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Effect of a mixture of conjugated linoleic acid isomers on growth performance and antibody production in broiler chicks

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The effect of dietary conjugated linoleic acid (CLA) isomers mixture on antibody titres against sheep blood erythrocytes (SRBC) and immunoglobulin (Ig) G concentration in plasma was studied in broiler chickens. In experiment 1, male and female broiler chicks (11 d of age, Cobb strain) were fed a diet supplemented with 10 g CLA or 10 g safflower-seed oil/kg diet for 2 weeks. An SRBC suspension (5:100, v/v) in a phosphate buffer was intravenously injected at 18 d of age and a blood sample was taken from the wing vein at 25 d of age. Chicks fed the CLA-supplemented diet had enhanced first antibody titres in plasma to SRBC as compared with those fed the safflower-seed oil-supplemented diet, irrespective of sex differences. In experiment 2, male broiler chicks (8 d of age, Ross strain) were fed a basal diet or a diet containing 10 g CLA/kg diet for 3 weeks. CLA in the CLA diet partially replaced the soyabean oil in the basal diet. The SRBC suspension was intravenously injected at 15 and 25 d of age and a blood sample was obtained at 21 and 29 d of age. The first antibody titres against SRBC were higher in chicks fed the CLA diet than those in chicks fed the basal diet, but the second titres were not. Plasma IgG concentrations in chicks fed the CLA diet were higher than those in chicks fed the basal diet on both sampling days. The results showed that dietary CLA enhanced antibody production in broiler chickens.

Dietary conjugated linoleic acid: Antibody production: Growth: Broiler chickens

Conjugated linoleic acids (CLA) are an isomeric mixture of 18 : 2 fatty acids that have conjugated double bonds (Fritsche & Steinhart, 1998). There is great interest in these fatty acid isomers because CLA have several unique proprieties that control physiological and metabolic responses, for example, anti-hypercholesterolaemic and anti-atherogenic effects in rabbits and hamsters, inhibition of cancer cell proliferation *in vitro*, suppression of mammary tumours in mice, and increased immune responses. These effects of CLA in animals have been reviewed by Fritsche & Steinhart (1998) and Pariza *et al.* (2000, 2001). CLA also have great impact on growth performance and lipid metabolism in rats, mice and pigs (Chin *et al.* 1994; Dugan *et al.* 1997; Park *et al.* 1997; West *et al.* 1998; DeLany *et al.* 1999; Ostrowska *et al.* 1999; Stangl, 2000), but the effect appears to be less effective in chickens (Szymczyk *et al.* 2001; Du & Ahn, 2002). It has also been suggested that CLA protects the catabolic responses against endotoxin in chicks and mammals (Cook *et al.* 1993; Miller *et al.* 1994; Takahashi *et al.* 2002). Although some studies in mammals suggest that CLA affects certain aspects of the immune response such as lymphocyte proliferation (Chew *et al.* 1997; Wong *et al.* 1997) and interleukin-2 production in mice (Hayek *et al.* 1999), the effects on antibody production are not clear. Dietary CLA enhanced immunoglobulin (Ig) production in immunocompetent organs and plasma IgG concentration in rats (Sugano *et al.* 1998). Yamasaki *et al.* (2000) observed that CLA enhanced Ig production in spleen but did not affect serum IgG levels in rats. Cook *et al.* (1993) showed that antibody production to sheep blood erythrocytes (SRBC) was not affected by feeding CLA in chicks. Therefore, the effect of dietary CLA on antibody production in broiler chickens was evaluated in the present experiment.

Materials and methods

Animals, diets and immune stimulation

In experiment 1, twenty each of male and female chicks (11 d of age, Cobb strain) were used and they were randomly

Abbreviations: CLA, conjugated linoleic acid; Ig, immunoglobulin; SRBC, sheep blood erythrocytes.

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assigned to two groups of twenty chicks (ten male and ten female), five replicates of two chicks in a cage. The birds were fed a commercial diet supplemented with 10 g safflower-seed oil or 10 gtCLA/kg diet for 14 d *ad libitum*. The commercial broiler chick diet (21 g crude protein, 2 g crude fat and 12·8 MJ metabolizable energy/kg diet) mainly consisted of maize, soyabean meal, and maize gluten meal and satisfied the nutrient demand for broiler chicks (Japanese Feeding Standard for Poultry, 1997). The safflower-seed oil supplemented diet was considered as a basal diet in experiment 1. At 18 d of age, 0·5 ml SRBC suspension (5:100, v/v) in a 0·01 M-phosphate buffer (pH 7·2) was intravenously injected. A blood sample was obtained via the wing vein at 25 d of age. CLA used in the present experiment consisted of 46·6 g *cis*-9, *trans*-11/*trans*-9, *cis*-11, 48·2 g *trans*-10, *cis*-12, 3·2 g *cis*-9, *cis*-12/*cis*-10, *cis*-12 and 2·0 g *trans*-9, *trans*-11/*trans*-10, *trans*-12 of linoleic acid/100 g CLA mixture (data from Rinoru Oil Mills Co. Ltd., Tokyo, Japan). The fatty acid composition of experimental diets is shown in Table 1. Body weight and feed intakes from 11 to 24 d of age were recorded to determine growth performance.

In experiment 2, forty male chicks (8 d of age, Ross strain) were used. They were randomly assigned to two groups of twenty chicks, with ten replicates of two chicks in a cage, and given either 0 or 10 g CLA/kg diet for 21 d *ad libitum*. The basal diet consisted of 452·6 g maize, 330 g soyabean meal, 100 g glucose, 41 g soyabean oil, 31 g soya protein, 12 g $CaCO_3$, 18 g $CaHPO_4.2H_2O$, 4·3 g NaCl, 3 gtDL-methionine, 0·1 g L-threonine, 4 g vitamin mixture and 4 g trace mineral mixture/kg diet. CLA in the CLA diet replaced part of the soyabean oil contained in the basal diet, which satisfied the nutrient demand for broiler chicks (Japanese Feeding Standard for Poultry, 1997). At 15 and 25 d of age, the SRBC suspension was injected in the same way as in experiment 1, and a blood sample was taken via the wing vein at 21 and 29 d of age. Body weight and feed intake from 8 to 29 d of age were recorded to determine growth performance. The Animal Care and Use Committee of the Graduate School of Agriculture of Tohoku University approved all procedures.

Analysis

Plasma was collected by centrifugation at 500 ***g*** for 10 min. For determination of antibody titres to SRBC, plasma was heated at 56°C for 30 min. The plasma samples were stored at −20°C until analysis. Antibody titres to SRBC were determined by the method of Isakov *et al.* (1982). Briefly two-fold serial dilutions of the tested plasma (25 μl each) were made with the phosphate buffer using ninety-six-well plates. The wells of plates for determination of total haemagglutinin titres were supplemented with 25 μl phosphate buffer. The plates were incubated at 37°C for 2 h and placed at 4°C overnight. The haemagglutinin titres were expressed as $\log_2$ of highest dilution showing visible agglutination. Plasma IgG concentration was determined by single radial immune diffusion methods; briefly, agarose gel (3:100, w/v) containing rabbit anti-chicken IgG serum was prepared in a plastic container, and 2·5 mm-diameter wells were then punched out of the gel. Plasma samples were placed into each well (5 μl/well). After 48–72 h at 37°C in a humid chamber, the diameters of the precipitin ring were measured with 0·1 mm accuracy using a calibrated digital viewer. The calibration curve was essentially linear between 0 to 2 mg purified chicken IgG/ml.

In experiment 1, a 2 (dietary treatments) by 2 (sexes) ANOVA was applied using SAS (SAS Institute, Cary, NC) with mean separation by Duncan's multiple range test. In experiment 2, the data of body-weight gain, feed intake and weight gain:feed intake ratio were analysed by the Student's test using SAS. For determination of the data of plasma IgG concentration and SRBC titres, a 2 (dietary treatments) by 2 (sampling times) ANOVA was applied using SAS (SAS Institute, Cary, NC) with mean separation by Duncan's multiple range test. In both experiments, the analyses for feed intake, and weight gain:feed intake ratio were based on cage replication. For analyses of data of body-weight gain and plasma IgG concentration

Table 1. Body-weight gain, feed intake and weight gain:feed intake ratio in male and female chicks fed the experimental diet supplemented with safflower-seed oil (basal) or conjugated linoleic acid (CLA) from 11 to 24 d of age (experiment 1) and those in chicks fed diets containing 0 (basal) or 10 g CLA/kg diet from 7 to 29 d of age (experiment 2)

(Mean values with their standard errors for ten chickens)

		Experiment 1						Experiment 2					
		Feed intake (g/14 d)		Body-weight gain (g/14 d)		Gain:feed intake		Feed intake (g/21 d)		Body-weight gain (g/21 d)		Gain:feed intake	
Diet	Sex	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Basal	Male	1004	25	586	18	0·566	0·010	1420	20	1008	22	0·697	0·006
CLA	Male	975	16	565	10	0·577	0·011	1391	20	997	23	0·717	0·012
Basal	Female	976	23	562	15	0·576	0·002	–	–	–	–	–	–
CLA	Female	964	21	556	18	0·563	0·008	–	–	–	–	–	–
Probability													
Diet		NS		NS		NS		NS		NS		NS	
Sex		NS		NS		NS		–		–		–	
Diet × Sex		NS		NS		NS		–		–		–	

NS, $P > 0·1$.

and SRBC titres, individual chicks were considered as experimental units.

Results and discussion

The present experiment showed that chicks fed the CLA diets had higher first haemagglutinin titres against SRBC and IgG concentration in plasma than chicks fed the basal diets containing soyabean oil or safflower-seed oil, irrespective of sex differences or strain (Fig. 1 (A), (B) and (C)). The effects of CLA on humoral immunity were not in agreement among the earlier experiments. Dietary CLA at level of 10 g/kg diet enhanced Ig production in immunocompetent organs and plasma IgG concentration in rats (Sugano *et al.* 1998). Yamasaki *et al.* (2000) observed that CLA enhanced Ig production in spleen at levels from 0·5 to 5 g/kg in diets in a dose-dependent manner, but did not affect serum IgG level in rats. Cook *et al.* (1993) found that dietary CLA did not affect antibody production to SRBC when chicks fed on a 5 g lard/kg or CLA-supplemented diet for 3 weeks were administrated SRBC into the peritoneal cavity. It has been demonstrated that the route of SRBC injection affects the rate and amount of antibody against SRBC and that the injection of an antigen into the peritoneal cavity has less potency to produce antibodies compared with an injection into the vein (van der Zijpp *et al.* 1986). Therefore, a reason for the difference in the results between Cook *et al.* (1993) and the present experiment is probably due to route of antigen administration. Another possible explanation is that dietary CLA concentration or fatty acid composition in the diet used may affect the effect of CLA on antibody production, since the immune modulation effect of CLA was significantly changed by dietary fat sources (Turek *et al.* 1998). Koga *et al.* (1997) showed that rats fed a diet containing elaidic acid (*trans*) produced higher antibody production than rats fed a diet with oleic acid (*cis*). Thus, it appears that *trans*-fatty acids including CLA used in the present experiment may have the property of enhancing antibody production relative to *cis*-fatty acids.

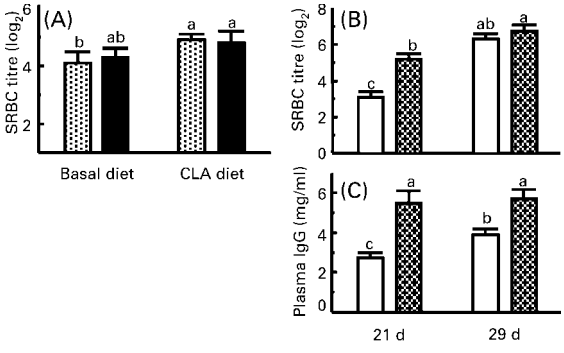

Fig. 1. (A), Antibody titres to sheep erythrocytes (SRBC) in male (■) and female (▤) broiler chicks fed diets supplemented with 10 g conjugated linoleic acid (CLA) or safflower-seed oil/kg in experiment 1. Diet effect $P<0·05$, sex effect $P>0·1$, their interaction $P>0·1$. (B), Antibody titres to SRBC with basal diet (□) and CLA-supplemented diet (▨) in 21- and 29-d-old chicks (diet effect $P<0·01$, age effect $P<0·05$, their interaction $P<0·1$) and (C) plasma immunoglobulin G (IgG) concentration (diet effect $P<0·01$, age effect $P<0·05$, their interaction $P<0·05$) in male broiler chicks in experiment 2. [a,b,c]Mean values with unlike letters were significantly different ($P<0·05$). Standard errors are represented by vertical bars.

The effect of dietary CLA on antibody production reported in the present and some of the previous experiments are comparable with the effects of *n*-3 fatty acids. Fritsche *et al.* (1991) showed that antibody production against SRBC in chicks fed a fish oil which was relatively rich in eicosapentaenoic acid and docosahexaenoic acid (*n*-3 polyunsaturated fatty acids) was significantly elevated as compared with that in chicks fed a diet rich in maize oil or rapeseed oil which consists of mainly *n*-6 fatty acids such as linoleic acid. The mode of action of *n*-3 fatty acids has been estimated to be due to changes in prostaglandin E_2 or eicosanoid production, which is at least in part induced by changes in fatty acid composition in the plasma membrane (Grimble, 1998). CLA itself hardly becomes incorporated into the phospholipids fraction, suggesting very little effect on eicosanoid production, although Whigham *et al.* (2002) suggested that dietary CLA reduces antigen-induced eicosanoid release in guinea-pigs. In addition, the effects of CLA on prostaglandin production vary with experimental condition (Li & Watkins, 1998; Liu & Belury, 1998; Sugano *et al.* 1998; Turek *et al.* 1998; Hayek *et al.* 1999). Thus the effect of CLA on eicosanoid production is still controversial. It remains to be clarified how CLA affect antibody production.

Feeding CLA at levels of 5 to 10 g/kg diet improved feed efficiency, growth and/or meat production in rats, mice and pigs (Chin *et al.* 1994; Dugan *et al.* 1997; West *et al.* 1998; DeLany *et al.* 1999; Ostrowska *et al.* 1999; Yamasaki *et al.* 1999). In contrast to mammals, Szymczyk *et al.* (2001) observed that feed intake, body-weight gain and feed conversion in chicks fed on diets containing 5 and 10 g CLA/kg diet had no significant effect. The present result of growth performance (Table 1) showed that feeding CLA at a concentration of 10 g/kg diet for 2 (experiment 1) or 3 weeks (experiment 2) did not affect body-weight gain, feed intake or feed efficiency. This is similar to the results of Szymczyk *et al.* (2001). Recently, Du & Ahn (2002) showed that dietary CLA at levels of 20 and 30 g/kg diet for 5 weeks reduced whole fat content without significant reduction in body-weight gain, but feeding 10 g CLA/kg diet for 3 weeks did not affect growth, abdominal and whole fat content in broiler chicks. This suggests that dietary CLA is less effective in changing body composition in chickens. Pariza *et al.* (2001) noted that the *cis*-9/*trans*-11 CLA isomer which enhances growth and probably feed efficiency in young rodents, and the *trans*-10/*cis*-12 CLA isomer which changes body composition use separate biochemical mechanisms. Therefore, feeding periods, dietary concentration, and type of isomer of CLA may be factors affecting growth performance and body fat content.

In conclusion, dietary CLA (10 g/kg diet) enhances antibody production in broiler chickens, regardless of sex, strain of chicks and dietary fat sources.

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