Effects of dietary essential amino acid deficiencies on immunological variables in broiler chickens

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Two experiments were conducted to determine the effects of essential amino acid deficiencies on several immunological variables in male broiler chickens. Essential amino acids were classified into five groups as follows: S-containing amino acids (SAA; methionine + cysteine), aromatic amino acids (AAA; phenylalanine + tyrosine), branched-chain amino acids (BCAA; isoleucine + leucine + valine), arginine plus lysine (Arg + Lys), and other essential amino acids (OEAA; glycine + serine + histidine + threonine + tryptophan). Chickens were fed *ad libitum* from 10 to 24 d of age on a control diet or amino-acid-deficient diets formulated to contain each amino acid group at 50 % and 16 % (Expt 1) at 50 % (Expt 2) of the recommended requirements (National Research Council, 1984). Effects of feed consumption on immune responses were also considered by setting pair-feeding (Expt 1) or restricted-feeding (Expt 2) groups fed on the control diet. In Expt 1, changes in lymphoid organ weights varied with the type and degree of deficiency of amino acid groups, with BCAA deficiency markedly decreasing weights. The haemagglutinin titres against sheep erythrocytes did not change in any amino-acid-deficient chickens except that the titres were lower in chickens fed on the 50 %- and 16 %-BCAA diets as compared with their pair-fed counterparts. In Expt 2, the splenocyte proliferative response to concanavalin A was higher in the chickens fed on the BCAA- and Arg + Lys-deficient diets and lower in chickens fed on the SAA- and AAA-deficient diets than the control chickens, independent of feed consumption. These results suggest that the effects of specific amino acid deficiencies on immune responses cannot be generalized, and that BCAA have the greatest potential to modulate immune responses among the amino acids in chickens.

Amino acid deficiency: Immune responses: Broiler chickens

It has been shown in mammals that nutritional deficiency or excess of protein or specific essential amino acids causes changes in several immune responses, e.g. antibody titre and number of plaque-forming cells against selected antigens (Gershoff *et al*. 1968; Kenney *et al*. 1970; Bounous & Kongshavn, 1978; Lotan *et al*. 1980), lymphocyte proliferative response to mitogens (Barbul *et al*. 1980; Petro & Bhattacharjee, 1981; Nauss *et al*. 1982), delayed-type hypersensitivity response (Bounous & Kongshavn, 1978) and allograft rejection (Lotan *et al*. 1980). Jose & Good (1973) observed that leucine deficiency (50 % and 25 % of a standard level) depressed cytotoxic cell-mediated immunity, whereas deficiencies of arginine, lysine or histidine affected, to a small extent, cytotoxic cell-mediated immunity, serum blocking activity, and haemagglutinin titre in tumour-bearing mice. These results suggested that each amino acid has specific effects on immune responses.

In chickens as well as mammals, it has been shown that deficiency or excess of dietary protein (Glick *et al*. 1981, 1983; Payne *et al*. 1990) or amino acids (Bhargava *et al*. 1970, 1971; Tsiagbe *et al*. 1987*a*,*b*) alters immune responses. A few investigations have been made on the effects of several essential amino acids on immune responses, yet the effects of all essential amino acids have not been studied in either chickens or mammals. Chickens have different metabolic properties and requirements for amino acids compared with mammals. For example, chickens are characterized by poor ability to synthesize arginine and glycine, and consequently require both amino acids from the diet. Therefore, the changes of immune responses induced by dietary amino acids in mammals may not always be applicable to chickens.

The present study was conducted to determine whether specific amino acid deficiencies affect lymphoid organ weights, antibody production to sheep erythrocytes (SRBC) and splenocyte proliferative responses to concanavalin A

Abbreviations: AAA, aromatic amino acids; BCAA, branched-chain amino acids; Con A, concanavalin A; IgG, immunoglobulin G; OEAA, other essential amino acids; SAA, sulfur-containing amino acids; SRBC, sheep erythrocytes.

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(Con A), and to clarify which amino acids have the most potential to modulate the immune responses in broiler chickens. To avoid the possible influence of the interactions among amino acids on immune responses (Aschkenasy, 1979), essential amino acids were tentatively classified from the metabolic viewpoint into five groups, i.e. S-containing amino acids (SAA; methionine + cysteine), aromatic amino acids (AAA; phenylalanine + tyrosine), branched-chain amino acids (BCAA; leucine + isoleucine + valine), arginine + lysine (Arg + Lys), and other essential amino acids (OEAA; glycine + serine + histidine + threonine + tryptophan). Dietary levels of the selected amino acids in each group were reduced at a constant ratio. Because feed consumption may affect immune responses (Klasing, 1988; Umezawa *et al.* 1990), the effects of decreased feed consumption involved in amino acid deficiency were also considered.

Materials and methods

Animals and diets

Unvaccinated 1-d-old male broiler chickens (Ross) obtained from a local hatchery were used in all experiments. They were housed in electrically heated battery brooders and fed on a commercial starter diet (220 g crude protein/kg and 12·55 MJ metabolizable energy/kg) *ad libitum* for 7 d. When they were 7 d old, chickens were individually weighed, assigned to dietary groups so that mean body weights were as uniform as possible, and reared in stainless-steel wire cages with two birds per cage in a temperature- (22–24°) and light- (24 h/d) controlled room. There followed a 3 d adaptation period where chickens were provided with mixtures (2 : 1, 1 : 1 and 1 : 2 w/w) of ground commercial starter diet and a control diet (Table 1). Water was provided *ad libitum*.

The control diet (Table 1) was formulated to contain all essential amino acids at recommended levels (National Research Council, 1984). Amino-acid-deficient diets were formulated to contain the selected essential amino acids (i.e. SAA, AAA, BCAA, Arg + Lys, or OEAA) at 50 % or 16 % of the amount in the control diet with all remaining essential amino acids as in the control diet. Glutamic acid, glucose, and soyabean oil were adjusted so that all experimental diets were isonitrogenous (230 g crude protein/kg) and isoenergetic (13·39 MJ metabolizable energy/kg).

Table 1. Composition of control diet (g/kg)

Ingredients	
Amino acid mixture*	101·6
L-Glutamic acid	195·4
Maize (83 g crude protein/kg)	200·0
Soyabean oil	46·3
Glucose	350·9
Cellulose	27·0
Vitamin mixture†	12·0
Mineral mixture‡	66·8
Calculated composition	
Crude protein	230·0
Metabolizable energy (MJ/kg)	13·39
Arginine	14·4
Glycine + serine	15·0
Histidine	3·5
Isoleucine	8·0
Leucine	13·5
Lysine	12·0
Methionine + cysteine	9·4
Phenylalanine + tyrosine	13·4
Threonine	8·0
Tryptophan	2·3
Valine	8·2
Calcium	11·9
Phosphorus (available)	6·6

* The amino acid mixture provided (g/kg diet): L-arginine 13·6, glycine 6·9, L-serine 6·7, L-histidine 3·0, L-isoleucine 7·4, L-leucine 11·7, L-lysine-HCl 14·8, DL-methionine 4·9, L-cysteine 4·0, L-phenylalanine 6·4, L-tyrosine 5·6, L-threonine 7·4, L-tryptophan 2·1, L-valine 7·4.
† See Baker *et al.* (1979).
‡ See Scott *et al.* (1982).

Expt 1

The effects of deficiencies of SAA, AAA, BCAA, Arg + Lys and OEAA on performance, lymphoid organ weights (thymus, spleen and bursa of Fabricius) and antibody production against SRBC were investigated in five experiments (i.e. Expts 1A–1E) in turn.

Experimental design. In Expt 1A, forty-eight chickens were divided into four treatment groups with six replications of two birds per cage. In Expts 1B–1D, sixty chickens were assigned to five treatment groups with six replications of two birds per cage. In Expt 1E, forty chickens were assigned to five treatment groups with four replications of two birds per cage. In Expts 1A–1E, three out of the four or five groups were fed *ad libitum* on either the control diet or amino-acid-deficient diets containing selected amino acids at 50 % or 16 % of the level in the control diet. The remaining groups were provided with the control diet in an amount equal to that consumed by chickens fed on the diets containing selected amino acids at the 50 % and 16 % levels in Expts 1B–1E, or at the 16 % level in Expt 1A. Each of the treatment pens was given an amount of feed equal to the average of that eaten by the *ad libitum* controls. Chickens under pair-feeding conditions were provided the diet three times per day in the ratio of 1 : 1 : 2 (by wt) at 09.00, 15.00, and 21.00 hours, respectively.

Lymphoid organ weights and haemagglutinin titres. At 17 d of age, eight chickens from each treatment were selected as having body weights close to the group mean, and injected intravenously with 0·5 ml of a suspension of SRBC in 0·01 M-PBS pH 7·2 (5 : 100, v/v). Immunizations were done after feeding. At 7 d after the challenge, chickens were killed following blood sampling. Thymus, spleen, and bursa of Fabricius were removed from five immunized chickens per group and weighed. Lymphoid organ weights were expressed in mg/100 g body weight. Plasma was collected by centrifugation at 500 ***g*** for 10 min and heat-inactivated at 56° for 30 min. The plasma samples were stored at −20° until analysis.

Antibody titres to SRBC were determined by the method of Isakov *et al.* (1982) with slight modifications. Briefly, two-fold serial dilutions of the tested plasma (25 μl each) were made with PBS containing 2 g bovine serum albumin/l, using ninety-six-well plates. The wells of plates for determination of immunoglobulin G (IgG) titres were supplemented with 25 μl PBS containing 0·2 M-2-mercaptoethanol, and those for determination of total haemagglutinin titres were

supplemented with 25 μl PBS alone. At 1 h after incubation at 37°, 50 μl of a suspension of SRBC in PBS containing the bovine serum albumin (5 : 1000, v/v) was added to each well. Thereafter, the plates were incubated at 37° for 2 h and placed at 4° overnight. The haemagglutinin titres were expressed as the $\log_2$ of highest dilution showing visible agglutination.

Expt 2

The effects of deficiencies of dietary essential amino acids on splenocyte proliferative responses to Con A, a T-cell mitogen, were determined.

Experimental design. Fifty-four chickens were assigned to nine treatment groups with three replications of two birds per cage. Six groups were fed *ad libitum* on the control diet or diets containing test amino acids at the 50 % level. To assess the effect of feed consumption, three groups were fed on the control diet in an amount equal to 80, 60 or 40 % of that consumed by chickens fed on the control diet *ad libitum*. Chickens in restricted-fed groups were provided the diet three times per day as described in Expt 1.

Splenocyte proliferative responses. At the end of the experimental period, four chickens with body weights close to the group mean were selected from each group and killed. Spleens were removed aseptically and placed in 5 ml RPMI 1640 medium containing 2 mM-L-glutamine, 48 mM-$NaHCO_3$, 100 U/ml penicillin, 100 μg/ml streptomycin, and 25 mM-HEPES. Blood was collected, allowed to clot at room temperature for 2–3 h, and then centrifuged at 500 ***g*** for 10 min. The sera from the four selected chickens in each group were pooled, heat-inactivated at 56° for 30 min, and sterile filtered.

Splenocytes were obtained by forcing the spleens through a 100-mesh stainless-steel screen, and then passed through sterile nylon mesh, to remove debris. Cell suspensions were layered over Ficoll-Isopaque solution (Wako Pure Chemicals Co. Ltd, Osaka, Japan) (d = 1·070), and centrifuged at 500 ***g*** for 30 min. The mononuclear cell layer was collected and washed three times with the medium. Erythrocytes were removed by resuspending cell pellets in 83 g NH_4Cl in 1000 ml 0·17 M-Tris buffer (pH 7·65). The washed spleen mononuclear cells were re-suspended in the RPMI 1640 medium. Preliminary experiments established the optimal cell densities, Con A concentration, serum concentration and incubation times for maximal proliferation of chick mononuclear cells. Cultures (200 μl) of 2×10^5 cells were cultured in ninety-six-well plates with or without the addition of Con A (12·5 μg/ml medium) for 48 h, in five replications, in a CO_2–air (5 : 95, v/v) humidified atmosphere at 37°. These cultures all contained autologous chick serum (50 ml/l). During the last 8 h of the incubation, 37 kBq [^{3}H]thymidine (specific activity, 185 GBq/mmol) was added to each well. After the incubation, cells were harvested onto glass-fibre mats and incorporation of the [^{3}H]thymidine, represented as disintegrations per minute (d.p.m.), was determined by liquid scintillation counting. Results were expressed as the stimulation index (d.p.m. stimulated/d.p.m. unstimulated).

Statistical analysis

Data were subjected to one-way ANOVA within the control group and amino-acid-deficient groups or within the control group and pair-fed groups or feed-restricted groups in each experiment. The analysis for feed consumption was done based on cage replications, and for the analysis for other measurements, individual chickens were considered as units. In Expt 1, when differences were significant ($P < 0·05$), means of treatment groups were separated by Duncan's multiple range test. To analyse the difference between each amino-acid-deficient group and its pair-fed counterpart, means were compared using Student's *t* test. In Expt 2, when differences were significant ($P < 0·05$), means of treatment groups were separated by Duncan's multiple range test).

Results and discussion

Performance

Tables 2 and 3 show body-weight gain and feed consumption in Expts 1 and 2 respectively. Body-weight gain was significantly lower in chickens fed on amino-acid-deficient diets than that in chickens fed on the control diet *ad libitum* (control chickens). In Expt 1, the body-weight gains of chickens fed on the diets 50 % deficient in SAA, AAA, BCAA, Arg + Lys, and OEAA were 60·0, 39·7, 27·6, 37·7, and 28·2 (pooled SEM 2·2) % of their *ad libitum* controls respectively. At the 16 % of control level in Expt 1, body-weight gains of SAA-, AAA-, BCAA-, Arg + Lys-, and OEAA-deficient chickens were 4·0, −4·2, −2·0, 1·7, and −4·7 (pooled SEM 0·6) % of their *ad libitum* controls respectively. The body-weight gains expressed as a percentage of control, of chickens fed on SAA-deficient diets were significantly greater than those of birds fed on other amino-acid-deficient diets, whereas the gains of chickens fed on BCAA- or OEAA-deficient diets were lower than others although the differences were not always significant. Similar observations were found in Expt 2 (Table 3). The results of the present study were similar to the observations of Okumura & Mori (1979) who noted that body-weight gain was reduced most severely by isoleucine deficiency and moderately by SAA deficiency in White Leghorn male chicks fed on diets 50 % deficient in a single essential amino acid.

Lymphoid organ weights

Table 4 shows relative weights of thymus, spleen and bursa of Fabricius of chickens in Expt 1. When the amino acid level in the diets was 50 % of the control, only BCAA deficiency significantly depressed relative weights of thymus and bursa as compared with both the control group and the pair-fed counterparts. No amino acid deficiency at 50 % of the control level affected the relative spleen weight. The organ weights in chickens fed on diets with only 16 % of the recommended level of amino acids were generally lower than those fed on the control diet or diets with 50 % of the recommended level. Of groups fed with 16 % of the control level, the relative weights of thymus or bursa were significantly lower in chickens fed on SAA-, AAA-, BCAA-, and OEAA-deficient diets and relative spleen weight was lower in chickens fed on SAA- and BCAA-deficient diets than those in the control chickens.

Table 2. Expt 1. The performance of broiler chickens fed on various amino-acid-deficient diets from 10 to 24 d of age
(Mean values with their standard errors for four to twelve observations)

		Amino acid level (% recommended level†) and feeding condition									
		100 % *ad libitum* (control)		50 % *ad libitum*		100 % pair-fed to 50 % diet		16 % *ad libitum*		100 % pair-fed to 16 % diet	
Experiment	Deficient amino acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Feed consumption (g)‡											
1A	SAA	818^{a}	22	632^{b}	50	ND		296^{c}	27	–	
1B	AAA	771^{a}	39	465^{b}	20	–		195^{c}	10	–	
1C	BCAA	772^{a}	24	417^{b}	9	–		205^{c}	8	–	
1D	Arg + Lys	795^{a}	46	530^{b}	18	–		242^{c}	16	–	
1E	OEAA	750^{a}	32	391^{b}	16	–		184^{c}	13	–	
Body-weight gain (g)§											
1A	SAA	510^{ax}	18	335^{b}	50	ND		20^{c***}	10	120^{y}	10
1B	AAA	474^{ax}	26	188^{b***}	9	231^{y}	9	–20^{c***}	3	35^{z}	5
1C	BCAA	470^{ax}	21	130^{b***}	9	180^{y}	8	–9^{c***}	4	30^{z}	4
1D	Arg + Lys	523^{ax}	29	197^{b***}	11	307^{y}	11	9^{c***}	7	77^{z}	5
1E	OEAA	477^{ax}	28	135^{b***}	9	201^{y}	12	–22^{c***}	5	57^{z}	6

SAA, S-containing amino acids (methionine + cysteine); AAA, aromatic amino acids (phenylalanine + tyrosine); BCAA, branched-chain amino acids (leucine + isoleucine + valine); OEAA, other essential amino acids (glycine + serine + histidine + threonine + tryptophan); ND, not determined.
^{a,b,c} Mean values for control and amino-acid-deficient groups within a row not sharing a common superscript letter were significantly different: $P < 0{\cdot}05$.
^{x,y,z} Mean values for control and pair-fed groups within a row not sharing a common superscript letter were significantly different: $P < 0{\cdot}05$.
Mean values were significantly different from those for the pair-fed counterparts: *** $P < 0{\cdot}001$.
†National Research Council (1984).
‡ Mean values for six (Expts 1A–1D) or four (Expt 1E) observations.
§ Mean values for twelve (Expts 1A–1D) or eight (Expt 1E) observations.

The present results suggest that weights of lymphoid organs are modified by either the type of essential amino acids or the degree of deficiency, and that weights of thymus and bursa are more susceptible to dietary amino acid deficiencies than weight of spleen. Our findings also showed that feeding the BCAA-deficient diet caused the most severe decrease in both thymus and bursa weights in chickens. Supporting our results is the observation by Aschkenasy (1975) that deficiencies of isoleucine and valine inhibited leucopoiesis, with involution of lymphoid organs, especially the thymus, and a dramatic drop in the blood lymphocyte numbers in rats. It is, therefore, possible that the relative requirement of BCAA for development of lymphoid organs is greater than that of other amino acids in

Table 3. Expt 2. The performance of broiler chickens fed *ad libitum* on various amino-acid-deficient diets or fed on the control diet under restricted-feeding conditions from 10 to 24 d of age*
(Mean values with their standard errors for three (feed consumption) or six (body-weight gain) observations)

		Feed consumption (g)		Body-weight gain (g)	
Diet	Feeding condition	Mean	SE	Mean	SE
Treatments 1: amino acid deficiencies					
Control	*ad libitum*	755^{a}	23	485^{a}	56
SAA-deficient	*ad libitum*	703^{a}	67	352^{b}	55
AAA-deficient	*ad libitum*	339^{c}	13	120^{c}	8
BCAA-deficient	*ad libitum*	342^{c}	29	106^{c}	17
Arg + Lys-deficient	*ad libitum*	462^{b}	36	187^{c}	22
OEAA-deficient	*ad libitum*	376^{bc}	18	124^{c}	12
Treatments 2: feed restriction					
Control	*ad libitum*	755	23	485^{a}	56
Control	80 % of *ad libitum*	(593)		329^{b}	23
Control	60 % of *ad libitum*	(439)		223^{c}	17
Control	40 % of *ad libitum*	(266)		104^{d}	19

SAA, S-containing amino acids (methionine + cysteine); AAA, aromatic amino acids (phenylalanine + tyrosine); BCAA, branched-chain amino acids (leucine + isoleucine + valine); OEAA, other essential amino acids (glycine + serine + histidine + threonine + tryptophan).
^{a,b,c,d} Mean values within a column not sharing a common superscript letter were significantly different: $P < 0{\cdot}05$.
* Amino-acid-deficient diets were formulated to contain the selected amino acids at 50 % of the recommended level (National Research Council, 1984).

Table 4. Expt 1. Relative weights of thymus, spleen and bursa of Fabricius of broiler chickens fed on various amino-acid-deficient diets from 10 to 24 d of age

(Mean values with their standard errors for five observations)

		Amino acid level (% recommended level†) and feeding condition									
		100 % *ad libitum* (control)		50 % *ad libitum*		100 % pair-fed to 50 % diet		16 % *ad libitum*		100 % pair-fed to 16 % diet	
Experiment	Deficient amino acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Thymus (mg/kg body wt)											
1A	SAA	270^{a}	9	257^{a}	33	ND		135^{b}*	18	236	39
1B	AAA	288^{a}	37	279^{a}	13	239	44	116^{b}	8	186	25
1C	BCAA	298^{ax}	17	182^{b}**	20	279^{x}	20	160^{b}	18	169^{y}	12
1D	Arg + Lys	254	33	221*	11	276	18	165**	24	282	24
1E	OEAA	232^{ay}	29	242^{a}	27	311^{x}	23	152^{b}	8	185^{y}	14
Spleen (mg/kg body wt)											
1A	SAA	100^{a}	9	91^{a}	7	ND		65^{b}	5	91	13
1B	AAA	112	11	101	7	106	11	79	8	94	17
1C	BCAA	85^{a}	5	80^{a}	4	74	6	60^{b}	5	83	10
1D	Arg + Lys	99	15	145	22	97	6	130	6	105	8
1E	OEAA	132	18	117	8	145	15	115	11	115	7
Bursa of Fabricius (mg/kg body wt)											
1A	SAA	224^{a}	12	251^{a}	30	ND		152^{b}	17	219	27
1B	AAA	240^{a}	16	247^{a}	33	261	12	154^{b}	14	206	39
1C	BCAA	244^{a}	20	173^{b}*	21	280	36	133^{b}	23	187	22
1D	Arg + Lys	187^{by}	11	235^{ab}	11	242^{xy}	22	250^{a}	22	273^{x}	28
1E	OEAA	245	36	277	41	264	25	167*	19	255	22

SAA, S-containing amino acids (methionine + cysteine); AAA, aromatic amino acids (phenylalanine + tyrosine); BCAA, branched-chain amino acids (leucine + isoleucine + valine); OEAA, other essential amino acids (glycine + serine + histidine + threonine + tryptophan); ND, not determined.

^{a,b} Mean values for control and amino-acid-deficient groups within a row not sharing a common superscript letter were significantly different: $P < 0{\cdot}05$.

^{x,y} Mean values for control and pair-fed groups within a row not sharing a common superscript letter were significantly different: $P < 0{\cdot}05$.

Mean values were significantly different from those for the pair-fed counterparts: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

† National Research Council (1984).

chickens. The pair-feeding of the control diet (i.e. restriction of feed consumption) did not always reduce relative lymphoid organ weights, substantiating the consideration that lymphoid organs may respond to amino acid deficiencies, *per se*, unrelated to feed consumption.

Haemagglutinin titres

Table 5 shows total haemagglutinin titre and 2-mercaptoethanol-resistant antibody (IgG) titre against SRBC of chickens in Expt 1. Total haemagglutinin titres against SRBC of chickens fed on each amino-acid-deficient diet were not different from that of chickens fed on the control diet *ad libitum*. However, as compared with those in their pair-fed counterparts, the total haemagglutinin titres against SRBC tended to be lower in chickens fed on the 50 %- and 16 %-BCAA diets ($P = 0{\cdot}054$ and $P < 0{\cdot}05$ respectively) and tended to be higher ($P = 0{\cdot}063$) in chickens fed on the 50 %-OEAA diet. These results indicate that one or some of the BCAA and OEAA may have potential to change antibody production.

It has been shown that moderate or severe deficiency of specific essential amino acids causes changes in antibody production in chickens and mammals. Bhargava *et al*. (1970, 1971) observed that deficiencies of valine and threonine reduced antibody production against Newcastle disease virus in chickens. Tsiagbe *et al*. (1987*a*,*b*) found that a low level of SAA (77 % of the recommended level) lowered antibody production to SRBC in broiler chickens. On the other hand, Takahashi *et al*. (1993, 1994) reported that marginal excesses or deficiencies of dietary methionine or threonine did not affect primary antibody production against SRBC or *Brucella abortus* in broiler chickens. In mammals, Gershoff *et al*. (1968) reported that deficiencies of phenylalanine and tryptophan depressed antibody production against SRBC in rats, whereas Bounous & Kongshavn (1978) observed that deficiency of phenylalanine + tyrosine enhanced antibody production against SRBC in rats. Jose & Good (1973) found in mice that a 50 % deficiency of a single amino acid did not affect the haemagglutinin titre determined by the polyvinylpyrrolidone method. Thus, the effects of amino acid deficiency on antibody production are still controversial. At any rate, dietary amino acid deficiency even at 16 % of the control level had little effect on the primary antibody titre against SRBC under the present experimental conditions in chickens.

The IgG titres against SRBC, estimated by 2-mercaptoethanol-resistant antibody titre, were generally higher in chickens fed on the amino-acid-deficient diets than those in the control groups, although significant differences were found in chickens fed on the SAA- and AAA-deficient diets (Table 5). The IgG titre increased gradually with a decrease in consumption of the control diet and the titres in the amino-acid-deficient group were not different from those found in their pair-fed counterparts. Therefore, changes in feed consumption may have been mainly responsible for the

Table 5. Expt 1. Total and 2-mercaptoethanol-resistant (immunoglobulin G; IgG) antibody titres against sheep erythrocytes in broiler chickens fed on various amino-acid-deficient diets from 10 to 24 d of age

(Mean values with their standard errors for eight observations)

		Amino acid level (% recommended level†) and feeding condition									
		100 % *ad libitum* (control)		50 % *ad libitum*		100 % pair-fed to 50 % diet		16 % *ad libitum*		100 % pair-fed to 16 % diet	
Experiment	Deficient amino acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Total antibody titre (log_2)											
1A	SAA	5·6	6·6	5·9	0·5	ND		4·8	0·6	5·0	0·4
1B	AAA	6·9	0·7	7·0	0·6	7·4	0·4	5·6*	0·6	6·4	0·5
1C	BCAA	6·6	0·6	6·3	0·5	7·9	0·6	6·0	0·5	7·4	0·4
1D	Arg + Lys	5·9	0·4	5·9	0·4	6·4	0·5	6·6	0·4	5·8	0·8
1E	OEAA	6·9	0·7	8·6	0·3	7·5	0·5	7·6	0·6	8·0	0·6
IgG titre (log_2)											
1A	SAA	$0·3^{by}$	0·3	1·4	$0·7^{ab}$	ND		$2·5^{a}$	0·2	$2·9^{x}$	0·3
1B	AAA	$0·8^{by}$	0·4	1·6	$0·4^{ab}$	$2·0^{x}$	0·3	$2·5^{a}$	0·2	$2·6^{x}$	0·2
1C	BCAA	$1·0^{y}$	0·5	1·6	0·5	$2·0^{xy}$	0·5	1·9	0·4	$2·8^{x}$	0·3
1D	Arg + Lys	1·6	0·5	1·4	0·4	1·6	0·5	2·0	0·3	2·1	0·4
1E	OEAA	1·5	0·5	2·5	0·4	2·4	0·4	2·6	0·4	2·3	0·5

SAA, S-containing amino acids (methionine + cysteine); AAA, aromatic amino acids (phenylalanine + tyrosine); BCAA, branched-chain amino acids (leucine + isoleucine + valine); OEAA, other essential amino acids (glycine + serine + histidine + threonine + tryptophan); ND, not determined.

[a,b] Mean values for control and amino-acid-deficient groups within a row not sharing a common superscript letter were significantly different: $P < 0·05$.

[x,y] Mean values for control and pair-fed groups within a row not sharing a common superscript letter were significantly different: $P < 0·05$.

Mean values were significantly different from those for the pair-fed counterparts: * $P < 0·05$.

† National Research Council (1984).

increase of IgG titre in amino-acid-deficient groups in the present study.

Splenocyte proliferative response

Fig. 1 shows the splenocyte proliferative responses to Con A, an index of cell-mediated immunity *in vitro*, in chickens fed on amino-acid-deficient diets in Expt 2. The splenocyte proliferative responses in chickens fed on the BCAA- and Arg + Lys-deficient diets were significantly higher than those in chickens fed *ad libitum* on the control diet. In contrast, the responses in chickens fed on SAA- and AAA-deficient diets were significantly lower than those fed *ad libitum* on the control diet. No significant difference in the proliferative response was observed between *ad libitum* controls and chickens fed on the OEAA-deficient diet. As shown in Fig. 1, the proliferative responses decreased slightly with a decrease in consumption of the control diet, suggesting that the feed consumption may also affect the proliferative response. Furthermore, splenocyte proliferative responses to Con A in the amino-acid-deficient groups differed from those in chickens fed on the control diet at any level (40–100 % of *ad libitum* feeding), suggesting that each amino acid probably has a specific effect on the splenocyte proliferative response to Con A, independent of feed consumption.

The stimulation of splenocyte proliferation by feeding BCAA- and Arg + Lys-deficient diets in the present study is similar to observations in mice by Petro & Bhattacharjee (1981) who showed that feeding isoleucine- and lysine-deficient diets significantly increased splenocyte proliferative responses to phytohaemagglutinin, a T-cell mitogen. Nauss *et al.* (1982) reported that marginal methionine–choline deficiency reduced thymic, splenic and mesenteric

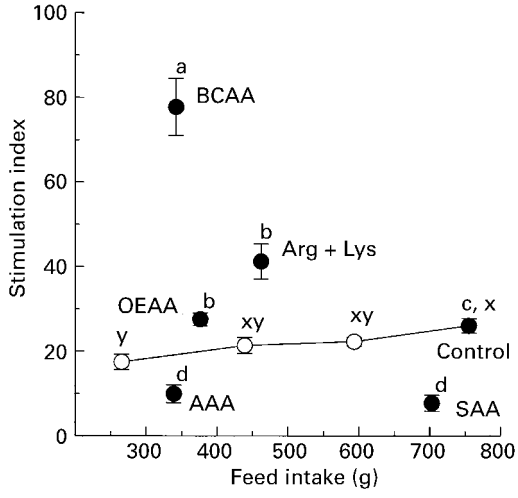

Fig. 1. The splenocyte proliferative response to concanavalin A (expressed as the stimulation index, see p. 451) of broiler chickens fed *ad libitum* on a control diet or diets deficient (50 % of the value recommended by National Research Council, 1984) in S-containing amino acids (SAA), aromatic amino acids (AAA), branched-chain amino acids (BCAA), arginine + lysine (Arg + Lys) or other essential amino acids (OEAA) (all ●) from 10 to 24 d of age, or given the control diet in amounts equal to 80, 60 or 40 % of that consumed by chickens under *ad libitum* conditions (all ○). Values are means for four chickens with their standard errors represented by vertical bars. [a,b,c] Mean values for control and amino-acid-deficient groups bearing different letters were significantly different ($P < 0·05$). [x,y,z] Mean values for control and feed-restricted groups not sharing a common letter were significantly different ($P < 0·05$).

lymphocyte proliferative responses to phytohaemagglutinin and Con A in young rats. Tsiagbe *et al.* (1987*a*,*b*) observed a decrease in delayed-type sensitivity response to phytohaemagglutinin, an index of cell-mediated immune response *in vivo*, in broiler chickens fed on a SAA-deficient diet. These observations are in accord with our present results. In contrast to these observations, Jose & Good (1973) reported that 50 % deficiencies of any amino acid except leucine did not alter cell-mediated lysis of allogeneic tumour cells in rats. With this conflict in mind, it is possible to conclude that certain amino acids, i.e. BCAA, Arg + Lys, SAA and AAA, modify cell-mediated immune response in chickens.

In the present study, autologous chicken serum was used to culture the splenocytes. Thus, it is possible that a serum component(s) is responsible for the changes of proliferative response due to dietary amino acid deficiency. In fact, it has been shown that the proliferative response of lymphocytes was modulated by serum components, e.g. hormones (reviewed by Marsh & Scanes, 1994), metabolites (Kollmorgen *et al.* 1979; De Deckere *et al.* 1988; Kuhlman *et al.* 1988; Fritsche *et al.* 1991), and immunomodulators or inhibitors (reviewed by Barta, 1983). Holt (1992*a*,*b*) reported that experimentally induced moulting decreased splenic and peripheral $CD4^+$ T cells (helper T cells) and increased splenic $CD8^+$ T cells (suppressor-cytotoxic T cells) in White Leghorn hens, and the moulting decreased the delayed-type hypersensitivity response in hens. Furthermore, Barbul (1990) observed that daily oral arginine–HCl supplements (30 g for 1 or 2 weeks increased the lymphocyte proliferation to mitogen-stimulation in human subjects, and decreased the number and percentage of $CD8^+$ T cells. Thus, possible changes in the T-cell subpopulation may also be responsible for changes in the proliferative response due to dietary amino acid deficiencies. In addition, because macrophages modulate lymphocyte proliferation responses to mitogen (Folch *et al.* 1973; Bell *et al.* 1986; Mills, 1991) and because splenocytes used in the present study contain macrophages, changes in number or function of macrophages may also be responsible for the change in mitogen-induced lymphocyte proliferation in amino-acid-deficient chickens. Further studies are needed to clarify how dietary amino acid deficiencies change the splenocyte proliferative responses to Con A.

The present study indicates that BCAA have the greatest potential to modulate the immune system among the amino acids tested in chickens. BCAA make up 30 % of the dietary requirement for essential amino acids and serve as building blocks for tissue proteins. During periods of decreased protein intake, BCAA availability could limit protein synthesis, because these amino acids are contained in nearly all body proteins. Therefore, optimal conservation of BCAA during times of limited dietary amino acid intake depends on precise control of their catabolism. The initial step in BCAA metabolism is a reversible transamination with α-ketoglutarate forming glutamate and the respective branched-chain α-keto acid (Taylor *et al.* 1970). Subsequently, the branched-chain α-keto acids are decarboxylated resulting in irreversible loss of these amino acids. Thus, during periods of limited protein intake, BCAA C atoms could be conserved by decreasing the rate of irreversible loss or by altering the interconversion of leucine and α-ketoisocaproate (Nissen & Haymond, 1981). Kuhlman *et al.* (1988) indicated that feeding leucine may adversely affect immune function by suppressing lymphocyte activity, whereas oral administration of α-ketoisocaproate has a positive influence on immune function in sheep by increasing lymphocyte activity. In general, the amino acid composition of immunoglobulin parallels that of the tissues and dietary requirement in chickens, but there are a few exceptions. Leucine is considerably higher in the globulin than in the tissues and valine is also higher in the globulin than the dietary requirement (Hill, 1982). Hence, it is likely that an increase in α-ketoisocaproate production during periods of limitation of BCAA intake is associated with increasing splenocyte proliferation, and lack of BCAA supply from diet and tissues may result in decreased globulin synthesis and lymphoid organ weights. However, further research is needed to elucidate the causes for the specific effects of BCAA in the immune system in chickens observed in the present study.

In conclusion, immune responses in broiler chickens varied with either the type or the degree of deficiency of amino acids, and BCAA appeared to have greatest potential to modulate the immune response among the amino acids studied. The results of the present study also indicate that amino acid deficiencies may preferentially affect cell-mediated immune responses relative to development of the lymphoid organs and antibody production in chickens.

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