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Conjugated linoleic acid enhanced the immune function in broiler chicks

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This study was undertaken to investigate the growth performance and immune responses of broiler chicks fed diets supplemented with conjugated linoleic acid (CLA). Two hundred and forty day-old Arbor Acre male broiler chicks were randomly allotted into four dietary treatments with different inclusion levels of CLA (0, 2·5, 5·0 or 10·0 g pure CLA/kg) for 6 weeks. Growth performance, lysozyme activity, peripheral blood mononuclear cell (PBMC) proliferation, prostaglandin E_2 (PGE_2) synthesis and antibody production were investigated. There were no significant differences in growth performance among treatments ($P > 0·05$). Chicks fed 10·0 g CLA/kg diet produced 40 % and 49 % more lysozyme activity in serum and spleen than the control group at 21 d of age ($P < 0·05$). Dietary CLA enhanced the PBMC proliferation in response to concanavalin A at the age of 21 and 42 d ($P < 0·05$). Systemic and peripheral blood lymphocytic synthesis of PGE_2 in chicks fed 10·0 g CLA/kg diet was significantly decreased by 57 % and 42 % compared to chicks fed control diet ($P < 0·05$). Antibody production to sheep red blood cell and bovine serum albumin were elevated in either 2·5 or 10·0 g CLA/kg dietary treatments ($P < 0·05$). The results indicated dietary CLA could enhance the immune response in broiler chicks, but did not alter the growth performance.

Conjugated linoleic acid: Broiler chickens: Growth: Antibody production: Prostaglandin E_2

Conjugated linoleic acid (CLA) is a collective name for the mixture of 18:2 fatty acids that have conjugated double bonds. CLA was found to act as a growth factor in rats (Chin *et al.* 1994) and pigs (Ostrowska *et al.* 1999; Szymczyk *et al.* 2000), and a potential inhibitor of body fat accumulation (West *et al.* 1998; Ostrowska *et al.* 1999; Yamasaki *et al.* 2003). Furthermore, it displayed anticarcinogenic, antiatherogenic, antidiabetic and immunomodulatory properties (Ip *et al.* 1991; Lee *et al.* 1994; Pariza *et al.* 2000, 2001; Belury, 2002). It has already been reported that CLA increased lymphocyte proliferation (Chew *et al.* 1997; Wong *et al.* 1997) and IL-2 levels in mice (Hayek *et al.* 1999), and immunoglobin production in rats (Sugano *et al.* 1998); CLA also mediates protection against immune-induced wasting (Cook *et al.* 1993; Miller *et al*. 1994), mucosal damage and growth failure in experimental colitis (Hontecillas *et al.* 2002) and antigen-induced type 1 hypersensitivity response (Whigham *et al.* 2001, 2002). A considerable amount of evidence concerning the immunomodulatory function of CLA has been found (O'Shea *et al.* 2004), and an immuno-enhancing effect of CLA has been proposed.

Some of the properties of CLA are similar to those of *n*-3 PUFA. It was hypothesized that *n*-3 PUFA could enhance immune responses and disease resistance by reducing eicosanoid production, particularly prostaglandin E_2 (PGE_2). Because CLA is an analogue of linoleic acid, it affects the metabolism of *n*-6 PUFA, and may alter arachidonic acid-derived eicosanoid formation. However, it appears that CLA modulation of eicosanoid production is species specific (Belury, 2002), and modulation in birds has not been investigated.

The effects of CLA on mammalian immune responses have been extensively studied but little is known about the effects of dietary CLA on chicken immunity. Though some studies in mammals demonstrate that dietary CLA enhances Ig production (Sugano *et al.* 1998; Yamasaki *et al.* 2000), the effects of CLA on antibody production of chicks are still uncertain. Cook *et al.* (1993) showed that antibody production in chicks against sheep red blood cell (SRBC) was not affected by feeding CLA, while Takahashi *et al.* (2003) reported that dietary CLA enhanced anti-SRBC antibody production in broilers. Limited information is available on other measures of immune response in birds.

Modulation of the immune status of chickens may bring beneficial effects and provide a new avenue in improving poultry health and production. Therefore, it is important to understand the effects and mechanisms of dietary CLA on chicken immune responses. The purpose of the current study was to examine the effect of diets supplemented with CLA on the immune response of broiler chicks and to explore whether the immune modulation of CLA is through alteration of PGE_2 production. So, in this study we used CLA substituted for maize oil (rich in *n*-6 PUFA, i.e. linoleic acid), and examined dietary CLA (or different ratios of *n*-6 PUFA and CLA) on the growth performance, lysozyme activity in serum and spleen, peripheral blood mononuclear cell

Abbreviations: BSA, bovine serum albumin; CLA, conjugated linoleic acid; ConA, concanavalin A; PBMC, peripheral blood mononuclear cell; PGE_2, prostaglandin E_2; SRBC, sheep red blood cell.

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(PBMC) proliferation, PGE_2 in circulation, and PBMC and antibody production in broiler chickens.

Materials and methods

Dietary treatments and animal management

Two hundred and forty day-old male Arbor Acre broiler chickens were assigned to four diets containing 0 (control), 2·5, 5·0 or 10·0 g pure CLA/kg. Diets were prepared by adding 0 (control), 3·12, 6·24 or 12·48 g of a commercial CLA (Auhai Biotech Ltd, Qingdao, P.R. China) containing 80·1 % CLA (c9, t11 = 39·2; t10, c12 = 38·9; other CLA isomers = 2·0) in the form of NEFA into every kilogram of diet. A corn–soyabean meal diet was used, and energy was adjusted using maize oil. Each treatment had six replications with ten birds each. Birds were fed for 6 weeks. Chicks had free access to food and water and were housed in wire cages and maintained on a 24 h constant-light programme. Temperatures in the chicken house were set to 33°C for the first 3 d and were reduced by 3°C each consecutive week until they reached 24°C. Compositions of the control diets and nutrient levels for starters (1–21 d) and growers (22–42 d) are presented in Table 1. Body weights were recorded for each replicate on days 1, 21 and 42 of age, and feed intake was measured over these periods in order to calculate feed conversion ratio. All management of birds was in accordance with the guidelines of raising Arbor Acre broilers (Wang, 2000). All procedures were approved by the Animal Care and Use Committee of the China Agricultural University.

Table 1. The composition of control diet and nutrient levels

	Starter	Grower
Ingredient (%)		
Corn	57·55	63·59
Soyabean meal (43 % crude protein)	30·22	22·86
Corn gluten meal	6·84	8·57
Maize oil*	1·25	1·25
Limestone	1·34	1·43
Dicalcium phosphate	1·78	1·34
L-Lysine (99 %)	0·14	0·20
DL-Methionine (98)	0·19	0·07
NaCl	0·35	0·35
Choline chloride (50 %)	0·10	0·10
Mineral premix†	0·20	0·20
Vitamin premix‡	0·02	0·02
Ethoxyquin (66 %)	0·02	0·02
Nutrient levels§		
Metabolizable energy (MJ/kg)	12·34	12·55
Crude protein (%)	21·00	19·00
Ca (%)	1·00	0·90
Available P (%)	0·45	0·35
Lysine (%)	1·10	1·00
Methionine (%)	0·50	0·38
Methionine + cystine (%)	0·95	0·75

* Conjugated linoleic acid (CLA) diet: maize oil was replaced by commercial CLA and balanced to total 1·25 %.

† The mineral premix supplied the following per kg of complete feed: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; Se, 0·15 mg; I, 0·35 mg.

‡ The vitamin premix supplied the following per kg of complete feed: vitamin A, 25 mg; vitamin D_3, 62·5 μg; vitamin K_3, 2·65 mg; vitamin B_1, 2 mg; vitamin B_2, 6 mg; vitamin B_{12}, 0·025 mg; vitamin E, 30 mg; biotin, 0·0325 mg; folic acid, 1·25 mg; pantothenic acid, 12 mg; niacin, 50 mg.

§ Calculated composition.

Sample collection and analysis

Lysozyme assay

At the age of 21 and 42 d, one bird from each replication was selected to supply blood from the wing vein. Serum was collected to determine lysozyme activity according to the method of Kreukniet *et al.* (1994) with some modifications. A series of concentrations of crystalline lysozyme (L6876; Sigma, Shanghai) dissolved in phosphate buffer was used to make the standard curve. The standard dilution series of crystalline lysozyme and serum samples were measured for their lysozyme activity in the lysis of *Micrococcus lysodeikticus* (purchased from the Institute of Microbiology, Chinese Academy of Science, Beijing). For the determination of lysozyme activity in the spleen, one bird from each replication was killed by cervix dislocation and the spleen was removed. After the fat and membrane were trimmed, spleen tissue (0·5 g) was homogenized in 5 ml ice-cold saline water (0·85 %) and the homogenate was diluted to make a concentration of 1 mg/ml. The diluted homogenate was determined for lysozyme activity as that of serum.

Proliferation assay of peripheral blood mononuclear cell

In vitro PBMC proliferation response was determined using a previously described method (Mosmann, 1983; Lin, 1999) at the age of 21 and 42 d. The heparinized (20 U/ml) blood sample obtained by wing-vein puncture was added to the same volume of sterile Hanks balanced salt solution (HBSS) without Ca^{2+} and Mg^{2+}. The diluted blood mixture was layered over half its volume of sterile lymphocyte separation medium (density = 1·077–1·080, Academy of Military Medical Science, Beijing), and separated by density-gradient centrifugation at 400 ***g*** for 30 min at 4°C to recover PBMC. PBMC were collected at the interface and washed with HBSS three times, then were suspended in 2 ml sterile RPMI 1640 media with $NaHCO_3$ (24 mM), L-glutamine (2 mM), sodium pyruvate (1 mM), HEPES (10 mM), penicillin (100 U/ml) and streptomycin (0·1 mg/ml). The live cells were detected by trpan blue dye exclusion using a microscope. Cell suspensions were diluted to a final concentration of 1×10^7 cells/ml in RPMI 1640 medium. One hundred microlitres of cell suspension, and 100 μl RPMI 1640 in the absence or presence of 90 μg/ml concanavalin A (ConA; C2613, Sigma, Shanghai) or 50 μg/ml lipopolysaccharide (L3129, Sigma, Shanghai) were added into a 96-well plate (Costar 3599). The cultures were set up in triplicate. After 56 h incubation in a 5 % CO_2 incubator at 41°C, 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium salts (MTT, M2128, Sigma) were added to the cell culture to make a final concentration 5 μg/ml. The cells were incubated for a further 4 h, then 100 μl acidified isopropyl alcohol was added to the culture and vibrated for at least 10 min to dissolve the coloured material fully. The absorbance of each sample was read via an automated ELISA reader (Bio-Rad, Model 550) at 570 nm.

Prostaglandin E_2 assay

Serum and culture medium of PBMC from birds at the age of 21 and 42 d were determined for PGE_2 synthesis. PBMC was prepared as described above (see section on proliferation of PBMC). Lymphocytes (1×10^7 per well) were cultured in 24-well culture plates (Costar 3524). Cells were coincubated with 45 μg/ml ConA for 48 h at 40°C in a 5 % CO_2 atmosphere. At the end of incubation,

cells and medium were collected and centrifuged at 2000 ***g*** for 10 min at 4°C. The supernatant fluid was stored at about 30°C until assayed. PGE_2 of the culture medium of PBMC and serum was determined by RIA. An ^{125}I-PGE_2 kit with manual was purchased from the Institute of Atomic Energy Application, Chinese Academy of Agricultural Sciences, Beijing.

Antibody production

Six chicks at the age of 14 and 28 d fed on each diet were injected intraperitoneally with 1 ml of a 7 % SRBC. Serum antibody titres against SRBC were measured on the 6th, 10th and 14th day after the first injection and on the 5th, 9th and 13th day after the second injection by active haemagglutination test (Isakov *et al.* 1982). Titres were expressed as the $\log_2$ values of the highest dilution giving a positive reaction.

For bovine serum albumin (BSA), chickens were immunized with 2 ml of 0·5 % BSA in the thigh muscle on the 14th day and the 28th day of age. Serum total antibody titres to BSA were determined by ELISA from six birds for each treatment on the 6th, 10th and 14th day after primary sensitization, and on the 5th, 9th and 13th day after secondary immunization. Briefly, 96-well plates were coated with 4 μg/ml BSA. After subsequent washing with PBS and 0·05 % Tween, the plates were incubated with 1:80 serum (preliminary experiments had shown that in the assay a serum dilution of 1/80 resulted in absorbance in the linear part of the dilution–absorbance curve). Binding of BSA-specific IgG was determined using 1:20 000 peroxidase-labelled polyclonal antibody against chicken IgG (Sigma). After washing, tetramethylbenzidine and 0·05 % hydrogen peroxide were added and incubated for 10 min at room temperature. The reaction was stopped with 1·25 M-sulphuric acid. The colour was measured at 405 nm using an ELISA plate reader (Bio-Rad, Model 550) against the negative control (PBS replaced the diluted serum).

Statistical analysis

Data were reported as means and standard deviations and analysed by one-way ANOVA of SPSS 10.0. The significance of differences among different groups was evaluated by a least significant difference *post hoc* multiple comparisons test. The correlation between PGE_2 and the proliferation of PBMC was analysed by the Pearson procedure of SPSS 10·0.

Results

Growth performance

Table 2 shows the effects of dietary CLA on growth performance in broiler chicks.The differences in feed intake, body weight gain and feed conversion rate among treatments were not statistically significant across different growth periods ($P>0·05$).

Lysozyme activity

Table 3 shows the results of dietary CLA on lysozyme activity in chicks. At the age of 21 d, chicks fed the highest level of CLA diet had significantly higher lysozyme activity compared with chicks fed control diet ($P<0·05$); the spleen lysozyme activities of chicks in the highest level of CLA treatment group were significantly higher than those of the control group and other CLA groups ($P<0·05$). However, at the age of 42 d, no differences in lysozyme activities were observed among treatments in either serum or spleen.

Peripheral blood lymphocyte proliferation

Table 4 shows the results of the effects of dietary CLA on proliferation of peripheral blood lymphocyte in young chicks. At the age of 21 d, when mitogen (lipopolysaccharide or ConA) was added into the culture media, PBMC proliferations in chicks fed 10·0 g CLA/kg diet were significant enhanced than in chicks fed control diet or other CLA diets ($P<0·05$). At the age of 42 d, PBMC proliferations in response to ConA were enhanced in chicks fed 5·0 and 10·0 g CLA/kg diet compared to chicks fed control diet ($P<0·05$). Proliferation of the unstimulated cells did not vary among treatments at both 21 and 42 d of age.

Prostaglandin E_2 synthesis

Table 5 shows the results of PGE_2 concentration in serum and culture medium of PBMC. Systemic and peripheral blood lymphocytic PGE_2 synthesis in birds fed 10·0 g CLA/kg diet was

Table 2. Growth performance of chicks (n 6)*

	Diet conjugated linoleic acid							
	0 (control)		2·5 g/kg		5·0 g/kg		10·0 g/kg	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Body weight gain (g)								
0–3 wk	577·9	24·6	591·3	27·5	571·5	29·4	539·1	26·5
3–6 wk	1285·3	93·8	1319·5	98·2	13 223·4	107·6	1345·8	82·7
0–6 wk	1858·5	117·3	1910·8	96·8	1894·9	113·6	1890·2	75·3
Feed intake (g)								
0–3 wk	785·5	76·2	787·1	42·1	776·9	65·8	759·4	36·2
3–6 wk	2680·3	168·5	2645·0	171·2	2713·7	239·8	2583·0	173·9
0–6 wk	3441·8	226·4	3432·1	181·3	3468·6	248·6	3377·5	163·5
Feed conversion ratio								
0–3 wk	1·328	0·101	1·334	0·097	1·358	0·055	1·408	0·088
3–6 wk	2·054	0·025	2·011	0·048	2·072	0·086	1·928	0·079
0–6 wk	1·868	0·043	1·800	0·066	1·849	0·104	1·795	0·033

* For details of procedures, see p. 747.

Table 3. Lysozyme activity in serum and spleen of chicks (*n* 6)*

	Serum (μg/ml)				Spleen (μg/mg tissue)			
	21 d		42 d		21 d		42 d	
Diet CLA	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (control)	2·80[b]	0·60	2·55	0·46	2·84[b]	0·33	2·82	0·30
2·5 g/kg	3·57[ab]	0·46	3·09	0·46	3·04[b]	0·43	2·84	0·59
5·0 g/kg	3·51[ab]	0·67	2·54	0·26	2·91[b]	0·24	3·58	0·88
10·0 g/kg	3·93[a]	1·00	2·91	0·61	4·22[a]	0·48	3·18	0·75

CLA, conjugated linoleic acid.
[a,b] Mean values within a column with unlike superscripts were significantly different ($P<0·05$).
* For details of procedures, see p. 747.

significantly decreased compared with birds fed control diet at both 21 and 42 d of age ($P<0·05$). The influence of dietary CLA on PGE_2 in circulation and PBMC were similar except that serum PGE_2 in chicks fed 2·5 g CLA/kg diet was significantly lower than in those in the control group at the age of 21 d ($P<0·05$).

Antibody production

Tables 6 and 7 show the effects of dietary CLA on the antibody production of primary and secondary challenge in chicks. Anti-SRBC titres in birds fed 2·5 g CLA/kg diet were higher than birds given control diet 6 d after primary immunization ($P<0·05$). Enhancement of anti-SRBC titres was observed in birds of the 10·0 g CLA/kg diet treatment group compared to those of the control group on 5d and 9 d after secondary immunization ($P<0·05$).

Higher anti-BSA titres were found at 10 d after primary immunization in birds given CLA diets than birds fed control diet ($P<0·05$). Anti-BSA titres in chickens fed 2·5 g CLA/kg diet on days 10 and 4 after first challenge and day 5 after secondary challenge were significantly higher than those in the control group ($P<0·05$).

Discussion

Some researchers have shown that feeding CLA at levels of 5–10 g/kg diet improved feed efficiency, growth and/or meat production in rats (Chin *et al.* 1994), mice (Dugan *et al.* 1997; West *et al.* 1998) and pigs (Ostrowska *et al.* 1999). Our results demonstrated that dietary supplementation of CLA has no effect on growth performance in young chicks. This finding was similar to those of Simon *et al.* (2000), Du & Ahn (2002) and An *et al.* (2003), i.e. dietary CLA had no significant effects on body gain, feed intake and feed efficiency in broiler chicks. Other researchers found that feeding CLA at levels of above 10 g/kg diet decreased the growth rate of mice (Belury & Kempa-steczko, 1997), rats (Szymczyk *et al.* 2000), broilers (Szymczyk *et al.* 2001; Badinga *et al.* 2003) and striped bass (Twibell *et al.* 2000). The discrepancy of effects of CLA on animal growth performance could be ascribed to the differences in animal species, dietary CLA concentration, type of isomers of CLA, feeding periods and nutritional status of the animals. West *et al.* (1998) pointed out that decreased growth performance of mice accompanied by high dose (above 10 g/kg) of dietary CLA was due to the acceleration of fatty acid oxidation and enhancement of metabolic rate.

Lysozyme present in external secretions, polymorphonuclear leukocytes and macrophages is highly active against Gram-positive bacteria. After the internalization of antigens, destruction is likely accomplished by the lysosomal reservoir of hydrolytic enzymes and by the respiratory burst (i.e. O_2^- and H_2O_2; Melnick *et al.* 1985). Hydrolytic enzymes, i.e. lysozyme and acid phosphatase found in the serum, can destroy the glucosidic bond in the cell wall of *Escherichia coli* and *Staphylococcus* as a result of the phagocytic activity (Wang *et al.* 1995). The phagocyte is an important kind of accessory cell in immune response and antigen presentation. Higher levels of lysozyme activity suggested that CLA could stimulate the activation or antigen presentation of the phagocyte, thus enhancing the antibacterial defence of the body. The mechanism might be the decreased

Table 4. Proliferation of peripheral mononuclear cells in chicks (*n* 6)*†

	21 d						42 d					
	Unstimulated		ConA		LPS		Unstimulated		ConA		LPS	
Diet	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 CLA (control)	0·217	0·011	0·418[b]	0·007	0·426[b]	0·035	0·250	0·018	0·500[b]	0·011	0·476	0·070
0·25 % CLA	0·228	0·013	0·484[ab]	0·067	0·481[b]	0·043	0·238	0·023	0·501[b]	0·021	0·536	0·011
0·5 % CLA	0·232	0·050	0·508[ab]	0·110	0·490[b]	0·044	0·249	0·031	0·594[a]	0·070	0·539	0·020
1 % CLA	0·258	0·012	0·580[a]	0·023	0·594[a]	0·021	0·263	0·012	0·627[a]	0·022	0·564	0·079

CLA, conjugated linoleic acid; ConA, concanavalin A; LPS, lipopolysaccharide.
[a,b] Mean values within a column with unlike superscripts were significantly different ($P<0·05$).
* Data are expressed as optical density values at a wavelength of 570 nm.
† For details of procedures, see p. 747.

Table 5. Prostaglandin E_2 in serum and culture medium of peripheral blood mononuclear cells (PBMC) in chicks (n 6)*

	Serum (pg/ml)				PBMC (pg/ml)			
	21 d		42 d		21 d		42 d	
Diet CLA	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (control)	265·15[a]	54·18	481·36[a]	142·65	23·16[a]	5·92	30·09[a]	6·06
2·5 g/kg	118·52[b]	20·26	434·99[ab]	153·07	17·88[ab]	3·90	24·95[ab]	1·53
5·0 g/kg	129·23[ab]	29·03	389·14[ab]	71·42	18·02[ab]	5·20	24·11[ab]	8·00
10·0 g/kg	83·69[b]	23·12	264·23[b]	61·47	12·36[b]	3·32	19·03[b]	7·21

CLA, conjugated linoleic acid.
[a,b] Mean values within a column with unlike superscripts were significantly different ($P<0·05$).
* For details of procedures, see p. 747.

production of metabolites of n-6 fatty acids, such as PGE_2, and changed membrane compositions of immune cells.

Our results showed that CLA enhanced ConA-induced lymphocyte proliferation in broiler chicks. This is in agreement with the reports of Michal *et al.* (1992) and Cook *et al.* (1993) who noted that CLA-supplemented chicks challenged with lipopolysaccharide had higher T cell blastogenic response to phytohaemagglutinin.

Reported effects of CLA on immune cell functions varied from stimulation, inhibition to no influence (Wong *et al.* 1997; Hayek *et al.* 1999; Bassaganya-Riera *et al.* 2001; Kelly *et al.* 2002). These discrepancies may be due to culture conditions (serum type and concentration, mitogen and culture period), cell type (peritoneal *v.* splenocyte *v.* blood cells), composition of the diets, animal species and age of the animal. The differences in isomer composition of the CLA mixture may not be important, because it was reported that c9, t11-CLA and t10, c12-CLA had similar effects on immune function in mice (Kelly *et al.* 2002).

Our results indicated that CLA would enhance ConA-induced T cell stimulation. The immune enhancement may be beneficial to fast-growing broiler chicks that are highly responsive to stress caused by vaccination, population density, ammonia concentration and environmental temperature. These beneficial effects are yet to be determined.

PGE_2, one of the most important eicosanoids, is an endogenous inhibitor of immune response. PGE_2 inhibits proliferation of T cells and production of IL-2 and γ-interferon from T cells (Hasler *et al.* 1983; Betz & Fox, 1991). By using the Pearson procedure, negative correlations were found between PGE_2 synthesis of PBMC and proliferation of T cells at the age of 21 d (r^2 − 0·975, P=0·013) and 42 d (r^2 − 0·706, P=0·160). The reduction of PGE_2 was consistent with the observed enhanced proliferation of T cells in our study.

Dietary CLA has also been shown to decrease prostanoid levels in serum (Sugano *et al.* 1997, 1998), bone (Li & Watkins, 1998), spleen (Sugano *et al.* 1997) and cultured keratinocytes (Liu & Belury, 1997). A number of reports have provided either direct or indirect evidence suggesting that CLA may interfere with prostaglandin production through a decrease in the supply of arachidonic acid precursor (Cook *et al.* 1993; Belury & Kempasteczko, 1997; Li & Watkins, 1998; Turek *et al.* 1998; Banni *et al.* 1999). Arachidonic acid is the precursor for PGE_2; thus, increased CLA intake may decrease PGE_2 production. But, CLA replacement of phospholipid archidonic acid might not be adequate to cause a significant change in PGE_2 production. Other studies reported that CLA had no significant effect on PGE_2 production (Turek *et al.* 1998; Hayek *et al.* 1999). Furthermore, dietary CLA reduced accumulation of the lipoxgenase products leukotriene-B4 and leukotriene-C4 in spleen and lung (Sugano *et al.* 1998) but not [^{14}C]hydroxyeicosatetraenoic acid in cultured human platelets (Truitt *et al.* 1999). Further studies are needed to determine whether the effect of CLA on PGE_2 production is species-, organ- or tissue-specific.

The reduction of arachidonate-derived eicosanoids by dietary CLA has been explained by at least three hypothesized mechanisms (Belury, 2002): first, CLA displaces arachidonate in phospholipids; secondly, CLA reduces the expression of cyclooxygenase (the rate-limiting enzyme which catalyses the conversion of prostanoids from arachidonic acid); and thirdly, CLA or its metabolites act as

Table 6. Anti-sheep blood red cell antibody production in chicks (n 6)*†

	Primary response						Secondary response					
	6 d		10 d		14 d		5 d		9 d		13 d	
Diet CLA	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (control)	1·67[b]	0·51	3·08	1·02	2·92	0·58	2·17[b]	0·41	2·75[b]	0·59	2·67	0·82
2·5 g/kg	2·67[a]	0·87	2·92	0·86	2·67	0·26	2·33[ab]	0·41	2·25[b]	0·38	2·67	0·52
5·0 g/kg	1·67[b]	0·26	3·17	0·93	2·67	0·41	2·42[ab]	0·49	2·83[b]	0·68	2·17	0·41
10·0 g/kg	2·25[ab]	0·69	4·08	1·33	2·67	0·26	3·08[a]	1·02	3·63[a]	0·74	2·83	0·75

CLA, conjugated linoleic acid.
[a,b] Mean values within a column with unlike superscripts were significantly different ($P<0·05$).
* Data are expressed as the $\log_2$ values of the highest dilution giving a positive reaction.
† For details of procedures, see p. 748.

Table 7. Anti-bovine serum albumin antibody production in chicks (*n* 6)*†

	Primary response						Secondary response					
	6 d		10 d		14 d		5 d		9 d		13 d	
Diet CLA	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0 (control)	0·540	0·018	0·515^{c}	0·019	0·520^{b}	0·018	0·506^{b}	0·044	0·535	0·029	0·475	0·042
2·5 g/kg	0·550	0·018	0·566^{a}	0·020	0·551^{a}	0·020	0·551^{a}	0·025	0·553	0·036	0·474	0·033
5·0 g/kg	0·526	0·025	0·540^{b}	0·021	0·522^{b}	0·018	0·505^{b}	0·020	0·542	0·032	0·476	0·026
10·0 g/kg	0·526	0·022	0·548^{ab}	0·016	0·503^{b}	0·029	0·535^{ab}	0·016	0·542	0·030	0·504	0·028

CLA, conjugated linoleic acid.
^{a,b} Mean values within a column with unlike superscripts were significantly different ($P<0·05$).
* Data are expressed as optical density values at a wavelength of 405 nm.
† For details of procedures, see p. 748.

substrates or antagonists for cyclooxygenase. Our study confirmed that CLA could decrease PGE_2 synthesis in chickens. The exact mechanism remains to be clarified in the future. Another possible explanation by which CLA decreases prostanoid may be down-regulation of enzyme activity by phospholipase A_2 the enzyme responsible for cleaving precursor fatty acids from the phospholipid before cyclooxygenation or lipoxygenation.

The kinetics of antibody production in our study was not observed. It is possible that the modern chicks are more resistant to environment stress. However, the effects of dietary CLA on thymus-dependent antigen SRBC and BSA were elevated in the sample periods. The effects of CLA on humoral immunity were not in agreement among the earlier experiments. Antibody response in chicks to SRBC or rats to BSA was not affected by feeding CLA (Cook *et al.* 1993). Takahashi *et al.* (2003) found that the first antibody titres against SRBC were higher in CLA-fed chicks, but the second titres were not. Our study examined the kinetics of antibody response after primary and secondary immunization with three CLA dose supplementations and enhancement of anti-SRBC titres after primary and secondary immunization were observed. The reasons for the differences among experimental results are probably due to the route of antigen administration, dietary CLA concentration or fatty acid composition in the diet used (Takahashi *et al.* 2003).

The higher antibody titres against BSA appeared on day 10 and day 9 after primary and secondary challenge, respectively. No dose-dependent response of dietary CLA on antibody production was found. In our study, it seemed that 2·5 g CLA/kg diet was optimal to enhance anti-BSA titres. So far, the effects of dietary CLA on antibody titres to BSA have been less clearly observed in birds.

The effects of dietary CLA on antibody production in the present study are comparable with the effects of *n*-3 fatty acids (Xia *et al.* 2003). The mode of action of *n*-3 fatty acids has been estimated to be due to changes in PGE_2 or eicosanoid production (Xia *et al.* 2003; Guo *et al.* 2004). However, comparing the effects on antibody production and PGE_2 synthesis, it seems that the enhancement of antibody production of dietary CLA may not be due to changes in eicosanoid production since the reduction of PGE_2 did not parallel with the elevation of antibody production. It remains elusive how CLA affects antibody production. Anyway, the present results in our study suggested that dietary CLA has the potential to elevate antibody production in chicks.

In conclusion, dietary CLA (< 10 g/kg diet) enhanced lysozyme activity, stimulated T lymphocyte proliferation, decreased PGE_2 synthesis and potentially elevated antibody production in male broiler chickens. The underlying mechanism is not clear. However, the young broiler chick offers a useful animal model to study the effect of CLA. Furthermore, the results of this series of studies will help not only the health of the animal but also the production of the broiler industry.

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