Arginine in poultry nutrition

1. Dietary requirement for arginine

By D. LEWIS, G. H. SMITH and C. G. PAYNE

University of Nottingham School of Agriculture, Sutton Bonington, Loughborough

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Attempts to determine the minimal dietary requirements of the chick for amino acids are usually based on an assessment of weight gain in relation to the amino acid composition of a series of diets. Generally one basal diet of known composition is used and supplemented with graded amounts of free amino acids. Grau (1947) used amino acid mixtures in studies of phenylalanine and tyrosine utilization by the chick and concluded that weight gain was the most important criterion of dietary adequacy with respect to amino acid supply. Nitrogen balance methods may be used as an alternative guide to dietary adequacy. Frost (1950) pointed out, however, that maintenance of N balance alone is not a guarantee of amino acid adequacy. It would appear that until the composition of the chick, e.g. bone, fat or lean meat content, becomes commercially important, live-weight gain must still be regarded as the best index of dietary quality. In the studies here described the rate of weight gain of chicks and the efficiency of their N retention have been measured.

There is still some uncertainty regarding the arginine requirement of the young chick. Almquist & Merritt (1950), using purified diets in which casein was the main source of protein, suggested that the requirement of the chick for arginine is 1·2% of the diet or 6% of the protein (in diets containing 20% protein). They stated that the arginine requirement was 0·9, 1·2 and 1·8% of the diet when it contained 15, 20 and 25% protein respectively. These values are still accepted ((USA) National Research Council, 1960), though subsequent studies have shown that the requirement can vary considerably with the nature of the ingredients in the diet.

Snyder, Morrison & Scott (1956) found that the requirement for arginine was 1·7% of a diet based upon casein, whereas it was around 1·1% of a diet mainly containing maize and soya-bean meal. Krautmann, Hauge, Mertz & Carrick (1957) confirmed the figure of 1·7% for a purified diet and reported that it was reduced to 1·4% when some gelatin was included. They also quoted a figure of 1·2% for a maize–casein diet and one of around 0·9% for a diet based on maize and soya-bean meal. The work of Klain, Scott & Johnson (1958, 1959, 1960) has shown that a similar range of values for the arginine requirement is found with purified diets based upon mixtures of individual amino acids. They reported values within the range from 1·0 to 2·1%, the higher figures being encountered when the mixture simulated the composition of casein. Particularly high values, around 2·3%, have been proposed by Hogan, Craghead, Savage, Cole & O'Dell (1957). The findings of Klain *et al.* (1959, 1960) and of

Anderson & Dobson (1959) established that the variability in the requirement for arginine is a function of the overall amino acid composition of the diet.

The experiments described here were designed to examine arginine requirement under standard conditions of husbandry with diets of a type that might be used in the UK, containing some ingredients other than maize and soya-bean meal. Every effort has been made to ensure that the diets supplied all nutrients other than arginine at a level that is optimal for the growth of chicks. The recommendations of the (USA) National Research Council (1960) regarding amino acids, vitamins and minerals have been observed. In all diets the amino acid composition has been determined, but for most vitamins and minerals it was necessary to use standard values from the literature (e.g. those of Albritton, 1954). With these values a margin of safety was adopted that was more than adequate to cover any expected variability. The diets also included standard quantities of antibiotic, coccidiostat and antioxidant agents.

To examine the adequacy of a diet in terms of its arginine content it is also necessary to ensure that growth is not being limited by an inadequate level of alleged unidentified growth factors. It seems to be generally accepted that there remain some growth factors needed by the chick that have not yet been identified (Menge, Combs, Hsu & Shorb, 1952; Tsang & Schaible, 1960; Wakelam & Jaffe, 1961). There is, however, considerable confusion with regard to the nature of these substances and their mode of action, but they are usually associated with the inclusion of the following materials in the diets: dried whey, dried liver, fish by-products, dried yeast, distillers' by-products and forage materials. In some instances it has been found that the growth response is partly due to mineral constituents, and it has also been shown (see Lillie, Sizemore & Bird, 1953) that an assay procedure for an unidentified factor can be developed with a basal diet complete in all known factors. Rasmussen, Luthy, Van Lanen & Boruff (1957) have reviewed the evidence for the existence of more than one such factor and Tsang & Schaible (1960) have commented upon the various factors that can influence the chick's reponse. It is surprising that work during the last decade has not led to an identification of these factors if they are in fact nutrients. In the work now described the preliminary objective was to determine which of the materials listed above was the most appropriate to use.

The second objective was to examine the adequacy of a series of diets in terms of the supply of arginine and related dietary constituents. The basal diets contained graded levels of protein; the ratio of arginine to protein was also graded. The final objective was to determine a minimal adequate arginine content with different diets of a constant protein content. An attempt was also made to show that with diets of this type the productive performance of the birds is not regulated by some other dietary constituents in a manner that makes the evaluation of the arginine requirement invalid. Since amino acids were mainly supplied as intact protein and since the food was offered *ad lib.* the availability of the amino acids was probably similar to that in normal practice.

EXPERIMENTAL

Housing of chicks

Chicks were housed in a windowless room, measuring 15 ft × 12 ft and 8 ft high. This room was thermally insulated and the air temperature maintained by thermostatically controlled electric tubular heaters of 4 kW capacity. During the 1st week of the life of the chicks the temperature was controlled at 90 °F, and was subsequently reduced by 5 °F each week until 65 °F was reached. Ventilation was by seepage only during the 1st week in summer and the first 2 weeks in winter. Subsequently ventilation was increased, by an electric extractor fan, to 150 ft^3/min in the winter and to 250 ft^3/min in the summer. Relative humidity in the room varied more than did temperature. It was usually 5% higher at night than during the day and 15% lower in summer than in winter.

Six blocks, each of four cages mounted vertically above each other, were housed in the room. The cages, 4 ft long, 2·5 ft wide and 15 in. high, were made of galvanized wire mesh. Food and water troughs were sited along the front of each cage, and were designed to minimize spilling. The floors of the cages were changed from 0·5 in. mesh to 0·75 in. mesh when the chicks reached 5 weeks of age. Beneath the floor of each cage was a galvanized-iron droppings tray. During the 1st week the chicks were confined to an area immediately behind the water trough by a barrier food trough extending across the width of the cage.

Lighting was by three 60 W white electric lamps and was continuous for 20 h daily.

Management

At the beginning of an experiment fifteen day-old cockerels were placed in each cage. The birds were a broiler cross type based on White Rock × Light Sussex. Each chick was weighed and wingbanded; they all received a control diet during the 1st week. Then each chick was again weighed and the numbers per pen were reduced to ten in such a way that the mean weights for all the pens were the same and the extremes (heavy and light) were eliminated. The birds were weighed individually weekly.

All diets were given in meal form and food and water were always available to the birds. Weighed quantities of food were added to each trough as required and any residue of uneaten food was weighed weekly. All the birds were debeaked when 3 weeks old by removing one half of their upper mandibles.

Procedure

Nitrogen balance. Food intake was measured and all excreta were collected for a period of 3 days. At the beginning of this period a known weight of food was placed in the trough; the residues and any additions were recorded daily. The droppings tray was cleaned at the beginning and covered with a layer of greaseproof paper. The paper was replaced at 24 h intervals and the daily total of droppings for each cage was collected. The droppings were homogenized and weighed and a 100 g sample was taken to determine dry-matter content. The dried droppings were ground and a 2 g sample was used for N determination by a standard Kjeldahl procedure.

Carcass analysis. Individual birds from several of the experiments were analysed. After killing by dislocation of the neck, the feathers, alimentary tract, head and feet were removed. The carcass was cut into pieces of 10–20 g and placed in a Waring Blendor with a known volume of water. The mixture was homogenized until it attained a uniform consistency, and samples were taken for analysis.

Conventional analyses. Crude protein, ether extract, crude fibre, ash and moisture were determined by the standard methods of the Association of Official Agricultural Chemists (1960).

Table 1. *Expt A* 1. *Composition of basal diet*

(Amino acid values were calculated from the values in Table 7. The other results were obtained by analysis of the diets. The experimental diets were prepared by replacing 2 % maize and 1 % wheat with 3 % of the material alleged to supply an unidentified growth factor. The composition of the supplement is given in Table 2, expressed in terms of the final concentrations contributed to the diet by the supplement when 5 % of it is included)

Ingredient (%)	
Maize meal	30
Wheat meal	29
Barley meal	10
Soya-bean meal	18
White-fish meal	8
Supplement	5
Proximate composition (%)	
Dry matter	88·00
Nitrogen	3·75
Ash	7·55
Ether extract	3·40
Crude fibre	4·40

Amino acid content (%)	
Aspartic acid	1·82
Threonine	0·84
Serine	1·15
Proline	1·22
Glutamic acid	4·02
Glycine	1·42
Alanine	1·07
Valine	1·05
Isoleucine	0·89
Leucine	1·64
Tyrosine	0·75
Phenylalanine	0·90
Lysine	1·26
Histidine	0·48
Arginine	1·38
Cystine	0·42
Methionine	0·53
Tryptophan	0·25

Amino acids. Individual amino acids were determined by a modification of the method of Moore & Stein (1954). No detergent was used in the buffer and the flow rate ranged between 8 and 18 ml/h. Most of the modifications of the method described by Bidmead & Ley (1958) were adopted. Several series of analyses were also carried out by Degussa Ltd, Frankfurt, and their results were in close agreement.

Diets and experimental design

Expt A 1 was designed to test the adequacy of the general procedures adopted and to measure any response to the inclusion in the diet of various materials that might supply unidentified growth factors. The ingredients and composition of the basal diet are given in Table 1. To supply adequate quantities of minerals and vitamins the basal ration in this and subsequent experiments contained a supplement (Table 2). The different experimental diets were made by replacing 2% maize and 1 % wheat in the basal diet with 3% of the material alleged to supply an unidentified growth factor: control (*A*), malt distillers' solubles (*B*), dried yeast (*C*), dried whey (*D*), molasses distillers' solubles (*E*) and dried lucerne (*F*).

Expts A 2–A 6 were designed to test the adequacy of the quantity of arginine supplied by various diets containing different quantities of N. The ingredients used and the results of the conventional analyses are given in Table 3. When the arginine content of the diet is 6% of the protein it is generally regarded as satisfying the requirements of the young chick (see (USA) National Research Council, 1960): in these experiments it varied from 6·2 to 4·1%. In the preparation of these diets some barley was introduced

Table 2. *Expts A 1–A-11. Composition of the mineral and vitamin supplement, expressed in terms of the final concentrations contributed to the diet by the supplement when included as 5% of the diet*

Constituent	Form in which supplied	Final level
Calcium	Limestone	0·85%
Phosphorus	Dicalcium phosphate	0·35%
Sodium	Common salt	0·12%
Chloride	Common salt	0·18%
Manganese	Manganese monoxide	80 mg/kg
Zinc	Zinc carbonate	50 mg/kg
Iron	Saccharated iron carbonate (BP)	20 mg/kg
Copper	Copper sulphate	2 mg/kg
Molybdenum	Sodium molybdate	2 mg/kg
Iodine	Potassium iodide	1 mg/kg
Cobalt	Cobalt carbonate	0·2 mg/kg
Selenium	Sodium selenite	0·1 mg/kg
Vitamin A	Concentrate (50000 i.u./g)	7000 i.u./kg
Vitamin D_3	Concentrate (100000 i.u./g)	1500 i.u./kg
Vitamin E	Supplied as such	5 i.u./kg
Vitamin K	Sodium menaphthone bisulphite	10 mg/kg
Choline chloride	25% choline chloride	500 mg/kg
Riboflavin	98% riboflavin	4 mg/kg
Calcium pantothenate	Supplied as such	8 mg/kg
Nicotinic acid	Supplied as such	20 mg/kg
Folic acid	Supplied as such	0·5 mg/kg
Vitamin B_{12}	Concentrate (64 mg/lb)	0·015 mg/kg
DL-methionine	Supplied as such	0·1%
Procaine penicillin	Concentrate, 5 g procaine benzyl penicillin/lb	20 mg/kg
BHT (antioxidant)	Butylated hydroxytoluene	0·0125%
Amprolium (coccidiostat)	Ampromix (Merck, Sharp & Dohme Ltd, Hoddesden)	0·0125%

into those containing less protein (A 4, A 5 and A 6) and, to reduce the arginine content, soya-bean meal was progressively replaced by maize-gluten meal. The composition of the supplement was the same as in Expt A 1. Since the basal diets in these experiments were inadequate with respect to several individual amino acids an additional supplement was included (Table 4) which replaced wheat meal. The samples taken for conventional analysis included this supplement. In the course of each experiment four groups of ten birds were assigned to each of six treatments in which the basal diets were supplemented with arginine or related compounds. The treatments were: basal diet alone, basal plus arginine, basal plus glycine, basal plus arginine and glycine, basal plus guanidoacetic acid, and basal plus creatine.

In Expts A 7–A 10 a series of basal diets was prepared with a constant protein and a progressively decreasing arginine content. The arginine content was reduced from

5·9% of the protein in Expt A7 to 3·6% in Expt A10 by progressively replacing soya-bean meal with maize-gluten meal (Table 5). The same main supplement as in the earlier experiments was used. The additional amino acids included are listed in Table 6.

Table 3. *Expts A2–A6. Ingredients of basal diets*

(Composition is expressed as percentage of dry matter, except for protein which is expressed on an air-dry basis)

	A2	A3	A4	A5	A6
Wheat meal	32	37	41	43	45
Maize meal	28	28	19	20	22
Barley meal	0	0	12	12	10
Soya-bean meal	18	18	0	0	0
Maize-gluten meal	0	0	8	11	12
Fish meal	14	9	12	6	3
Dried whey	3	3	3	3	3
Supplement	5	5	5	5	5
Ash	8·2	7·2	7·4	6·6	6·0
Ether extract	3·5	3·4	3·2	3·1	3·0
Crude fibre	2·6	2·9	2·8	3·1	3·2
Protein (N × 6·25)	24·0	21·9	19·1	17·6	15·9
Arginine: % of diet	1·49	1·27	1·06	0·79	0·65
% of protein	6·2	5·8	5·5	4·5	4·1

Table 4. *Expts A2–A6. Additional amino acids* (%) *included in the basal diets*

Supplement	A2	A3	A4	A5	A6
DL-methionine	0·2	0·2	0·15	0·1	0·1
DL-tryptophan	0·05	0·05	0·1	0·1	0·1
L-tyrosine	0·1	0·1	0·2	0·1	0·1
L-lysine	—	—	0·2	0·4	0·5
DL-valine	—	—	—	—	0·1
Glycine	—	—	—	0·1	0·2

Table 5. *Expts A7–A10. Ingredients of basal diets*

(Composition is expressed as percentage of dry matter, except for protein which is expressed on an air-dry basis)

	A7	A8	A9	A10
Maize meal	60	57	57	55
Soya-bean meal	24	15	8	0
Maize-gluten meal	8	20	27	37
Dried whey	3	3	3	3
Supplement	5	5	5	5
Ash	6·6	6·8	6·8	7·0
Ether extract	3·6	3·4	3·3	3·1
Crude fibre	4·3	4·5	4·2	3·9
Protein (N × 6·25)	21·7	22·0	21·5	21·6
Arginine: % of diet	1·29	1·13	0·97	0·79
% of protein	5·9	5·1	4·5	3·6

In these experiments six groups of ten birds were allocated to each of four treatments which tested the effects of supplementing the basal diet with arginine and related compounds, the treatments being: basal diet alone, basal plus arginine, basal plus arginine and glycine, and basal plus guanidoacetic acid.

Expt A11 was designed to examine the effect of the lysine content of the diet upon the arginine requirement. The work of Harper (1959) and of Anderson & Dobson (1959) showed clearly that addition of an excess of a second limiting amino acid can lead to an inhibition of growth. The inhibition can be reversed by adding the first limiting amino acid. This experiment therefore served to determine whether a marginal arginine deficiency might be made more clearly apparent by the addition of excess lysine. The same basal diet as in Expt A10 was used, including also the same amino acid supplement (Tables 5 and 6). The treatments were: basal diet alone, basal plus arginine, basal plus lysine, and basal plus arginine and lysine. Six groups of ten birds were again used in each of the four treatments in this experiment.

All experiments were conducted in the form of randomized block designs and analyses of variance were performed on the results. N retention was measured at the 2- and 5-week stage in Expts A2–A6 and at the 5-week stage in Expts A7–A10.

Table 6. *Expts A7–A10. Additional amino acids* (%) *included in the basal diets*

	A7	A8	A9	A10
DL-methionine	0·2	0·15	0·1	0·1
DL-tryptophan	0·05	0·1	0·15	0·2
L-tyrosine	0·2	0·2	0·2	0·2
L-lysine	0·2	0·4	0·5	0·7
Glycine	—	—	0·2	0·3
Total	0·65	0·85	1·15	1·5

RESULTS

Amino acid composition of the diets

Each constituent of the basal diets was analysed after acid hydrolysis for individual amino acids by ion-exchange chromatography (Table 7). The values obtained (Table 8) were used to calculate the amino acid composition of the basal diets.

Since it was particularly important to define the arginine contents of the diets, they were assayed for arginine by the specific colorimetric method of Rosenberg, Ennor & Morrison (1956). The results are given in Table 9 and compared with the values obtained by ion-exchange chromatography.

Expt A1. Unidentified growth factors

A selection of the results is given in Table 10. The birds grew well, with a mean weight at 10 weeks of almost 4 lb (around 1·8 kg). The food conversion efficiency (lb food eaten/lb live-weight gain) was around 2·6 and also showed that the diets were not unduly inadequate in any respect. Analysis of variance showed that treatment differences were not statistically significant ($P > 0{\cdot}05$). Only when a supplement of dried whey was given were the live weights of the experimental birds greater than those of the control group receiving the basal diet, and the food conversion ratio lower. Because of this finding dried whey was included in the basal diet in all the subsequent experiments.

Expts A2–A6. Diets with varying N and arginine contents

The results are given in Tables 11 and 12. As the protein content of the basal diet decreased (from Expt A2 to Expt A6) the live weights of the chicks both at 3 weeks and 6 weeks also decreased (Table 11). In the same way the food conversion ratio increased at both the 3- and 6-week stages (Table 12). Since it is likely that the basal diets supplied adequate amounts of minerals, vitamins and amino acids other than

Table 7. *Amino acid composition (g/16 g nitrogen) of constituents used in the preparation of the experimental diets*

(Analyses by ion-exchange chromatography. Each figure represents the mean of three replicate determinations. The values for tryptophan were determined separately by a colorimetric method using *p*-dimethylaminobenzaldehyde (Snell & Snell, 1954))

Amino acid	Wheat meal	Maize meal	Barley meal	Milo meal	Fish meal	Soya-bean meal	Maize-gluten meal	Dried whey	Dried yeast
Aspartic acid	4·1	9·0	5·6	6·4	9·4	9·8	5·1	11·8	9·8
Threonine	3·1	4·5	3·0	3·3	4·2	4·1	3·7	6·4	6·4
Serine	4·8	4·8	4·0	4·6	5·3	6·0	4·2	6·2	6·9
Proline	7·6	8·0	8·1	7·7	3·8	5·2	4·9	2·1	4·0
Glutamic acid	28·4	16·5	19·3	20·3	12·5	19·5	18·9	17·7	12·6
Glycine	5·6	4·1	4·1	3·0	9·5	7·5	4·0	3·1	4·7
Alanine	5·1	8·7	4·3	8·4	5·2	3·9	7·3	3·8	8·0
Valine	3·9	5·9	4·4	4·7	5·2	4·8	4·8	5·8	6·4
Isoleucine	4·5	3·7	3·9	3·8	3·8	4·4	4·0	5·3	4·7
Leucine	8·8	12·1	6·5	12·2	6·8	6·6	14·4	9·4	7·0
Tyrosine	3·6	4·2	3·0	3·3	2·7	3·6	2·5	4·0	2·9
Phenylalanine	4·9	4·4	4·9	4·7	4·0	4·0	5·4	4·3	4·9
Lysine	2·9	2·7	3·1	2·4	8·5	6·8	1·9	3·7	6·4
Histidine	2·0	2·9	2·0	2·3	3·0	1·9	1·9	1·1	2·2
Arginine	4·2	5·0	4·6	3·8	7·2	7·2	3·5	2·3	2·9
Cystine	2·9	2·0	1·9	2·5	1·6	1·8	2·1	2·5	1·1
Methionine	2·7	2·0	1·5	1·7	2·7	1·4	2·2	0·9	1·5
Tryptophan	1·2	0·7	1·2	1·0	0·9	1·2	0·6	1·4	0·6
Total	100·3	101·2	85·4	96·1	96·3	99·7	91·5	90·5	93·0

Table 8. *Expts A2–A10. Amino acid composition (%) of basal diet used, calculated from the content in the ingredients*

(These values do not include the supplementary amino acids)

Amino acid	A2	A3	A4	A5	A6	A7	A8	A9	A10
Arginine	1·49	1·27	1·06	0·79	0·65	1·29	1·13	0·97	0·79
Glycine	1·88	1·53	1·25	0·98	0·81	1·29	1·14	0·99	0·85
Histidine	0·59	0·49	0·46	0·39	0·34	0·49	0·48	0·48	0·46
Leucine	1·87	1·67	1·73	1·81	1·66	1·96	2·36	2·56	2·89
Isoleucine	1·02	0·90	0·76	0·71	0·64	0·88	0·91	0·83	0·82
Lysine	1·58	1·27	0·95	0·67	0·51	1·05	0·82	0·64	0·42
Methionine	0·52	0·44	0·46	0·41	0·36	0·44	0·48	0·50	0·53
Cystine	0·47	0·43	0·39	0·38	0·35	0·41	0·43	0·46	0·44
Phenylalanine	1·03	0·91	0·86	0·84	0·79	0·93	1·01	1·03	1·09
Tyrosine	0·82	0·74	0·58	0·54	0·49	0·77	0·72	0·67	0·62
Threonine	1·00	0·85	0·73	0·66	0·59	0·90	0·92	0·85	0·84
Tryptophan	0·25	0·23	0·18	0·15	0·14	0·21	0·13	0·16	0·14
Valine	1·22	1·04	0·92	0·83	0·73	1·09	1·10	1·08	1·08

Table 9. *Expts A2–A10. Arginine content (% air-dry material) of basal diets*

(Values obtained by ion-exchange chromatography are compared with those by a colorimetric method (Rosenberg *et al.* 1956))

Basal diet	Chromatographic method	Colorimetric method
A2	1·49	1·41
A3	1·27	1·24
A4	1·06	1·09
A5	0·79	0·81
A6	0·65	0·67
A7	1·29	1·21
A8	1·13	1·09
A9	0·97	0·95
A10	0·79	0·77

Table 10. *Expt A1. Live weights (g) and food conversion efficiency* (FCE),* *for groups of ten birds at* 3, 6 *and* 10 *weeks of age given different supplements* (B–F) *with the basal diet*

(The figures are the means of the four replicates per treatment)

Week	Measurement	*A* Control	*B* Dried distillers' solubles	*C* Dried yeast	*D* Dried whey	*E* Molasses distillers' solubles	*F* Dried lucerne	SE
3	Live weight	326	323	305	332	324	307	±6·32
	FCE	1·85	1·95	1·98	1·82	1·88	1·97	±0·028
6	Live weight	871	847	853	869	858	847	±11·4
	FCE	2·20	2·21	2·29	2·18	2·27	2·27	±0·050
10	Live weight	1808	1752	1776	1846	1781	1771	±25·0
	FCE	2·56	2·54	2·60	2·47	2·57	2·60	±0·053

SE, standard error of the mean. * g food eaten/g weight gain.

Table 11. *Mean live weights (g) of four replicate groups of ten birds at* 3 *and* 6 *weeks*

(Groups of fifteen chicks placed in pens at day-old, groups reduced to ten at 7 days and experimental diets given)

Treatment	A2	A3	A4	A5	A6
		At 3 weeks			
A, control	307	316	266	236	230
B, 0·2% L-arginine	294	303	256	236	237
C, 0·2% glycine	315	304	254	235	227
D, 0·2% L-arginine + 0·2% glycine	313	287	249	234	220
E, 0·2% guanidoacetic acid	305	305	246	226	230
F, 0·2% creatine	314	307	248	225	228
S.E	±12·3	±9·1	±3·9	±6·8	±7·0
		At 6 weeks			
A	862	914	825	666	630
B	850	867	815	662	698
C	848	865	800	666	651
D	881	847	798	657	662
E	821	862	798	630	695
F	840	873	793	651	657
SE	±19·2	±29·9	±17·5	±19·5	±17·9

SE, standard error of the mean.

arginine it would appear that in general the protein or 'energy' level was the factor that limited growth.

In Expts A2, A3, A4, and A5 no significant improvement in rate of weight gain resulted from the addition of arginine or related compounds to the basal diet. In fact in most instances the mean weights of birds receiving any supplement were lower than those of the controls. In Expt A4 there was a significant reduction ($P < 0.05$) in growth rate in response to supplementation with arginine or glycine or both, which implies there may even have been excess of these amino acids present. In any event there was no indication on the basis of either live-weight gain or efficiency of food conversion that the arginine required exceeded that supplied in the basal diet.

Table 12. *Food conversion efficiency* (FCE),* *of birds within the periods of* 1–3 *weeks and* 1–6 *weeks*

(For experimental details see Table 11)

Treatment	A2	A3	A4	A5	A6
		1–3 weeks			
A, control	1·52	1·68	1·68	1·84	1·98
B, 0·2% L-arginine	1·52	1·67	1·73	1·83	1·99
C, 0·2% glycine	1·47	1·69	1·70	1·84	1·97
D, 0·2% L-arginine +0·2% glycine	1·52	1·72	1·69	1·81	2·01
E, 0·2% guanidoacetic acid	1·53	1·67	1·67	1·83	1·99
F, 0·2% creatine	1·52	1·68	1·65	1·90	1·92
SE	±0·025	±0·031	±0·025	±0·028	±0·032
		1–6 weeks			
A	2·23	2·26	2·26	2·60	2·64
B	2·14	2·33	2·13	2·61	2·46
C	2·11	2·33	2·10	2·60	2·61
D	2·20	2·25	2·23	2·72	2·52
E	2·19	2·34	2·29	2·65	2·44
F	2·27	2·31	2·11	2·70	2·56
SE	±0·070	±0·094	±0·110	±0·073	±0·076

SE, standard error of the mean. * g food eaten/g weight gain.

In Expt A6 there were significant growth responses to arginine and to guanidoacetic acid (for each, $P < 0.05$). The food conversion ratios also showed improvement which approached significance at the same level. The basal diet used in this experiment contained 0·64% arginine, corresponding to 4·1% in the protein. The magnitude of the improvement in growth and food utilization suggests that the basal diet contained only marginally less arginine than the requirement for optimal growth.

The results of N balance determinations are given in Table 13. The N retained at 2 weeks was always within the range of 50–60% of that consumed and there was no significant difference after supplementation of the diets with arginine or related compounds. It would be expected that if the added amino acids were correcting an inadequacy or imbalance there would be an improvement in the N retention. The N retained at the 5-week stage was always around 40% of that consumed and there were

no differences on supplementing with arginine or related compounds. The N balance results confirmed that in none of the diets was the arginine content seriously inadequate.

Expts A7–A10. *Diets with constant N and decreasing arginine contents*

The results are given in Tables 14 and 15. There was again a definite decrease (from Expt A7 to Expt A10) in the live weights of the chicks (Table 14) at both 3 and 6 weeks of age. There was, however, a less marked change in the food conversion ratio (Table 15) and a reduced food consumption in Expt A10 compared with that in Expt A7.

Table 13. *Nitrogen retention at 2 and 5 weeks of age of birds given different supplements*

(Mean values for four replicate groups of ten birds in three successive 24 h collections)

	2 weeks			5 weeks		
		N retained			N retained	
Treatment	N eaten (g/day)	g/day	%	N eaten (g/day)	g/day	%
		Expt A2				
A, basal diet only	1·01	0·59	58	2·77	1·08	39
B, 0·2% L-arginine	0·93	0·55	60	2·83	1·20	42
C, 0·2% glycine	1·12	0·66	58	3·08	1·23	40
D, 0·2% L-arginine + 0·2% glycine	1·18	0·69	58	2·80	1·19	42
E, 0·2% guanidoacetic acid	1·06	0·63	59	2·69	1·00	37
F, 0·2% creatine	1·06	0·61	57	2·89	1·19	41
		Expt A3				
A	1·00	0·57	57	2·70	1·13	42
B	0·96	0·51	53	2·70	1·09	40
C	0·98	0·54	55	2·69	1·05	39
D	0·94	0·49	52	2·56	1·06	41
E	0·97	0·51	53	2·64	1·12	42
F	0·94	0·53	56	2·79	1·13	40
		Expt A5				
A	0·81	0·49	60	2·30	0·95	41
B	0·76	0·43	57	2·28	1·00	44
C	0·76	0·42	54	2·30	1·05	45
D	0·76	0·44	58	2·18	0·97	44
E	0·72	0·41	57	2·26	1·01	44
F	0·75	0·41	55	2·15	0·93	43
		Expt A6				
A	0·90	0·48	53	1·67	0·69	41
B	0·85	0·45	53	1·69	0·64	38
C	0·83	0·46	53	1·65	0·66	40
D	0·88	0·47	54	1·75	0·68	39
E	0·90	0·47	52	1·51	0·57	38
F	0·88	0·45	51	1·50	0·55	36

There was no significant stimulation of growth or improvement in food conversion ratio on supplementation of the basal diets in Expts A7, A8 and A9 with arginine, glycine or guanidoacetic acid. There is every reason to believe that in all diets the arginine content was adequate to support optimal growth and that live-weight gain

was only limited by the overall N or 'energy' content of the diet. Analysis of variance showed that treatment differences were not statistically significant ($P > 0.05$).

In Expt A 10, however, there was a significant increase ($P < 0.05$) in gain in weight at 6 weeks when the basal diet was supplemented with arginine either alone

Table 14. *Mean live weights (g) of four replicate groups of ten birds at 3 and 6 weeks*

(Groups of fifteen chicks placed in pens at day-old, groups reduced to ten at 7 days and experimental diets given)

Treatment	A 7	A 8	A 9	A 10
	At 3 weeks			
A, basal diet only	294	262	236	236
B, 0·2 % L-arginine	306	261	231	234
C, 0·2 % L-arginine + 0·2 % glycine	295	261	231	233
D, 0·2 % guanidoacetic acid	303	260	232	235
SE	±8·1	±8·9	±5·2	±3·8
	At 6 weeks			
A	833	764	690	642
B	837	769	669	684
C	813	781	701	677
D	847	769	662	652
SE	±8·4	±16·0	±16·7	±11·4

SE, standard error of the mean.

Table 15. *Food conversion efficiency* (FCE),* *of birds within the periods of 1–3 weeks and 1–6 weeks*

(For experimental details see Table 14)

Treatment	A 7	A 8	A 9	A 10
	At 3 weeks			
A, basal diet only	1·50	1·58	1·76	1·75
B, 0·2 % L-arginine	1·46	1·62	1·74	1·76
C, 0·2 % L-arginine + 0·2 % glycine	1·45	1·56	1·73	1·71
D, 0·2 % guanidoacetic acid	1·44	1·58	1·69	1·77
SE	±0·030	±0·031	±0·041	±0·040
	At 6 weeks			
A	2·07	2·02	2·10	2·36
B	2·06	2·092	2·18	2·22
C	2·09	2·00	2·09	2·20
D	2·01	2·03	2·13	2 29
SE	±0·034	±0·034	±0·036	±0·035

SE, standard error of the mean. * g food eaten/g weight gain.

or in the presence of glycine. This increase was accompanied by an equivalent improvement in food conversion efficiency. The arginine content of the basal diet in Expt A 9 was 4·5 % of the protein, and there was no advantage in supplementing with arginine.

In Expt A 10 the arginine content was 3·6% of the protein, and this level appeared to be marginally inadequate.

The results for the mean daily retention of N at the 5-week stage in Expts A 7 and A 10 are given in Table 16. In these experiments the N retention (around 48%) was rather better at the 5-week stage than that in Expts A 2–A 6 (around 43%). There was, however, again no improvement when the basal diets were supplemented with arginine. It was disappointing to observe that there was a slight response in terms of live-weight gain to arginine supplementation in Expt 10 that was not reflected in N retention. This result may be accounted for by inadequacies in the procedures adopted for measuring N retention.

Table 16. *Nitrogen retention at 2 weeks and 5 weeks of age of birds given different supplements*

(Mean values for four replicate groups of ten birds in three successive 24 h collections)

Treatment	N eaten (g/day)	N retained	
		g/day	%
	Expt A 7		
A, basal diet only	1·81	0·86	47
B, 0·2% L-arginine	1·98	0·96	48
C, 0·2% L-arginine +0·2% glycine	1·92	0·95	49
D, 0·2% guanidoacetic acid	1·98	0·97	49
	Expt A 10		
A	2·51	1·16	46
B	2·70	1·29	48
C	2·66	1·27	48
D	2·65	1·25	47
	Expt A 11		
A	2·02	1·01	50
B	1·98	1·00	51
C	1·91	0·88	46
D	2·08	1·08	52

Expt A 11. *Arginine and lysine supplementation of the diets*

The results are given in Table 17. A significant ($P < 0·01$) growth inhibition, also reflected in reduced efficiency of food utilization, occurred in response to the addition of excess lysine. When arginine and lysine were added together, the rate of weight gain rose to the level obtained with the basal diet. The level of arginine required in the diet for optimal growth was therefore increased by the addition of lysine. However, the addition of only 0·2% of arginine was sufficient to correct the deficiency, and hence the increase in arginine requirement was comparatively small. Yet the growth inhibition in response to the relative inadequacy of arginine was severe, a feature that confirms the sensitivity of a procedure of this type to determine amino acid requirements. The growth inhibition resulting from addition of lysine was approximately paralleled by poorer N retention (Table 16).

Carcass composition

No consistent differences were found in relation to the treatments but, as expected, the birds of lower weight contained less fat and more protein. Typical values of 67% water, 20% protein, 9% ether extract and 3% ash agreed with the appropriate values of Donaldson, Combs & Romoser (1958).

Table 17. *Expt A11. Mean live weights and food conversion efficiency* of birds at 3 weeks and 6 weeks of age*

(The procedure was the same as in Expts A7–A10 (see Table 14) and the basal diet was identical to that used in Expt A10 (see Table 5). Mean values for six replicate groups of ten birds)

Treatment	3 weeks	6 weeks
	Weight (g)	
A, basal diet only	264	751
B, 0·2% L-arginine	267	738
C, 0·6% L-lysine	238	666
D, 0·2% L-arginine + 0·6% L-lysine	271	759
SE	±5·1	±12·0
	Food conversion efficiency	
A	1·76	2·25
B	1·71	2·23
C	1·83	2·39
D	1·69	2·18
SE	±0·027	±0·034

SE, standard error of the mean. * g food eaten/g weight gain.

Table 18. *Amino acid composition of basal diets in relation to dietary requirement of the young domestic chicken*

(The values are expressed as a percentage of the (USA) National Research Council (1960) requirement and include the added free amino acids)

Amino acid	NRC value (% of diet)	Expt A2	A3	A4	A5	A6	Expt A1	A7	A8	A9	A10
Arginine	1·2	124	106	88	66	54	115	108	94	81	66
Glycine	1·0	188	153	125	108	101	142	129	114	119	115
Histidine	0·3	196	163	153	130	113	160	163	160	160	153
Leucine	1·4	133	120	123	129	119	117	140	168	178	206
Isoleucine	0·6	170	150	127	119	107	148	147	152	138	137
Lysine	1·0	158	127	115	107	101	126	125	122	114	112
Methionine 0·45 / Cystine 0·35 } 0·8		149	134	125	111	101	119	131	132	132	134
Phenylalanine 0·70 / Tyrosine 0·70 } 1·4		139	125	117	105	99	118	135	137	135	136
Threonine	0·60	166	142	122	110	99	118	150	153	142	140
Tryptophan	0·20	150	140	140	125	120	125	130	140	155	170
Valine	0·80	152	130	115	104	104	131	136	137	135	135

DISCUSSION

It has been clearly demonstrated that, when conditions of amino acid imbalance as defined by Harper (1959) are induced, there is an apparent increase in the dietary requirement for arginine. Anderson & Dobson (1959) demonstrated that the addition to diets of amino acid mixtures or individual amino acids led to an increased requirement for arginine, the amino acid that under those conditions was the most limiting. It is, however, evident that this high requirement induced by imbalance cannot be regarded as a true optimal requirement, which must refer to circumstances of good overall balance.

Adequacy of amino acid balance can best be expressed with reference to the most appropriate values of requirement that are available. The composition of the diets used in Expts A1, A2–A6 and A7–A10 can thus be stated in terms of a percentage of the requirements listed by the (USA) National Research Council (1960). In the first series of experiments (Table 18) the balance of amino acids was good in relation to requirements. Except for arginine, the range in A2 was 133–196% whereas in A6 it was 99–119%. The demonstrated low requirement for arginine was associated with this relatively good amino acid balance. In the second series (A7–A10), when maize was a major component of the diet, the amino acid balance was also good (Table 18) except for leucine which was present in considerable excess. This excess, however, presumably did not induce an imbalance to such an extent that the requirement for arginine increased. In this series of experiments it was demonstrated that the arginine supply was only slightly inadequate at 3·6% of the protein in a diet containing 21·6% protein.

The results of Expt A11 confirmed that in general terms the absence of response to arginine supplementation was not a consequence of some other gross dietary inadequacy. It also firmly established that the arginine requirement was increased by inducing an imbalance. It is therefore unlikely that a diet containing an inadequate level of arginine can be prepared from conventional ingredients unless it is constituted in such a way that a severe amino acid imbalance is produced. Such an imbalance could be encouraged by a proportionally high level of lysine in the diet.

It is also necessary to comment upon the reduced rate of weight gain of the chicks consuming the basal diet alone in Expts A7–A10. The 6-week weight for the control group in Expt A7 was 833 g and in Expt A10 it was only 642 g. The corresponding food conversion ratios were 2·07 and 2·36 respectively. These results can perhaps be accounted for by a reduced acceptability of the diet. The drop in food intake would in itself lead to a deterioration in the efficiency of food utilization. The higher metabolizable energy of diet A10 as compared with A7 (3210 and 3070 kcal/kg respectively) might also contribute to the reduced food consumption. Also, the proteins of maize products are somewhat indigestible and the ratio of leucine to isoleucine might introduce a new factor of amino acid imbalance. No evidence is, however, available to decide which factors are involved.

The results show that the requirement of the young chick for arginine, under relatively normal conditions of feeding, is around 4% of the dietary protein. The fact

that there was a slight stimulation of growth when arginine was added to the basal diet in Expt A10 but no such increase in Expt A11 demonstrates that an arginine concentration of 3·6% of the protein is marginal. The values for N retention confirm this conclusion in that the only significant change was that encountered when an excess of lysine was added in Expt A11.

Provided there are no other gross amino acid imbalances, the arginine requirement of the young chick can be expressed as 4% of the protein. Any efficient system of nutrition must, however, eliminate imbalance.

SUMMARY

1. A series of trials has been carried out to examine the requirements of the young chick for arginine.

2. Diets were prepared that were adequate in all other respects but which contained decreasing levels of protein and arginine. In the first series the protein level decreased from 24 to 16% and the arginine fell from 1·5 to 0·65%. There was a slight improvement in growth performance when the diet containing 0·65% arginine was supplemented with arginine.

3. In a further series of experiments all the basal diets contained about 21% protein and the arginine content fell from 1·3 to 0·8%. Only at the lowest level was there any improvement in growth with addition of arginine. However, even in this instance it was demonstrated that the arginine content was only marginally inadequate. An arginine deficiency was aggravated by the addition of excess lysine.

4. It has thus been demonstrated that the arginine requirement of the chick is around 0·8% of the diet when it contains 21% protein, or rather less than 4% of the protein. Arginine inadequacy is unlikely to occur in practical chick diets in the United Kingdom unless conditions of extreme amino acid imbalance are induced.

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