Quantitative Aspects of the Nicotinic Acid–Tryptophan Interrelationship in the Chick

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The tryptophan requirement of the chick has been studied independently of the nicotinic-acid requirement by Almquist (1947), who reported a requirement of 0·25% L-tryptophan, and by Wilkening, Schweigert, Pearson & Sherwood (1947), who found a requirement of 0·18% of the diet. Glista (1951), using a forced-feeding technique with nitrogen balance as the criterion of amino-acid adequacy, reported an L-tryptophan requirement of 0·20%. He fed DL-tryptophan and assumed a 40% utilization of the D form.

Briggs, Mills, Elvehjem & Hart (1942) found the nicotinic-acid requirement for optimal growth and the prevention of chick black-tongue to be 1·8 mg/100 g diet. In later work, Briggs (1945) and Briggs, Groschke & Lillie (1946) concluded that tryptophan could replace nicotinic acid for the chick. It was also shown (Briggs *et al.* 1946; Groschke & Briggs, 1946; Briggs, 1945) that the type of dietary protein and the amino-acid balance were important factors in the estimation of the nicotinic-acid requirement of the chick.

West, Carrick, Hauge & Mertz (1952) and Childs, Carrick & Hauge (1952) have studied simultaneously the nicotinic-acid and tryptophan requirements of the chick. They report the tryptophan requirement as 0·19% and the nicotinic-acid requirement as 3 mg/100 g diet. In their experiments, which are difficult to interpret because of the question of utilization of D-tryptophan and the use of various proteins as sources for tryptophan, tryptophan failed to compensate for a nicotinic-acid deficiency in contradiction to the findings of Briggs (1945).

Sarma & Elvehjem (1946) reported an increased nicotinic-acid requirement needed to overcome a depression in chick growth due to corn grits. Scott, Singsen & Matterson (1946) also reported the need for added nicotinic acid in a simplified ration high in maize meal.

In a recent collaborative study on the nicotinic-acid requirement of chicks on practical starting rations, Matterson & Decker (1953), reporting for the collaborators, failed to obtain a clear-cut answer to this question.

In view of the known interrelationship between tryptophan and nicotinic acid in other animal species (Krehl, 1949) and the conflicting evidence with the chick, it seemed of importance to study this relationship further. In order to overcome some

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of the weaknesses of the earlier studies, a diet was designed free of both tryptophan and nicotinic acid, so that both nutrients could be studied simultaneously with the use of chemically pure compounds.

EXPERIMENTAL

Chicks and their management

Day-old New Hampshire × Columbian male chicks were used in all experiments. Except for Exp. 4 the chicks were housed in individual wire cages maintained in electrically heated batteries. All chicks were wing-banded, and weights were recorded at 1 day and at weekly intervals thereafter. A pair of chicks was placed in each cage and kept for 3 days, since it was found that in this way the chicks learned more rapidly to locate the water and feed dishes and thus got off to a better start. At the end of 3 days the poorer chick of each pair was discarded, and accurate feed-consumption records were kept from then on. All chicks were placed on their respective diets at 1 day of age.

Pilot experiment to determine the most suitable basal diet

The purified diet used was modified from one then in use in this laboratory for the study of unidentified growth factors which contained 22 % casein supplemented with 0·3 % DL-methionine, 0·6 % glycine and 0·3 % L-arginine hydrochloride, supplying about 1·2 % arginine in all. A salt-free hydrolysed casein (Hy-Case (*R*), a product of Sheffield Chemical Company, Inc., Norwich, N.Y.) was substituted for the whole casein, and in a pilot experiment the adequacy of this diet in amino-acids other than tryptophan was determined, 0·5 % tryptophan being added to replace that destroyed in the hydrolysis. Threonine and histidine were added at the levels indicated in Table 1,

Table 1. *Pilot experiment. Effect on growth of chicks of amino-acid supplementation of a hydrolysed-casein diet**

(Mean results for five chicks/treatment)

Supplement	Weight at 4 weeks (g)
None	100
0·2 % L-threonine	120
0·1 % L-histidine hydrochloride	110
0·6 % L-arginine hydrochloride	239
0·2 % L-threonine + 0·1 % L-histidine hydrochloride + 0·6 % L-arginine hydrochloride	223

* 22 % hydrolysed casein supplemented with 0·3 % DL-methionine, 0·6 % glycine, 0·3 % L-arginine hydrochloride, 0·5 % DL-tryptophan; other ingredients as in Table 2.

on the basis of Henderson's finding (Henderson, Koeppe & Zimmerman, 1953) that a similarly prepared hydrolysed casein was deficient for the rat in these amino-acids. Recent work on the arginine requirement of the chick (Young, Gillis & Norris, 1953; Snyder, Morrison & Scott, 1954; Wietlake, Hogan, O'Dell & Kempster, 1954) indicates that the (U.S.A.) National Research Council requirement figure of 1·2 % (Bird, Almquist, Cravens, Hill & McGinnis, 1954) is much too low and that 1·7 %

is more nearly adequate. It has been suggested that the arginine in whole casein is not completely available (Klose, Stokstad & Almquist, 1938). On these grounds arginine supplementation to the basal diet was also studied.

The results in Table 1 clearly demonstrated that arginine when fed at the 1·2% level recommended by the (U.S.A.) National Research Council was extremely limiting. This finding on a hydrolysed-casein diet confirms the higher arginine requirement established with whole casein, indicating that availability is not involved. Neither threonine nor histidine at the levels fed exerted any further growth improvement above that of arginine. On the basis of this preliminary investigation, the diet shown in Table 2 was employed in all the experiments with synthetic diets (Exps. 1, 2 and 3).

Table 2. *Hydrolysed-casein diet used in Exps.* 1, 2 *and* 3

Ingredient	Amount (%)	Vitamins added	Amount (mg/kg)
Hydrolysed casein*	22·00	Thiamine hydrochloride	25
Salts (Glista, 1951)†	5·34	Riboflavin	16
Maize oil	3·00	Calcium pantothenate	20
Choline chloride	0·20	Vitamin B_{12}	0·02
Cellulose (Ruffex)	3·00	Pyridoxin hydrochloride	6
DL-Methionine	0·30	Biotin	0·6
L-Arginine hydrochloride	1·00	Folic acid	4
Glycine	1·00	Inositol	100
Glucose (cerelose)	64·16	*p*-Aminobenzoic acid	2
		2-Methyl-1:4-naphthoquinone	5
		α-Tocopheryl acetate	20
		Ascorbic acid	250
		Vitamin A and D concentrate‡	1000

* Hy-Case (*R*), a product of Sheffield Chemical Company, Inc., Norwich, N.Y.

† Salt mixture: $CaCO_3$, 0·3000; $Ca_3(PO_4)_2$, 2·8000; K_2HPO_4, 0·9000; $MgSO_4.7H_2O$, 0·2500; $Fe(C_6H_5O_7)_2.6H_2O$, 0·1400; $ZnCl_2$, 0·0020; KI, 0·0040; $CuSO_4.5H_2O$, 0·0020; H_3BO_3, 0·0009; $CoSO_4.7H_2O$, 0·0001; $MnSO_4.H_2O$, 0·0650; NaCl, 0·8800 parts.

‡ Containing 10,000 i.u. vitamin A and 600 i.u. vitamin D_3 per g.

Design of experiments

Experiment 1. Exp. 1 was a 5 × 5 factorial design laid out in four complete Graeco-Latin squares. The cage racks were best suited to this design, since there were five cages to each row and five columns on each side of the battery, so that four blocks involved two complete batteries with four chicks to each treatment. The design, except for the Graeco-Latin-square arrangement, and the levels of nicotinic acid and tryptophan studied are shown in Table 3.

Experiment 2. As indicated in Table 4, Exp. 2 involved part of the same design as Exp. 1, with the important difference that to each diet there were added at the expense of glucose 0·3% L-histidine, 0·8% DL-threonine and 0·65% L-leucine. Anderson, Combs, Groschke & Briggs (1951) have shown that these amino-acids, among others, each fed as 4% of the diet, depress chick growth, and that this growth depression can be alleviated with nicotinic acid. The amounts of histidine, threonine and valine used to study their effect on the nicotinic acid–tryptophan requirements represent the differences in the calculated values for these amino-acids between the hydrolysed-casein diet employed in this study and a practical maize–soya-bean meal chick starting ration.

Experiment 3. Table 5 shows the design employed in Exp. 3 on the effect of maize on the nicotinic acid–tryptophan requirements. In series A maize replaced all the glucose, and the protein content of the ration was thus increased by the amount present in the added maize (6%). In series B the protein content was adjusted by reducing the amount of hydrolysed casein to the extent of the protein added by the maize (16% hydrolysed casein and 6% maize protein).

Experiment 4. The effect of additional nicotinic-acid supplementation to two practical chick starting rations was studied in Exp. 4. The compositions of the diets were:

Soya-bean meal (50% protein) (%)	—	30·00
Fish meal (menhaden, 65% protein) (%)	23·00	—
Maize meal (%)	65·30	53·67
Lucerne meal (%)	5·00	5·00
Distiller's solubles (%)	4·00	4·00
Whey (%)	2·00	2·00
Salt (%)	0·50	0·50
Vitamin A and D oily solution (%)	0·15	0·15
$MnSO_4.H_2O$ (%)	0·05	0·05
Dicalcium phosphate (%)	—	3·33
Limestone (%)	—	1·30
Riboflavin (mg/100 lb.)	200	200
Vitamin B_{12} (mg/100 lb.)	—	1

Unlike in Exps. 1, 2 and 3, which were conducted in individual-cage batteries with four chicks per treatment, fifteen chicks were placed in each lot of Exp. 4, which was carried out in group batteries. The levels of nicotinic acid added to the diets were as indicated in Table 6.

RESULTS

Experiment 1. The 3-week weights in Exp. 1 (Table 3) show a sharp break in growth at the zero nicotinic-acid level between 0·15 and 0·20% tryptophan as well as at the 0·15% tryptophan level between 0 and 2·5 mg/100 g diet of nicotinic acid. It thus appears that the L-tryptophan requirement of the chick is met by 0·15% in the presence of adequate nicotinic acid. At this level of tryptophan, 2·5 mg nicotinic acid/100 g diet appear to satisfy the nicotinic-acid requirement. In the absence of nicotinic acid, 0·20% L-tryptophan, or more, completely replaces nicotinic acid. On the other hand, no benefit is derived from higher levels of nicotinic acid at the sub-marginal L-tryptophan level of 0·10%.

Analysis of variance of these results is not appropriate owing to a lack of homogeneity; nor does this type of analysis yield information on points of maximum response. Instead, regression analysis was employed in fitting a curved surface to the results, which permits the construction of contour charts from which various combinations of nicotinic acid and tryptophan may be located that will yield given growth responses. The details of this analysis will be published separately.

Nine scattered cases of perosis were found among the 100 birds on experiment, and no clear connexion with nicotinic-acid adequacy or inadequacy could be established.

Table 3. *Exp.* 1 *Effect of nicotinic acid and* L-*tryptophan on growth of chicks and incidence of perosis. Individual weights (g) at* 3 *weeks and incidence of perosis for blocks of four chicks receiving nicotinic acid and tryptophan supplements to a basal diet**

Nicotinic acid level (%)	Block	L-tryptophan level (%) 0·10	0·15	0·20	0·30	0·40
0	1	54	61	130	109	151
	2	50	62	137	132	121
	3	48	84	138	139	106
	4	48	D	148	117	130
	Group mean	50	69	138	124	127
2·5	1	94P	D	137P	142	121
	2	67	118	102	122	139
	3	63	125	139	106	114
	4	80	121	124	117	150P
	Group mean	76	121	126	122	131
5·0	1	56	D	138	132P	158
	2	69	120	126	131	106
	3	D	149P	129	126	127
	4	73	130	134	157	131
	Group mean	66	133	132	137	130
10·0	1	59	145	117P	104	139
	2	45	100	147P	116	90
	3	59	141	123	156	221
	4	49	131	122	127	175
	Group mean	53	129	127	126	156
20·0	1	55	135	144	151P	168
	2	68	119	154	162	148
	3	77	119P	112	123	148
	4	63	152	170	139	128
	Group mean	66	131	145	144	148

P = perosis; D = died.

* See Table 2.

Experiment 2. The effect of the addition of histidine, threonine and leucine to the hydrolysed-casein diet was reflected in the large incidence of perosis (Table 4). The incidence with the amino-acid supplemented diets was highly significantly greater than that in Exp. 1 ($\chi^2_c = 9{\cdot}273$, $P < 0{\cdot}01$). The weight changes show the same general picture for nicotinic acid and tryptophan as in Exp. 1, but much greater amounts of nicotinic acid or tryptophan were required for protection against perosis. The perosis was so severe that the experiment had to be terminated at 19 days, since inability to move about seriously interfered with the intake of food and water and thus had begun to affect the weight gains of the afflicted birds.

Experiment 3. In view of the indicated L-tryptophan requirement of 0·15% in the presence of adequate nicotinic acid (see Exp. 1) levels of 0·15 and 0·20% were used in Exp. 3 to study the influence of maize on the nicotinic acid–tryptophan requirement. Statistical analysis of the results given in Table 5 revealed a highly significant effect on the nicotinic-acid requirement, whereas that on the tryptophan requirement was

Table 4. *Exp. 2. Influence of amino-acids on the nicotinic acid–tryptophan requirement of chicks. Individual weights (g) at 19 days and incidence of perosis for blocks of four chicks receiving nicotinic acid and tryptophan supplements to a basal diet* to which 0·3 % L-histidine, 0·8 % DL-threonine and 0·65 % L-leucine were added at the expense of glucose*

Nicotinic-acid level (%)	Block	L-tryptophan level (%)			
		0·10	0·15	0·20	0·30
0	1	60	103	111	148
	2	50	74	141	154
	3	44	65	155	91
	4	60	55	141 P	147 P
	Group mean	53	74	137	135
2·5	1	70	99	D P	D
	2	76	144 P	125 P	107
	3	76 P	117	105	148 P
	4	D	110 P	119	116
	Group mean	74	118	116	124
5·0	1	63	102 P	200 P	
	2	79 P	97 P	162	
	3	72	99	135	
	4	60	D P	118	
	Group mean	69	99	154	
10·0	1	75	106		
	2	66 P	106		
	3	66 P	110		
	4	66 P	177		
	Group mean	68	110		

P = perosis; D = died.

* See Table 2.

not significant. This finding indicates again that 0·15 % L-tryptophan fully meets the chick's requirement for that amino-acid in the presence of adequate nicotinic acid. Unlike in the earlier study (Matterson & Decker, 1953), the 0·20 % level of L-tryptophan did not meet the entire nicotinic-acid requirement in either series A or series B. This finding, together with the incidence of perosis encountered, indicates that maize does exert a stress condition that can be overcome with higher levels of nicotinic acid. The better growth attained by the chicks in series B receiving no nicotinic acid and 0·15 % tryptophan compared to those in series A (Table 5) is probably the result not only of the lower total protein in series B but also of the inferior protein quality of the maize that was replacing a good-quality casein protein, so that the total effective protein in series B may have been less than in the 22 % hydrolysed-casein diets previously used. Thus, more tryptophan would have become available for nicotinic-acid formation if the tryptophan requirement for protein synthesis had been lowered by the effective protein availability. The importance of the protein level, especially for the balance of amino-acids in these proteins, is further emphasized by the somewhat greater incidence of perosis in series A. If maize contains a toxic principle, as has been postulated (Borrow, Fowden, Stedman, Waterlow & Webb, 1948), then the degree of perosis should have been the same in both series A and series B since the same amount of maize was fed. Since this was not so, and in view of our earlier findings on the effect of certain amino-acids on the incidence of perosis, it appears that the stress condition induced by maize may be due to its amino-acid composition or balance. This experiment also was terminated early because of the severe crippling of birds by perosis. The nicotinic-acid requirement necessary for both optimum growth and prevention of perosis on the maize diet appeared to be about 10 mg/100 g diet.

Table 5. *Exp.* 3. *Effect of maize on the nicotinic acid–tryptophan requirement of chicks. Individual weights (g) at 17 days and incidence of perosis for blocks of four chicks receiving a basal diet* in which the glucose was replaced by maize*

Nicotinic-acid level (%)	Block	L-tryptophan level (%) Series A† 0·15	Series A† 0·20	Series B‡ 0·15	Series B‡ 0·20
0	1	88P	136P	166	119
	2	65	136P	137P	171P
	3	93P	171	151P	165
	4	87	D	138	138
	Group mean	83	148	148	148
1·25	1	139P	153	170	186P
	2	160P	165	153P	116
	3	167P	180	194	129
	4	140	180	155P	153P
	Group mean	152	170	168	146
2·5	1	133P	149P	194	162
	2	136P	210P	182P	165P
	3	168P	145	161	152P
	4	156P	147	176	200
	Group mean	148	163	178	170
5·0	1	181	141P	190	213
	2	D P	188	199	174P
	3	168	164	228	197
	4	158P	203	177	132
	Group mean	169	174	196	179
10·0	1	144	145	146	192
	2	170	185P	223	184
	3	215	168P	148	150
	4	212P	179	119P	171
	Group mean	185	169	159	174

P = perosis; D = died.
* See Table 2.
† 22 % hydrolysed casein + 6 % protein from maize.
‡ 16 % hydrolysed casein + 6 % protein from maize.

Experiment 4. The results of Exp. 4 given in Table 6 indicate clearly that neither a practical soya-bean nor a practical fish-meal diet was improved by the addition of nicotinic acid. No perosis was encountered, indicating that the content of either nicotinic acid or tryptophan or both is adequate in diets of this type for good growth and prevention of perosis.

Table 6. *Exp. 4. Influence of nicotinic-acid supplements on the growth of chicks on practical starting rations. Mean weights (g) at 3 weeks of groups of fifteen chicks*

Nicotinic-acid supplement (mg/100 g diet)	Soya-bean diet	Fish-meal diet
0	297	275
0·35	263	252
0·70	280	262
1·40	269	256
2·80	297	259
5·60	271	253

DISCUSSION

These studies have shown that the L-tryptophan requirement on a 20% protein diet is met by 0·15%. This value is in close agreement with the value of 0·18% reported by Wilkening *et al.* (1947), but is considerably lower than that of 0·25% reported by Almquist (1947). Differences such as these are undoubtedly due to differences in protein sources used in these studies and the effective protein level fed. Thus Almquist used a 12% oxidized-casein, 10% gelatin diet and considered the total protein level to be about 21%. He assumed that the protein of gelatin is equal to that of casein and does not seem to have considered that gelatin has a biological value of nought (Bender, Miller & Tunnah, 1953) and that 12% casein can hardly make up all the deficiencies in gelatin. The work of Briggs (Briggs, 1945; Groschke & Briggs, 1946; Briggs *et al.* 1946), moreover, shows very clearly that gelatin has a marked influence on the nicotinic-acid requirement of chicks, and it is not certain that these higher requirements were met by Almquist when he studied the tryptophan requirement on a diet containing 10% gelatin.

In our studies L-tryptophan could replace completely nicotinic acid, the finding confirming the work of Briggs *et al.* (1946), but contradicting that of Childs *et al.* (1952). It is possible that the unexplained high mortality in the experiments of Childs *et al.* was the result of some disorder which may have influenced the response of the chicks to the nutritional variables.

The results of Exp. 1 permit a rough estimation of the efficiency of the conversion of tryptophan to nicotinic acid. Since 0·15% L-tryptophan with 2·5 mg nicotinic acid/100 g diet was as effective as 0·20% L-tryptophan, one may calculate that 50 mg L-tryptophan are roughly equivalent to 2·5 mg nicotinic acid. This equivalence would indicate an efficiency of replacement of nicotinic acid by tryptophan of 1:20.

The nicotinic-acid requirement appears to be conditioned by the balance of certain amino-acids in the diet. On a good protein like casein the nicotinic-acid requirement is quite low, being met by 2·5 mg/100 g diet. Whereas marked growth depression was observed by Anderson *et al.* (1951) upon the addition of 4% of certain amino-acids, our findings illustrate that much smaller amounts of some of these amino-acids exert enough stress on chicks to increase the nicotinic-acid requirement for the prevention of perosis. In our experiments an optimum protein level of about 20% was given, so that an increase in any essential amino-acid could hardly make any other essential amino-acid limiting since the protein requirements had already been met. In other words, if the addition of histidine, threonine and leucine in Exp. 2 or of maize in Exp. 3 had made tryptophan the limiting amino-acid then it should be expected that in the presence of more tryptophan and adequate nicotinic acid better growth would have resulted. This was not so. Thus our results indicate that nicotinic acid is involved in the metabolism of these amino-acids directly and not, as suggested by Henderson *et al.* (1953), indirectly by making tryptophan the limiting amino-acid.

It is interesting to note that nicotinic acid at inadequate tryptophan levels does not appreciably improve growth. This finding implies a considerable conversion of tryptophan to nicotinic acid even at tryptophan levels insufficient and limiting for growth.

The addition of maize clearly produces a stress condition that responds to nicotinic-acid supplementation. Since the tryptophan requirement was met by 0·15% L-tryptophan under all conditions studied it appears that nicotinic acid is directly involved, as already indicated. The work of Groschke, Anderson & Briggs (1948) on the influence of amino-acids in zein protein on the nicotinic-acid requirement of the chick lends additional support to our findings, which suggest a direct involvement of nicotinic acid in the metabolism of certain amino-acids.

The excellent growth obtained with the maize-containing diets compared to that with the glucose and hydrolysed-casein diets may be explained on the basis of (1) unidentified growth factor(s) in maize as already suggested by Briggs *et al.* (1946), and (2) the physical properties of the purified diet. Hydrolysed casein is extremely hygroscopic, and at the 22% level, together with glucose (cerelose) it tends to stick to the beaks and tongues of the birds, creating a mechanical interference in food intake. The presence of maize meal largely prevented this interference. In our opinion the second explanation is the likely one.

The finding that nicotinic acid did not improve growth when added to either of two practical chick starting rations can be explained on the basis of the tryptophan content of these rations. Microbiological assays gave values of 0·31 and 0·26% L-tryptophan for the soya-bean and fish-meal diets respectively. These levels of tryptophan are sufficiently high, in view of our other results, to obviate the need for any nicotinic-acid supplementation, even if the diets did not contain any nicotinic acid at all. Calculation shows that they did contain about 3 mg/100 g diet. However, the availability of the nicotinic acid in plant materials is not well known, since it occurs mainly in a bound form (Chaudhuri & Kodicek, 1950), and there is much variation in nicotinic-acid content from sample to sample (Rodriguez, Hunt & Bethke, 1950).

SUMMARY

1. The nicotinic acid–tryptophan requirements of chicks have been studied in a series of three factorially designed experiments involving about 300 chicks individually fed on a basal diet with hydrolysed casein, devoid of both nutrients.

2. The L-tryptophan requirement of the chick was consistently met by 0·15% of this amino-acid in the diet in the presence of adequate nicotinic acid.

3. In the absence of nicotinic acid, levels of L-tryptophan of 0·20% or higher completely satisfied both the tryptophan and nicotinic-acid requirements.

4. At the marginal tryptophan level of 0·15% the nicotinic-acid requirement varied from 2·5 to 10 mg/100 g diet, depending upon certain stress factors.

5. Small amounts of histidine, threonine and leucine produced a high incidence of perosis which could be prevented by about 10 mg nicotinic acid/100 g diet.

6. The addition of maize to the diet did not affect the tryptophan requirement, but did increase the nicotinic-acid requirement to about 10 mg/100 g diet for optimum growth and prevention of perosis.

7. The stress induced by maize appears to be due to its amino-acid composition rather than to any toxic principle.

8. The amino-acid stress condition indicates a direct involvement of nicotinic acid in the metabolism of histidine, threonine and leucine.

9. Practical fish-meal and soya-bean meal rations were found adequate in nicotinic acid and tryptophan for optimum growth and prevention of perosis.

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