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Identification of Limiting Amino Acids and Determination of Requirement of Total Sulfur-containing Amino Acids in a Low Protein Diet in Young Chicks.

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어린병아리에서 低蛋白質飼料내 制限아미노산의 규명과 含硫黃아미노산의 요구량 결정

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摘 要

低蛋白質(13% C.P.) 사료는 어린 병아리에서 단백질급원의 Net Protein Utilization價를 조사하거나 또는 아미노산의 不均衡을 연구할 때 적절한 방법으로 인정되고 있다. 이때의 기준 단백질로서 精製된 대두단백을 많이 이용하고 있으나 이런 사료의 制限 아미노산에 대한 연구가 되어 있지 않다. 本硏究에서는 사료내 유일한 단백질급원으로서 isolated soy protein을 15% 수준으로 함유한 purified-type의 基礎사료에서 제한 아미노산을 규명하고 이의 요구량을 조사하였다.

어린 병아리의 기초사료에 methionine, lysine, threonine 및 tryptophan 등을 단독으로 또는 두개 이상을 같이 첨가해서 급여한 결과, methionine만이 결핍된 것으로 나타났다. 總含硫黃아미노산(TSAA)의 요구량을 dose-response curve 방법에 의해 反應線과 plateau線과의 상호교차점에서 구하였는데, 최대의 성장과 사료섭취량을 위한 요구량은 각각 사료단백질의 4.73%와 3.73% 수준이였다. 이들 數値를 TSAA의 섭취량을 기준으로 표시해 보면 최대성장율, 사료섭취량 및 사료효율을 위해 각각 167.7, 136.8 및 159.7mg/bird/day의 量이 필요하였다.

I. INTRODUCTION

A low dietary protein level has been known to enhance any amino acid deficiency in experimental animals (Harper et al., 1970). According to Summers and Fisher (1961), if too low a protein level was fed, the proportion of test protein available for growth might be so small that its true growth promoting value might be effectively masked by the disproportional utilization of certain amino acids for maintenance purposes. Therefore, the protein level should be high enough to permit growth, and sufficiently high to distinguish between the amino acids requirement for maintenance and growth of the animals. They also suggest that a 13% protein level would be reasonable to young growing chicks for this purpose.

However, no studies have been reported yet for amino acids required for optimum growth of young chicks fed such a low protein diet.

This study was conducted to determine the most limiting amino acid in a 13% protein diet containing 15% isolated-soy-protein and, also, to determine the requirement of methionine for optimum growth of young chicks fed the diets.

II. METHODS AND PROCEDURES

Experiment I

Experiments I A and I B were conducted to determine in young chicks the most limiting amino acid in a low protein basal diet. A purified type diet (Table 1) containing 15% of isolated-soy-protein to provide a 13% level of dietary protein was used as the basal diet. The durations for experiments I A and I B were from 28 days old to 36 days of age and from 7 days old to 23 days of age, respectively.

Experiment I A One hundred and twenty chicks, 4 weeks of age, were divided into 24 groups with 5 birds each per replication and 3 replications per treatment. The initial average body weight of the chicks was 261 grams.

The experimental design (Table 2) was a completely randomized block design with 8 treatments. To determine the most deficient amino acid in basal diet (Table 1), each individual amino acid such as DL-methionine, L-lysine HCl, L-tryptophan and L-threonine, or a mixture of different combinations of each amino acid was added to the basal diet at the expense of mono-Na-glutamate ("General Biochemicals") on a weight for weight basis. Thus, all diets were isonitrogenous. The levels of amino acids supplemented were to be in excess of NAS-NRC requirements (1971) for young chicks. Therefore, the levels of each amino acid were provided at 80% of the NAS-NRC requirement (1971), though the protein level of the basal diet (13.05%) was equivalent to 65% of the requirement (20%). Actually, calculations based on the data from Scott *et al.* (1969) showed that only methionine(TSAA)

Table 1. Composition of purified – type basal diet (Experiment I).

Ingredients	% of Diet
Isolated Soy Protein[1]	15.00
Starch, corn	20.00
Corn oil, stabilized[2]	4.00
Cellulose ("Sulka floc)[3]	3.50
Choline – Cl (50%)[4]	0.35
Vitamin mixture[5]	0.50
Salt mixture[6]	6.24
Mono-Na-glutamate	1.00
Glucose monohydrate ("Clintose")[7]	up to 100
Calculated analysis	
Crude protein (%)	13.05
Crude fat (%)	4.00
Metabolizable Energy (Kcal./g.)	3.42
Calcium: Phosphorus	1.0:0.6

1. Isolated Soy Protein (87% protein), General Biochemicals, Laboratory Park, Charin Falls, Ohio.
   In experiment II A, Isolated Soy Protein was replaced by Promine-D (Central Soya, North Laramie Avenue, Chicago, Illinois).
2. Stabilized with ethoxyquin at levels of 125 mg/kg diet.
3. Brown Company, Berlin, New Hampshire.
4. Cholfeed-S, N.V. Chemische Industrie Randstad, Soest, Holland.
5. Supplied the following per kg. of diet:
   Vitamin A, 10,000 I.U.; Vitamin D_3, 1,000 I.C.U.; Vitamin E, 10 I.U.; Vitamin K, 2.0 mg.; Thiamin, 3.0 mg.; Riboflavin, 10.0 mg.; Pantothenic acid, 15.0 mg.; Niacin, 100 mg.; Pyridoxine, 6.0 mg.; Biotin, 0.15 mg.; Folacin, 3.0 mg.; Vitamin B12, 0.015 mg.
6. Supplied the following per kg. of diet:
   $CaCO_3$, 18.0 g.; $CaHPO_4 \cdot H_2O$, 25.0 g.; K_2HPO_4, 9.0 g.; $MnSO_4 \cdot H_2O$, 169.23 mg.; MgO, 828.9 mg.; $FeSO_4 \cdot 7H_2O$, 398.2 mg.; $CuCl_2 . 2H_2O$, 10.73 mg.; $ZnSO_4 \cdot H_2O$, 137.25 mg.; KI, 0.46mg.; $Na_2MoO_4 \cdot 2H_2O$, 9.84 mg.; $Na_2SeO_4 \cdot 10H_2O$, 0.47 mg., $CoSO_4 \cdot 7H_2O$, 1.0 mg.; H_3BO_3, 9.0 mg.; NaCl, 8.8 mg.
7. "Clinton", Clinton Corn Processing Co., Clinton, Iowa.

Table 2. Effect of various amino acids on body weight gain and feed intake of young chicks fed a purified-type diet with supplemental amino acids. (Experiment I A.)

Diets	Weight gain [1] g./bird/day	Feed intake [1] g./bird/day
Basal diet	0.03 ± 1.1[3],a[4]	22.7 ± 2.2[3],a[4]
〃 + DL-met. (0.26%)[2]	14.00 ± 0.9 b	38.1 ± 1.8 b
〃 + L-try. (0.07%)	-0.13 ± 0.7 a	22.0 ± 2.6 a
〃 + L-thr. (0.13%)	-0.55 ± 0.9 a	20.4 ± 1.8 a
〃 + L-lys. HCl (0.19%)	-0.70 ± 0.4 a	22.4 ± 1.2 a
〃 + L-thr. + L-lys.HCl	-0.12 ± 0.7 a	22.6 ± 0.8 a
〃 + DL-met. + L-try.	13.90 ± 0.8 b	37.4 ± 1.7 b
〃 + DL-met. + L-try. 〃 + L-thr. + L-lys.HCl	14.60 ± 0.3 b	38.7 ± 0.5 b

1. Mean of 5 birds/rep. X 3 replications/treatment.
2. Amount of amino acids added to the basal diet.
3. Mean ± S.E.
4. Means not carrying the same subscript in each column are significantly different ($P<0.01$) in accordance with Duncan's Multiple Range Test.

and tryptophan were limiting, with methionine most limiting. Threonine was just on the borderline. Lysine was added to be certain of its adequacy. Sources of each amino acid are Calbiochem (DL-methionine, B grade), ICN Pharmaceuticals, Inc. (L-lysine HCl), General Biochemicals (L-tryptophan), and Nutritional Biochemicals, Inc. (L-threonine).

The basal diet (Table 1) was a purified-type diet with a protein content of 13.05% and a calculated metabolizable energy of 3.42 kcal./g. Glucose monohydrate ("Clintose") and corn starch were the major sources of dietary energy. Corn oil stabilized with ethoxyquin was added at 4% level to provide a sufficient amount of essential fatty acids. Choline chloride ("Cholfeed-S") as a 50% active compound was added to the diet at a level of 0.35%. The basal diet contained 0.144% methionine and 0.158% of cystine, according to calculations based on data published by Scott *et al.* (1969)

Experiment I B A week old, 240 chicks with average body weight of 59.5 gram were alloted into 10 birds per replication and 4 replications per treatment. For this experiment, mono-Na-glutamate was omitted from the basal diet (Table 1) because it was reported to give damage to various areas of brain (Robinzon *et al.*, 1975). Amino acids were replaced at the expense of "Clintose" on the same weight basis, instead of replacing mono-Na-glutamate.

Table 3. Effects of various amino acids on body weight gain and feed intake of young chicks fed a purified-type diet with or without supplemental amino acids (Experiment I B)

Supplemented amino acids[1]				Weight gain[2]	Feed intake[2]
0.26% DL-meth.	0.07% L-try.	0.13% L-thr.	0.19% L-lys.HCl	g./bird/day	g./bird/day
–	–	–	–	1.3 ± 0.1[3] a[4]	8.1 ± 0.7[3] a[4]
+	+	+	+	8.1 ± 0.2 b	18.1 ± 0.5 b
–	+	+	+	1.1 ± 0.1 a	7.2 ± 0.3 a
+	–	+	+	8.0 ± 0.2 b	18.1 ± 0.1 b
+	+	–	+	8.4 ± 0.4 b	18.5 ± 0.5 b
+	+	+	–	8.5 ± 0.2 b	18.4 ± 0.2 b

1. Purified type diet (Table 1) supplemented with the amino acids at the indicated levels.
2. Mean of 10 birds/rep. x 4 replications/treatment.
3. Mean ± S.E.
4. Means not carrying the same subscript in each column are significantly different ($P < 0.01$).

A different approach from that of experiment I A was used in present experiment to determine the most limiting amino acid in the basal diet. For a particular treatment, an amino acid was omitted from a mixture of four amino acids (Table 3). The levels of each amino acid supplemented were the same as in experiment I A.

For both of the experiments I A and I B, feed intake and body weight gain were measured as a group basis. Chicks were fasted for 8 hours before starting the experiments.

Experiment II

Experiments II A and II B were designed to determine the requirement of methionine for optimum growth of young chicks fed a diet of 13% protein level. Since methionine alone can meet all requirements of sulfur-containing amino acids for optimum growth (Baker, 1976), the requirement of cystine was not determined separately in the present experiment.

The chicks of 3 weeks of age were fed diets for 1 week or 4 days, respectively, in experiments II A or II B.

Experiment II A One hundred and thirty five chicks of relatively uniform body weight (average 188 gram) were randomly assigned to 27 pens (3 replications x 9 treatments) as groups of five birds.

Various levels of DL-methionine (Table 4), i.e. 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40 and 0.50% of diet, were supplemented to the basal diet at the expense of glucose on a weight for weight basis. One treatment involved feeding the unsupplemented basal.

The formulation of the basal diet was the same as in Table 1 except that no mono-Na-glutamate was added, and the level of glucose was increased to 50% instead of 49%. Also the soy-protein (trade name, "Promine D") from "Central Soya Co." replaced the isolated-soy-protein from "General Biochemicals" used for previous experiments.

Experiment II B The same experimental design as in experiment II A was used except that the levels of DL-methionine supplemented by replacing glucose on a weight basis were 0, 0.15, 0.20, 0.25, 0.30, 0.35 and 0.50% (Table 5). The basal diet was as described in experiment II A.

One hundred and sixty eight chicks with average body weight of 166 grams were randomly assigned as 6 birds/replication and 4 replications/treatment for a 4 day experiment.

Daily feed intake and beginning and final body weight were measured on a group basis in both of the experiments.

Table 4. Estimation of requirement for DL-methionine to obtain optimum growth of young chicks fed a purified-type diet containing a level of 13% protein (Experiment II A). Also see Figure 1.

Levels of DL-methionine added to basal %	Weight gain[1] g./bird/day	Feed intake [1] g./bird/day	Gain/Feed
0	- 1.2 ± 1.3[2] a[3]	16.5 ± 2.2[2] e[3]	–
0.10	4.8 ± 0.1 b	23.5 ± 0.9 f	0.21 ± 0.01[2] h[3]
0.15	6.8 ± 0.1 bc	26.6 ± 1.5 fg	0.26 ± 0.02 i
0.20	7.9 ± 0.3 cd	28.3 ± 1.2 g	0.28 ± 0.01 ij
0.25	9.2 ± 1.2 d	29.8 ± 2.1 g	0.31 ± 0.03 j
0.30	10.5 ± 0.7 d	28.6 ± 1.7 g	0.37 ± 0.01 k
0.35	10.8 ± 0.7 d	28.6 ± 0.8 g	0.38 ± 0.02 k
0.40	10.8 ± 0.6 d	29.6 ± 1.5 g	0.37 ± 0.01 k
0.50	10.6 ± 0.5 d	29.2 ± 0.3 g	0.36 ± 0.01 k

1. Means of 5 birds/rep. x 3 replications/treatment.
2. Mean ± S.E.
3. Means not carrying the same subscript in each column are significantly different ($P<0.05$) in accordance with Duncan's multiple range test.

Table 5. Weight gain, feed intake, and gain/feed ratio of young chicks fed diets of various levels of DL-methionine (Experiment II B)

Level of DL-methionine added to basal diet %	Weight gain[1] g./bird/day	Feed intake [1] g./bird/day	Gain/Feed
0	1.8 ± 1.1[2] a[3]	18.8 ± 1.4[2] f[3]	0.14 ± 0.02 h
0.15	8.5 ± 0.4 b	26.0 ± 0.7 g	0.33 ± 0.02 i
0.20	9.7 ± 0.8 bc	28.2 ± 2.1 g	0.34 ± 0.01 i
0.25	10.5 ± 0.3 cd	27.4 ± 0.9 g	0.38 ± 0.02 j
0.30	11.8 ± 0.5 de	28.8 ± 1.0 g	0.41 ± 0.01 j
0.35	10.6 ± 0.3 cd	26.6 ± 0.6 g	0.40 ± 0.01 j
0.50	12.5 ± 0.3 e	28.3 ± 1.5 g	0.44 ± 0.01 k

1. Means of 6 bird/rep. x 4 replications/treatment.
2. Mean ± S.E.
3. Means not carrying the same subscript in each column are significantly different ($P < 0.05$).

Analysis of variance using a completely randomized design was employed to test treatment differences and Duncan's Multiple Range Test (Little and Hills, 1975) was used to test the differences among the means. Curves were fitted to the data by the least square method.

III. RESULTS AND DISCUSSION

Experiment I

The effects of supplementation of four different essential amino acids to the basal diet on the weight gain and feed intake of chicks are presented in Table 2.

The results indicated that birds fed the diets supplemented with amino acids other than DL-methionine consumed significantly less feed ($P<0.01$) and grew much less ($P<0.01$) than those on the diet with DL-methionine added. The supplementation with any other amino acid, i.e. lysine, tryptophan and/or threonine to the basal diet did not bring about any improvement in growth rate and feed intake. No significant differences were observed in weight gain and feed intake between the group fed the mixture of four essential amino acids and the group on the diet supplemented with DL-methionine only.

The data in Table 3 showed that the growth rate and feed intake were depressed for groups fed diets without supplemental DL-methionine ($P<0.01$). At the same time, the omission of amino acids other than DL-methionine from the mixture did not give any influence on the growth rate and feed intake.

The results from present experiement, therefore, lead to the conclusion that DL-methionine (or TSAA) is (or are) the first limiting amino acid(s) in this low protein diet in which isolated-soy-protein is the only source of dietary protein. From the calculation, the second limiting amino acid might be tryptophan or threonine. However, the supplementations of DL-methionine along with L-tryptophan or L-threonine to the basal diet did not improve the body weight gain or feed intake of chicks (Table 2 and 3). This lack of responses in performances of chicks to the addition of the two amino acids may be due to the low dietary protein used in the present study. There would appear to be no second limiting amino acid at the protein level of 13.1% for this diet.

One of the observations obtained from the two experiments was that the basal diet permitted maintenance of body weight in young chicks from 7 days to 35 days old.

Experiment II

The effects on weight gain and feed intake of young chicks fed various levels of supplemental DL-methionine in diet with 13% protein, are shown by the data presented in table 4 and Figure 1.

A linear response in weight gain to supplemental methionine was observed and further supplementation failed to produce any significant increase (Figure 1). The requirement of DL-methionine was estimated as the point at which the growth-response curve intersected a line representing the plateau for maximum weight gain. The dose-response line for growth was $Y = 27.5X + 2.3$ and the horizontal line representing the plateau portion for growth was $Y = -1.4X + 11.3$, where Y = weight gain in grams/bird/day, and X = % of supplemental DL-methionine. That point corresponded to 0.311% of supplemental DL-methionine. Thus, the estimated TSAA requirement for maximal growth at a 13% protein level, taking into account the methionine (0.144%) and cystine (0.158%) supplied by isolated-soy-protein, was 0.615% of diet or 4.73% of dietary protein.

When the data on feed intake were plotted against the level of supplemental DL-methionine (Figure 1), a linear response was observed. The regression analysis revealed that this line was characterized by the equation, $Y = 67.7X + 16.6$, where Y = feed intake in grams/bird/day and X = % of supplemental methionine. The point of intersection with the horizontal line, $Y = 1.8X + 28.4$, where Y = feed intake in grams/bird/day and X = % of supplemental methionine, was at 0.18% of supplemental DL-methionine. Therefore, the requirement of TSAA for maximum feed intake was estimated to be 0.48% of diet or 3.72% of dietary protein, considering the methionine (0.144%) and cystine (0.158%) from isolated soy-protein. Thus, the requirement of TSAA for optimal growth was 27% higher than that for optimal feed intake.

Gain/feed ratio (Table 4) appeared to become more efficient as the supplemental

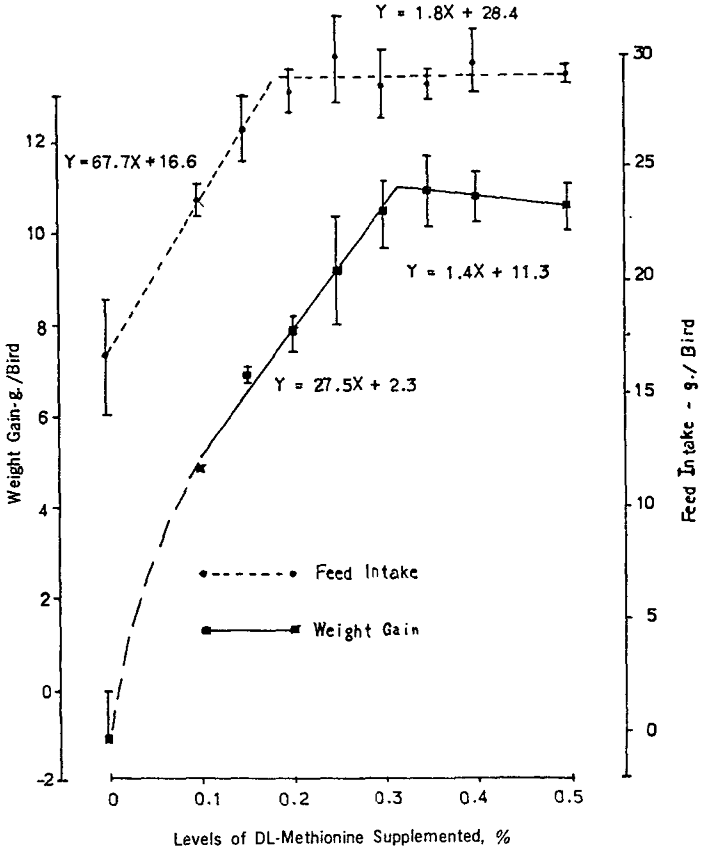

Figure 1.--Chick weight gain and feed intake as a function of dietary sulfur amino acids concentrations. The basal diet contained 0.144% methionine and 0.158% cystine (Experiment IIA).

levels of DL-methionine were increasing up to about 0.3% and thereafter, remained constant.

The results of weight gain, feed intake and gain/feed ratio for experiment II B are shown in Table 5. The data in this experiment were not as definitive in assessing optimum methionine requirement for feed intake and growth as in the first experiment. Variability was greater in the groups on the plateau portion. The estimate made for maximum growth appeared to be between 0.2 and 0.3% with the intersection occurring at 0.31%. The two equations for the growth responses were Y = 3.6X + 10.7 for the plateau portion and Y = 21.3X + 5.3 for the growing portion, where Y = average weight gain in grams/bird/day and X = % of supplemental DL-methionine.

The estimated optimum level of supplemental methionine to obtain maximum feed intake was 0.19%. The two equations for plateau and response were Y = 1.9X + 27.5 and Y = 47.2X + 18.8, respectively, where Y = average feed intake in grams/bird/day, and X = % of DL-methionine supplemented in the diet.

Thus, again considering the levels of methionine and cystine from isolated-soy-protein, the TSAA requirements for maximum growth and feed intake were 4.73% and 3.73%, of dietary protein, respectively.

The gain/feed ratio (Table 5) was plateaued at a point between 0.20% and 0.25% of DL-methionine level which was somewhat lower than the level of about 0.3% in experiment II A.

Because the two experiments were in close agreement (Tables 4 and 5), they were combined and the data were expressed in Table 6 and Figure 2 in terms of the amount of TSAA intake. The amount of TSAA intake required for maximum weight gain was 167.7 mg. TSAA. This value was based on the intersection of the two response lines, each defined by the equations, $Y = 0.0085X + 9.39$, and $Y = 0.077X - 2.10$, representing the plateau and response lines, respectively, where Y = weight gain in grams/bird/day, and X = amount of TSAA intake in mg./bird/day. The requirement of TSAA for maximum feed intake estimated in the same way, appeared to be 136.8 mg. TSAA/bird/day. The weight gain at this level of TSAA intake (136.8 mg.) was 76% of the maximum weight gain. The difference between the amounts of TSAA intake for maximum weight gain and feed intake was 30.9 mg. which was approximately 18.4% of the amount required for maximum gain. Thus, the 18.4% of TSAA intake above the point of maximum feed intake was used for chicks to grow an additional 24% to arrive at their maximum weight gain.

Gain/feed ratios in relation to TSAA intake were shown in Figure 2. Two equations calculated for that criterion were $Y = 0.00017X + 0.349$ for the horizontal line, and $Y = 0.0031X - 0.119$ for the response line, where Y = gain/feed ratio, and X = TSAA intake as

Table 6. Results of total sulfur amino acids (TSAA) intake related to weight gain, feed intake, and gain/feed from experiments II A and II B.

Levels of DL-methionine added %	TSAA intake mg./bird/day	Average results of expt. II A and II B[1]					
		Weight gain g./bird/day		Feed intake g./bird/day		Gain/Feed	
0	53.5	0.3		17.7		0.02	
0.10[2]	94.5	4.8		23.5		0.20	
0.15	118.9	7.7		26.3		0.29	
0.20	142.1	8.8		28.3		0.31	
0.25	157.9	9.9		28.6		0.35	
0.30	172.9	11.2		28.7		0.39	
0.35	180.0	10.7		27.6	28.6	0.39	0.39
0.40[2]	207.8	10.8	11.1	29.6		0.36	
0.50	231.0	11.6		28.8		0.40	

1. Data from Tables 4 and 5 were averaged.
2. Only from experiment II A.

mg./bird/day. The ratio was improved by addition of methionine to bring TSAA intake to 159. 7 mg./bird/day.

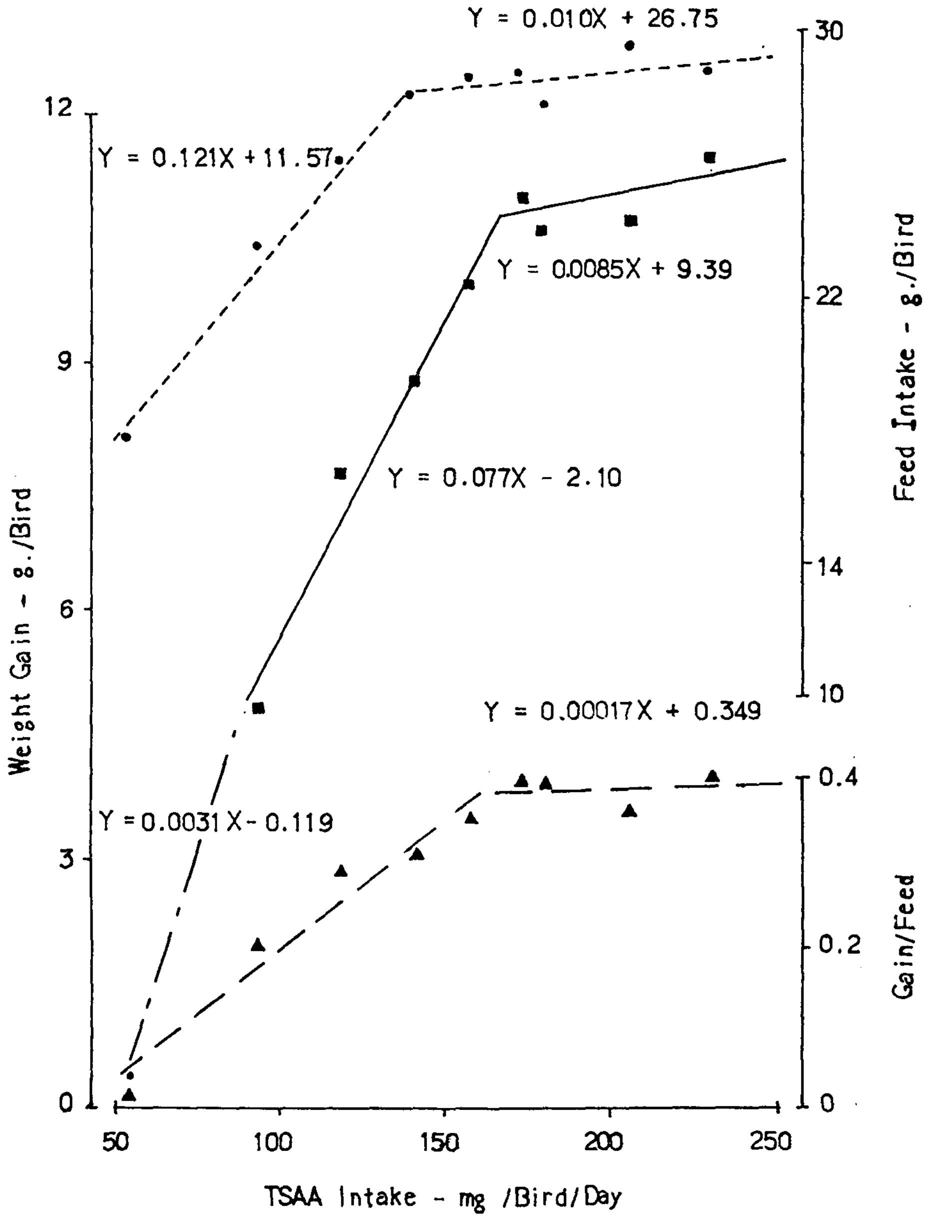

Figure 2.--Chick weight gain (■——■), feed intake (•-----•), and gain/feed (▲— —▲) as a function of dietary TSAA intake (Experiment II).

IV. SUMMARY

Since a 13% dietary protein level is generally accepted as a standard in evaluating net protein utilization values of protein sources in chicks, limiting amino acids in a 13% protein basal diet containing 15% isolated soy-protein as the only source of dietary protein, were identified.

Of such amino acids as methionine, lysine, threonine and tryptophan added to the basal diet singly or as a combination, methionine appeared as the only limiting amino acid for optimum growth of the chicks. When the requirement of total sulfur-containing acids (TSAA) was estimated as the point at which the dose-response curve intersected a line representing the plateau for maximum performance, the TSAA requirements for maximum growth and feed intake were 4.73% and 3.73% of dietary protein, respectively. The values, expressed in terms of TSAA intake, required for maximum weight gain, feed intake and gain/feed ratio were 167.7, 136.8 and 159.7 mg/bird/day, respectively.

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