet: riboflavin, 4.4 mg; Ca pantothenate, 8.8 mg; niacin, 20 mg; choline, 322 mg; vitamin B_{12} , 10 μg ; menadione, 2 mg; vitamin E, 11 IU; vitamin A, 5,200 IU; cholecalciferol, 1,000 IU.

growth depressions accompanied by significant reductions in feed intake compared to the control. Neither His, Trp, nor Val excesses significantly affected feed intake. Compared to the control, gain:feed was depressed only with Phe and Trp.

In Experiment 2 (Table 2), chicks fed excessive levels of four of the five amino acids (Arg, Lys, Met, and Thr) also had significantly poorer growth rates than the control. Leucine excess had no effect. Both Lys and Met excesses produced the slowest growth rates, which were significantly more depressed than the reductions caused by Arg and Thr. However, unlike results in Experiment 1, all growth depressions were accompanied by reductions in feed intake relative to the control, with Arg and Met excesses producing significant differences from each other. Chicks fed Met excess had the lowest feed intakes. Only Arg and Lys excesses caused significantly poorer gain:feed ratios compared to the control.

Thyroid Hormones

Chicks fed excesses of Ile and Val had significantly higher plasma T_3 values (25 and 19%, respectively) compared to the control in Experiment 1 (Table 2). All other plasma T_3 levels in both experiments (Table 2) were not significantly different. Plasma T_4 levels (Table 2) were not significantly different from the control in either experiment. There was, however, a significant difference in this value between Trp and Val, the latter being the lower one.

DISCUSSION

Many amino acids, when fed in excess to growing chickens, cause toxic effects such as depressions in

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TABLE 2. Effect of dietary amino acids excesses on feed intake, weight gain, gain:feed ratio, and blood plasma concentrations of triiodothyronine (T₃) and thyroxine (T₄) in 10- to 24-d-old broiler chicks

Diet ¹	Weight gain	Feed intake	Gain:feed ratio	T ₃ ²	T ₄ ²
	(g)		(g:g)	(ng/mL)	(μg/dL)
Experiment 1					
Control	729 ^a	1,036 ^a	0.70 ^a	2.20 ± 0.09 ^b (12)	3.89 ± 0.13 ^{ab} (15)
Histidine	732 ^a	1,053 ^a	0.69 ^{ab}	2.08 ± 0.09 ^b (13)	3.68 ± 0.12 ^{ab} (15)
Isoleucine	672 ^{bc}	972 ^b	0.69 ^{ab}	2.74 ± 0.23 ^a (12)	3.96 ± 0.12 ^{ab} (15)
Phenylalanine	663 ^c	970 ^b	0.68 ^b	2.44 ± 0.14 ^{ab} (13)	3.72 ± 0.13 ^{ab} (15)
Tryptophan	696 ^b	1,020 ^{ab}	0.68 ^b	2.11 ± 0.07 ^b (12)	4.02 ± 0.16 ^a (15)
Valine	692 ^b	1,006 ^{ab}	0.69 ^{ab}	2.62 ± 0.12 ^a (12)	3.57 ± 0.14 ^b (15)
SEM	14	28	0.009		
Experiment 2					
Control	732 ^a	1,063 ^a	0.69 ^{ab}	2.50 ± 0.16 ^a (15)	4.34 ± 0.08 ^a (15)
Arginine	630 ^b	956 ^{bc}	0.65 ^c	2.27 ± 0.14 ^a (15)	4.00 ± 0.16 ^a (15)
Leucine	719 ^a	1,020 ^{ab}	0.68 ^{ab}	2.25 ± 0.13 ^a (15)	4.05 ± 0.12 ^a (15)
Lysine	550 ^c	923 ^{cd}	0.60 ^d	2.71 ± 0.18 ^a (15)	4.15 ± 0.13 ^a (15)
Methionine	578 ^c	870 ^d	0.66 ^{bc}	2.44 ± 0.14 ^a (15)	4.30 ± 0.13 ^a (14)
Threonine	661 ^b	931 ^{cd}	0.71 ^a	2.57 ± 0.18 ^a (15)	4.12 ± 0.14 ^a (15)
SEM	29	42	0.02		

^{a-d}Means within a column and experiment with no common superscript, differ significantly ($P \leq 0.05$).

¹Amino acids added at a level of 284% above the National Research Council (1984) recommendation.

²Numbers in parentheses = numbers of chicks sampled.

growth, decreases in feed intake and increased mortality (Snetsinger and Scott, 1961; Dean and Scott, 1968; Edmonds and Baker, 1987; Han and Baker, 1993).

In the present study, chicks consuming diets with excess Ile, Phe, Trp, or Val (Experiment 1) and excess Arg, Lys, Met, or Thr (Experiment 2), grew at a slower rate than controls. Only His and Leu excesses had no effect on growth. These depressions in growth were not always accompanied by reductions in feed intake. The decreased growth with Trp or Val did not have a parallel reduction in feed intake, but such a reduction in feed intake did occur with Ile and Phe (Experiment 1) and Arg, Lys, Met, and Thr (Experiment 2). Thus, it cannot be stated as an absolute rule that growth depressions in chicks fed high levels of essential amino acids will be accompanied by reductions in feed intake. This result suggests that the mechanism of the growth depression is not a single mechanism related to decreases in feed intake but is also due to other causes that vary with the amino acid in question. Certain of these situations are well known, such as the antagonism between Lys and Arg. Excess Lys in the diet causes an increase in the Arg requirement (O'Dell and Savage, 1966), and chicks receiving excess Lys may exhibit signs of an Arg deficiency because Arg alleviates Lys toxicity (Dean and Scott, 1968).

In the present study, excess Lys caused the most severe reduction in both weight gain (-25%) and gain:feed ratio (-14%) and the second most severe reduction in feed intake (-13%; the worst was Met at -18%) compared to the control. Lysine has been reported to be among the more toxic of the amino acids (Snetsinger and Scott, 1961; Okumura and Yamaguchi, 1980; Edmonds and Baker, 1987), and our results agree with those reports.

Excess Met (1.92% dietary total) produced the most severe depression in feed intake (-18%) along with a severe reduction in growth (-21%) but was accompanied by no significant change in gain:feed ratio. This result agrees with those of Han and Baker (1993), who observed that between 1 and 2% added Met depresses both feed intake and weight gain, and closely agrees with Edmonds and Baker (1987), who reported that Met was the most toxic of all amino acids excesses when fed at the 4% level. Okumura and Yamaguchi (1980) also showed that 3% excess Met is the most toxic of all amino acids, but they used a protein-deficient diet (10% protein) in their studies and, therefore, these results are not equivalent to those of the present studies.

Excess dietary Leu has been reported to increase the need for Ile and Val and to result in growth retardation unless these two amino acids are supplemented along with Leu (Chi, 1984). However, in our study, excess Leu caused no changes in growth or feed intake, which agrees with the report by Edmonds and Baker (1987).

In the present study, excess Thr significantly reduced feed intake and growth rate, but not as severely as Lys and Met. Edmonds and Baker (1987) reported excess Thr to be somewhat more toxic than we did, and found that it reduced weight gain by 29%, whereas we observed only a 10% reduction. However, they used a slightly higher level than we did (4.0 vs 3.07%).

According to our method of studying amino acid excesses at 2.84× above the NRC requirement, the excesses produced the following significant reductions in growth (in percentage): Lys, 25; Met, 21; Thr, 10; Phe, 9; Ile, 8; Trp, 5; Val, 5. Excesses of His and Leu had no effect. Our observations that Leu and Val excesses had little or no effect on growth, and that Lys and Met had major effects, agree with the comprehensive study by

REFERENCES

Edmonds and Baker (1987). Regarding feed intake, the significant reductions were as follows (in percentage): Met, 18; Lys, 13; Thr, 12; Arg, 10; Ile, 6; Phe, 6. Excesses of His, Leu, Trp, and Val had no such effect.

Concerning plasma thyroid hormone levels, excesses of Ile and Val caused significant elevations in plasma T_3 ; the other amino acids had no effect. This result shows that amino acid excesses have differential effects on thyroid hormones, and may explain, in part, the depressive effects of these two amino acids on growth, as an elevation in T_3 is often accompanied by increased heat expenditure. However, heat production was not measured, and the other amino acid excesses depressed growth without affecting circulating T_3 levels. Furthermore, Ile but not Val excess also depressed feed intake. Therefore, changes in blood T_3 levels were not related to changes in feed intake.

Plasma T_4 levels were generally resistant to change, and the only significant difference observed was between chicks fed excesses of Trp and Val, the meaning of which is obscure. However, although the lower level of T_4 compared to control with the Val excess was not significantly different, this effect may represent reduced conversion of T_4 into the elevated plasma T_3 . Nevertheless, there is no suggestion of such a shift in the case of Ile.

Dietary deficiencies of individual essential amino acids can have marked effects on circulating levels of thyroid hormones (May, 1979; Elkin *et al.*, 1980; Carew *et al.*, 1983, 1997). The present data suggest that dietary excesses of essential amino acids also affect this endocrine system, but this effect is limited to two of the amino acids, namely Ile and Val.

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- Carew, L. B., F. A. Alster, D. C. Foss, and C. G. Scanes, 1983. Effect of a tryptophan deficiency on thyroid gland, growth hormone and testicular functions in chickens. *J. Nutr.* 113: 1756-1765.
- Carew, L. B., K. G. Evarts, and F. A. Alster, 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. *Poultry Sci.* 76: 1398-1404.
- Chi, M. S., 1984. Amino acid balance and its effect on the growth rate of white leghorn female chickens. *Nutr. Rep. Int.* 29:45-53.
- Dean, W. F., and H. M. Scott, 1968. Ability of arginine to reverse the growth depression induced by supplementing a crystalline amino acid diet with excess lysine. *Poultry Sci.* 47:341-342.
- Duncan, D. R., 1955. Multiple range and multiple F tests. *Biometrics* 11:1-42.
- Edmonds, M. S., and D. H. Baker, 1987. Comparative effects of individual amino acid excesses when added to a corn-soybean meal diet: Effects on growth and dietary choice in the chick. *J. Anim. Sci.* 65:699-705.
- Elkin, R. G., W. R. Featherston, and J. C. Rogler, 1980. Effects of dietary phenylalanine and tyrosine on circulating thyroid hormone levels and growth in the chick. *J. Nutr.* 110:130-138.
- Griminger, P., and H. Fisher, 1968. Methionine excess and chick growth. *Poultry Sci.* 47:1271-1273.
- Han, Y., and D. H. Baker, 1993. Effects of excess methionine or lysine for broilers fed a corn-soybean meal diet. *Poultry Sci.* 72:1070-1074.
- Kino, K., and J. Okumura, 1986. The effect of single essential amino acid deprivation on chick growth and nitrogen and energy balances at ad-libitum and equalized food intakes. *Poultry Sci.* 65:1728-1735.
- May, J. D., 1979. Dietary lysine and serum thyroid hormone concentration. *Poultry Sci.* 58:1084.
- National Research Council, 1984. *Nutrient Requirements of Poultry*. 8th rev. ed. National Academy Press, Washington, DC.
- O'Dell, B. L., and J. L. Savage, 1966. Arginine-lysine antagonism in the chick and its relationship to dietary cations. *J. Nutr.* 90:364-370.
- Okumura, J., and K. Yamaguchi, 1980. Effect of excess individual essential amino acids in diets on chicks. *Jpn. Poult. Sci.* 17:135-139.
- Ousterhout, L. E., 1960. Survival time and biochemical changes in chicks fed diets lacking different essential amino acids. *J. Nutr.* 70:226-234.
- Pastro, K. R., B. E. March, and J. Biely, 1969. Diminished thyroidal activity in chicks in response to lysine deficiency. *Can. J. Physiol. Pharmacol.* 47:645-647.
- Snetsinger, D. C., and H. M. Scott, 1961. Efficacy of glycine and arginine in alleviating the stress induced by dietary excesses of single amino acids. *Poultry Sci.* 40:1675-1680.