Arginine in poultry nutrition

3*. Agent and target in amino acid interactions

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1. A series of trials has been conducted to investigate interactions between amino acids in the diet of the growing chick. 2. Diets were prepared in which the level of arginine was limiting. To these diets were added excess levels of lysine, histidine, isoleucine and leucine. Of these amino acids lysine was most effective in reducing the growth rate. Growth rate was restored by adding arginine. 3. The addition of excess quantities of lysine to a diet in which the level of tryptophan was limiting and the level of arginine adequate did not reduce growth rate. 4. These results confirm the existence of a specific interaction between arginine and lysine. The concept is discussed that such specific interactions between pairs or among small groups of amino acids might underly many of the phenomenons of amino acid imbalance.

The uncertainty regarding the arginine requirement of the young chick (see Lewis, Smith & Payne, 1963) is likely to be a consequence of the widely different amino acid composition of the diets used (Klain, Scott & Johnson, 1958, 1959, 1960; Fisher, Shapiro & Griminger, 1960; Anderson & Dobson, 1959). It is well recognized that the requirements of chicks for individual essential amino acids to achieve optimum growth are minimal when excesses and deficiencies of all other amino acids are eliminated; i.e. when the amino acid pattern of the diet is balanced.

Amino acid imbalance was defined by Harper (1958) as that which is operative in 'those cases in which the addition of a relatively small amount (what might be called a supplemental amount) of an indispensable amino acid, or of a mixture of such amino acids, or of an unbalanced protein to a diet that is low in one or more amino acids causes a retardation of growth or some other adverse effect that can be prevented by the concomitant addition of small quantities of the limiting amino acids to the diet. The critical points are that the diet must contain a marginal amount of at least one indispensable amino acid and that the effect of the imbalance can be prevented by a small supplement of the most limiting amino acids in the diet.' This concept does not cover all effects produced by the addition of excess amino acids to the diet, since interactions between amino acids leading to a limitation of growth are not all of the same type. Thus whereas in some instances the addition of one or more amino acids may precipitate a deficiency of the most limiting amino acid, corresponding to the 'imbalance' of Harper (1958), in other instances the addition of one amino acid may be specifically antagonistic to another, and again there may be toxic effects which are not corrected by the addition of a single amino acid. Distinction between these types of interaction was made by Elvehjem (1956).

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It is not clear which of these effects is operative in increasing the arginine requirement of chicks when diets based on casein are used (see Lewis *et al.* 1963). It is possible that there is a relation between this increased requirement and the large amounts of lysine contributed by casein. It has been shown that addition of lysine to a diet marginally deficient in arginine results in poor growth which is alleviated by supplementation with arginine (Lewis *et al.* 1963), and there is some evidence for a specific antagonism (Jones, 1962; O'Dell, Limbaugh & Savage, 1962).

Experiments have been conducted which investigate such interactions in more detail. An attempt has been made to determine the specificity of such interactions, that is to ascertain whether the addition of amino acids other than lysine may precipitate an arginine deficiency and, conversely, whether the requirements for amino acids other than arginine are increased by the addition of excess lysine.

EXPERIMENTAL

Housing of chicks

Details of the room in which the chicks were housed, together with the lighting, heating and ventilation regime employed, have already been described (Lewis *et al.* 1963). In Expts A 12, A 13 and A 14, cages were used as previously described (Lewis *et al.* 1963), but these were modified in Expts A 13 and A 14 by dividing each into two cages with a vertical partition. This allowed the use of more groups of chicks. In Expt A 15, cages of a new type were used. These were 15 in long by 12 in wide by 10 in high and were mounted in blocks of two vertical groups of six. A group of seventeen such blocks was housed in the room described. The cages were made of galvanized wire mesh with removable fronts; the food and water troughs, designed to minimize spilling, were sited along the front of the cage. Beneath the floor of each cage was a polythene tray to collect all droppings.

Management

The birds used were of a broiler-cross type based on White Rock × Light Sussex. For the 1st week of their lives the birds were fed on a commercial broiler starter diet containing 22% protein. At the end of the 1st week the birds were weighed, and groups selected for uniformity of weight were wing-banded and allocated to the cages. The birds were weighed weekly.

All diets were given in finely ground form and food and water were always available to the birds. Weighed quantities of food were added to each trough as required and the residue of uneaten food was weighed weekly.

Procedure

The general procedures used have been previously described (Lewis *et al.* 1963). In Expt A 15 the procedure for determining nitrogen balance was modified as follows. Droppings were collected into 300 ml of 0·1% (w/v) H_2SO_4 contained in the polythene tray. At the end of the collection period the acid containing the droppings from

each cage was made up to a known volume and homogenized in an Atomix blender (M.S.E., Ltd, London). A portion was taken for determination of the dry matter and nitrogen contents.

Diets and experimental design

In Expts A 12, A 13 and A 14 the basal diet was composed essentially of maize and maize-gluten meal. The ingredients and the analysis of this diet are given in Table 1. The standard supplement, supplying adequate quantities of minerals and vitamins and added to all diets, has been described by Lewis *et al.* (1963). An additional supplement was added supplying amino acids, other than those under test, in which the basal diet was deficient as assessed by the recommendations of the (USA) National Research Council (1960). The composition of this supplement is also given in Table 1. The basal diet supplied 0·79% arginine (Table 2), which for a diet of 21·6% protein has been shown to be marginally inadequate (Lewis *et al.* 1963).

Table 1. *Expts A 12–A 15. Ingredients and composition of the basal diets and additional supplements*

(Compositions are expressed as percentages of the dry matter, except for protein which is expressed on an air-dry basis; the composition of the standard supplement was given by Lewis *et al.* (1963))

Expts ...	A 12–A 14	A 15
Basal diet		
Maize meal	55	65
Maize-gluten meal	37	20
Zein	—	7
Dried whey	3	3
Standard supplement	5	5
Protein (N × 6·25)	21·6	22·4
Ash	5·5	5·8
Ether extract	3·8	4·0
Crude fibre	1·9	1·7

Additional supplement

Expts ...	A 12–A 13	A 14	A 15
L-lysine	0·6	0·4	0·6
DL-tryptophan	0·2	0	0
L-arginine	0	0	0·34
L-tyrosine	0·2	0·2	0
Glycine	0·3	0·3	0·3

Expt A 12 was designed to determine whether histidine, like lysine, would produce a growth depression that could be alleviated by supplementing with arginine. The effects of addition of leucine and isoleucine were also tested. There were eight treatments, to each of which were assigned three groups of fifteen birds: basal diet alone; basal + lysine; basal + arginine; basal + lysine and arginine; basal + isoleucine; basal + leucine; basal + histidine; and basal + histidine and arginine. Expt A 13 was designed to repeat some of these treatments and to achieve greater replication in investigating any potential histidine–arginine relationship. In this trial, eight replicates (each of five birds) were assigned to each of four treatments: basal diet alone; basal + arginine; basal + histidine; and basal + arginine and histidine.

In Expt A 14 an attempt was made to ascertain whether a deficiency of an amino acid other than arginine could be precipitated by an excess of lysine. The basal diet used in this trial was deficient in tryptophan as judged by the recommendations of the (USA) National Research Council (1960), and no tryptophan was included in the supplement of amino acids. Four replicates of five birds were assigned to each of eight treatments: basal diet alone; basal + arginine; basal + lysine; basal + tryptophan; basal + arginine and lysine; basal + arginine and tryptophan; basal + lysine and tryptophan; and basal + arginine, lysine and tryptophan.

Table 2. *Expts A 12–A 15. Percentage amino acid composition of basal diets*

(The amino acid values are calculated from the determined composition of representative samples of typical ingredients (see Lewis *et al.* 1963). The values do not include the supplementary amino acids listed in Table 1)

Amino acid	Expts A 12–A 14	Expt A 15
Arginine	0·79	0·78
Glycine	0·88	0·59
Histidine	0·46	0·42
Leucine	2·89	3·66
Isoleucine	0·87	1·10
Lysine	0·45	0·34
Methionine	0·56	0·48
Cystine	0·46	0·39
Phenylalanine	1·10	1·19
Tyrosine	0·63	0·84
Threonine	0·84	0·82
Tryptophan	0·13	0·06
Valine	1·08	0·97

Expt A 15 was designed to obtain further evidence of any interaction between lysine and tryptophan. The basal diet was prepared mainly with maize and maize-gluten meal, but zein was included in place of some of the maize-gluten meal to achieve a tryptophan level well below that recommended by the (USA) National Research Council (1960). The composition of the basal diet is given in Table 1. Since in this experiment it was necessary to ensure that the arginine level was adequate, this amino acid was included in the supplement (Table 1). The total level of arginine in the basal diet of Expt A 15 was 1·13%. There were four replicates of five birds assigned to each of eight treatments: basal diet alone; basal + arginine; basal + lysine; basal + tryptophan; basal + arginine and lysine; basal + arginine and tryptophan; basal + lysine and tryptophan; and basal + arginine, lysine and tryptophan.

RESULTS

A selection of the results of Expt A 12 is shown in Table 3. The basal diet was clearly limiting in respect of arginine, since arginine supplementation caused a significant increase in weight gains ($P < 0·05$) after 3 weeks. Supplementation of the basal diet with lysine produced a marked growth depression ($P < 0·05$); this can be regarded as an aggravation of the arginine deficiency, since supplementing the diet with arginine in addition to lysine restored gain in weight to that on the basal diet and indeed gave a significant increase ($P < 0·01$) compared with the controls.

It might be expected that another basic amino acid, histidine, would cause a similar result. This was not so, however; although the basal diet contained a clear excess of this amino acid, the addition of further amounts did not produce a growth depression. The simultaneous supplementation of the diet with arginine and histidine gave a weight response which at 6 weeks was significantly higher than that with arginine alone.

Table 3. *Expt A* 12. *Mean live weight (g) of three replicate groups of fifteen chicks at* 3 *and* 6 *weeks*

Treatment	3 weeks	6 weeks
Control	256	740
0·3 % lysine	219	659
0·2 % arginine	285	773
0·3 % lysine + 0·2 % arginine	273	766
0·3 % isoleucine	250	725
0·5 % leucine	249	682
0·1 % histidine	253	720
0·1 % histidine + 0·2 % arginine	283	827
SE of means	±6·56	±20·9

Table 4. *Expt A* 13. *Mean live weight (g) and food conversion efficiency of eight replicate groups of five chicks at* 2 *and* 4 *weeks of age*

(The food conversion efficiency is expressed as g food eaten/g live-weight gain)

	2 weeks		4 weeks	
Treatment	Live weight	Food conversion efficiency	Live weight	Food conversion efficiency
Control	188	1·96	409	2·19
0·2 % arginine	203	1·81	456	1·96
0·15 % histidine	181	1·98	404	2·17
0·2 % arginine + 0·15 % histidine	205	1·79	464	2·00
SE of means	±4·7	±0·031	±6·3	±0·032
Factorial analysis: summary of effects				
Main effects:				
+0·2 % arginine	+19·8***	−0·17***	+53·5***	−0·2***
+0·15 % histidine	NS	NS	NS	NS
Interactions:				
+0·2 % arginine + +0·15 % histidine	NS	NS	NS	NS
SE of main effects and interactions	±4·7	±0·031	±6·3	±0·032
General mean	194·10	1·88	433·25	2·08

NS, not significant.
*** Significant at $P < 0.001$.

Although leucine was present in the basal diet in definite excess of the stated requirement, supplementation with leucine produced only a slight growth depression as compared with the depression produced by lysine. Increasing the level produced no

growth depression at 3 weeks, but at 6 weeks a growth depression was produced which was, however, smaller than that produced by lysine, even though the level of lysine in the basal diet was below that recommended by the (USA) National Research Council (1960).

Table 5. *Expt A* 14. *Mean live weight (g) and food conversion efficiency of four replicate groups of five chicks at 2 and 4 weeks of age*

(The food conversion efficiency is expressed as g food eaten/g live-weight gain)

	2 weeks		4 weeks	
Treatment	Live weight	Food conversion efficiency	Live weight	Food conversion efficiency
Control	177	1·6	345	2·8
0·2 % arginine	174	1·8	376	2·6
0·6 % lysine	141	2·6	223	4·5
0·2 % tryptophan	183	1·8	363	2·7
0·2 % arginine + 0·6 % lysine	162	2·0	306	2·9
0·2 % arginine + 0·2 % tryptophan	177	1·8	323	2·9
0·6 % lysine + 0·2 % tryptophan	135	2·5	180	5·3
0·6 % lysine + 0·2 % tryptophan + 0·2 % arginine	156	2·0	239	3·7
SE of means	±3·1	±0·11	±14·3	±0·27

Factorial analysis: summary of effects

	2 weeks		4 weeks	
Effect	Live weight	Food conversion efficiency	Live weight	Food conversion efficiency
Main effects:				
0·2 % arginine (A)	+8·4***	−0·22**	+33·2**	−0·80***
0·6 % lysine (L)	−29·2***	+0·5***	−114·8***	+1·32***
0·2 % tryptophan (T)	NS	NS	−36·2**	+0·45**
Interactions:				
A × L	+12·7***	−0·32***	+37·5**	−0·8***
A × T	NS	NS	−24·3*	+0·4*
L × T	−4·9*	NS	NS	NS
A × L × T	NS	NS	NS	NS
SE of main effects and interactions	±2·2	±0·07	±10·0	±0·18
General mean	163	2·0	294	3·4

NS, not significant.
* Significant at $P < 0·05$.
** Significant at $P < 0·01$.
*** Significant at $P < 0·001$.

The results of Expt A 13 are given in Table 4. It is again evident that the basal diet was deficient in arginine, since arginine supplementation produced a significant increase in weight gain ($P < 0·05$). There was a small, but not statistically significant, growth depression as a result of supplementing the diet with histidine. Supplementing

the diet with arginine and histidine again produced a significant increase in growth ($P < 0.05$), presumably due to the arginine.

The effects (Expt A 14) of supplementary amino acids when added to a diet deficient in tryptophan and arginine are shown in Table 5. The level of tryptophan in the basal diet was 0·13%. When tryptophan was added to the basal diet alone it produced a slight though not statistically significant growth response. When added together with other amino acids tryptophan produced a growth depression which becomes evident in the factorial analysis of the experiment. This is possibly the result of some as yet

Table 6. *Expt A 15. Mean live weight (g) and food conversion efficiency of four replicate groups of five chicks at 2 and 3 weeks of age*

(The food conversion efficiency is expressed as g food eaten/g live weight gain)

	2 weeks		3 weeks		
Treatment	Live weight	Food conversion efficiency	Live weight	Food conversion efficiency	N retained (%)
Basal diet	132	2·84	163	3·57	33·8
0·2% arginine	129	3·26	163	3·67	39·5
0·6% lysine	133	2·98	167	3·71	35·5
0·2% tryptophan	180	1·86	295	2·40	47·3
0·2% arginine + 0·6% lysine	131	2·83	165	4·01	33·5
0·2% arginine + 0·2% tryptophan	168	1·26	272	3·24	51·1
0·2% tryptophan + 0·6% lysine	185	1·68	305	2·23	46·7
0·6% lysine + 0·2% tryptophan + 0·2% arginine	186	1·73	314	1·83	50·6
SE of means	±2·93	±0·5	±5·6	±0·30	±2·6

Factorial analysis: summary of effects

	2 weeks		3 weeks	
Effect	Live weight	Food conversion efficiency	Live weight	Food conversion efficiency
Main effects:				
0·2% arginine (A)	NS	NS	NS	NS
0·6% lysine (L)	+6·6**	NS	+14·5***	NS
0·2% tryptophan (T)	+48·4***	−1·2***	+132***	−1·6*
Interactions:				
A × L	NS	NS	+7·5*	NS
A × T	NS	NS	NS	NS
L × T	+5·2*	NS	+11·5***	NS
A × L × T	NS	NS	+8·5**	NS
SE of main effects and interactions	±2·1	±0·11	±3·9	±0·21
General mean	155·6	2·4	230·7	2·96

NS, not significant.
* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

unexplained interaction. At 4 weeks a slight growth response to arginine was evident but again it did not reach statistical significance ($P > 0.05$). It is possible that the level of tryptophan recommended by the (USA) National Research Council (1960) is higher than is necessary to support optimum growth on this diet, but nevertheless the slight growth response to tryptophan suggests that the amino acid was supplied in quantities only slightly in excess of that which would limit growth. There was a very marked growth depression as a consequence of lysine addition, and this growth depression was alleviated by arginine supplementation, but not by tryptophan supplementation. In this experiment addition of arginine did not restore rate of growth to that of the controls, as in Expt A12. This was probably a consequence of the larger amount of lysine added in Expt A14.

Expt A15 was designed to confirm that the growth depression consequent upon the addition of excess lysine did not occur when tryptophan was the limiting amino acid. The basal diet was designed to be adequate in arginine, but definitely limiting in tryptophan. That this was achieved was evident in the results of adding arginine and tryptophan to the basal diet (Table 6). Factorial analysis of the experiment indicates that lysine produced an increased rate of growth and that there were positive interactions between arginine and lysine, between tryptophan and lysine, and among arginine, tryptophan and lysine. This was probably a consequence of the low levels of both arginine and lysine in the basal diet. When the growth limitation due to tryptophan deficiency is alleviated the addition of arginine and lysine produces a growth response.

As would be expected if both arginine and lysine are potentially limiting, when the deficiency of tryptophan is made good the double supplement of arginine and lysine is more effective than either alone.

DISCUSSION

The present results provide evidence of an interaction between lysine and arginine. One of the most effective analyses of the problem of amino acid interaction was carried out by Elvehjem (1956) who proposed three types of amino acid interaction. These interactions may be described in terms somewhat different to those of Elvehjem (1956) as follows: imbalances, in which the effect of the agent amino acid(s) is reversed by supplementing the diet with that amino acid limiting before the agent was added; antagonisms, in which the effect of the agent is reversed by supplementing the diet with an amino acid not necessarily limiting before the addition of the agent; and toxic effects, in which the effect of the agent is not directed towards any single amino acid.

In spite of the considerable attention which has been given to the study of amino acid imbalance, no mechanism has been proposed which can account for the observations. Although it may be possible to classify interactions into the three types described by Elvehjem (1956), there are still not sufficient examples of interaction which fall clearly into each of these types to justify the inference that three corresponding and disparate mechanisms are involved. It may therefore be preferable to abandon this classification and group examples of interaction in terms of the amino

acids which are involved, since each amino acid is involved in widely different pathways of metabolism. Investigation of common aspects of the patterns of metabolism may assist in resolving the mechanisms of interaction.

The amino acid that must be added to counteract the growth depression consequent upon the intake of an unbalanced diet may be defined as the target of some unknown mechanism which results in the requirement for that amino acid being increased. In certain instances it is evident that the agent of this mechanism is a specific amino acid which has been added to the diet in excess. It is suggested that even when a mixture of amino acids or a protein supplement precipitates an imbalance, this effect may be attributable to specific amino acid agent(s) within the supplement. An appraisal of the specificity of an interaction considered in these terms constitutes a logical development; namely, whether any other target amino acids are susceptible to the action of the agent or conversely whether other amino acids are as effective as agents against that target amino acid.

The present results have shown that the arginine requirement for growth is increased if lysine be added to the diet, and the specificity of this interaction has been examined in relation to target and agent. For example, the results of Expts A 14 and A 15 suggest that arginine but not tryptophan is a target in lysine-induced imbalance. Together with the findings of Snetsinger & Scott (1961) that excess lysine did not increase the requirement of the chick for methionine when this was the limiting amino acid, these results suggest that arginine may be the specific target of lysine.

In the same way the results of Expts A 12 and A 13 suggest that, when arginine is the target, lysine is the most effective agent. This lends weight to the suggestion that there is some specific antagonism between lysine and arginine, and confirms, the observations of Jones (1962) and of O'Dell *et al.* (1962). A similar antagonism appears to occur between threonine and tryptophan (Florentino & Pearson, 1962) and among leucine, isoleucine and valine (Harper, Benton & Elvehjem, 1955).

It is possible that many, if not all, of the phenomenons of interaction can be reduced to an antagonism between pairs of amino acids which are members of small groups among which interaction is possible. The nature and number of amino acids constituting each group, and the extent to which their antagonism is mutual, may provide some information which will enable the site of interaction of that group to be established.

Two general mechanisms have been suggested which might underly the interactions observed. The first of these proposes that the effect of the agent amino acids is to cause impaired utilization of the target amino acid, possibly by increased katabolism. The presence of excess amino acids is said to increase the activity of the physiological means for their disposal, and concomitantly some of the limiting amino acid itself may be lost (Salmon, 1954). Some evidence for this has been found in the interaction between threonine and tryptophan (Florentino & Pearson, 1962) but contradictory evidence was obtained for the same interaction by Wilson, Wortham, Benton & Henderson (1962). The concept that interaction occurs between pairs of amino acids is compatible with this mechanism, since there are several sites where one amino acid might interfere with another so as to cause impaired utilization: for example, at

sites of absorption from the intestine or from the blood stream into the cells. It is most interesting, in view of the original suggestion of Salmon (1954), that arginine and lysine share a common pathway for oxidative katabolism (Boulanger & Osteux, 1956; Lewis & Smith, 1963), and that direct oxidative katabolism is an important pathway in birds (Efimochkina, 1959). An adaptive increase in the activity of an amino acid oxidase of wide specificity in response to one of its substrates may lead to a concomitant increase in the katabolism of alternative substrates.

The second general mechanism that might underly amino acid interactions is that envisaged by Fisher & Shapiro (1961), who suggest that the depression of food intake which always results from the ingestion of imbalanced diets is by itself sufficient to account for the growth depression. In support of this view they have demonstrated that there is no difference in the efficiency with which lysine is utilized from 'balanced' and 'unbalanced' diets, provided that intake of each diet is made equal by adjusting the energy level of the diets.

This mechanism operating by reducing food intake is more difficult to reconcile with the observed specificity of the interaction between lysine and arginine. If the mechanism, which must be initiated by the nervous system, is operative in response to excess of the agent amino acid in the blood stream then there would seem no reason to suppose that it should be more effective against one amino acid target than upon another, nor that different amino acids should have differing potency as agents. However, the extent to which the plasma level of an amino acid will rise when the acid is present in excess in the diet depends upon the relation between its rate of absorption and its rate of entry into metabolic pathways and removal from the blood stream.

There are two factors which allow compatibility between the theory of Fisher & Shapiro (1961) and the present observations. Lysine may be more effective as an agent than other amino acids (Expts A 12 and A 13) because it is comparatively stable metabolically and is not easily removed from the blood. Arginine, on the other hand, may be the amino acid most effective in alleviating the growth depression because it acts as a specific protective agent. It may reduce the plasma level of lysine by interfering with its absorption or stimulating its katabolism, rather than by acting as the specific target of an arginine–lysine competition in which it is less effectively utilized.

The alternative interpretations illustrate the impossibility of drawing valid general conclusions until each instance of amino acid interaction has been studied in greater detail.

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