Asian-Aust. J. Anim. Sci.
Vol. 20, No. 6 : 954 - 961
June 2007

www.ajas.info

Effect of a Mixture of Conjugated Linoleic Acid (CLA) Isomers on T Cell Subpopulation and Responsiveness to Mitogen in Splenocytes of Male Broiler Chicks

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ABSTRACT : The experiments were conducted to determine effects of a mixture of conjugated linoleic acid (CLA) isomers on T cell subpopulations and responsiveness to mitogen of splenocytes in male broiler chicks. In experiment 1, birds (8-d old) were fed basal, CLA-(CLA) and safflower oil-supplemented (SA) diets which were formulated by supplementary 10 g CLA or safflower oil/kg to the basal diet for 14 d. Broiler starter diet, which mainly consisted of corn and soybean meal, was served as the basal diet. Proliferative response and interleukin (IL)-2-like activity stimulated by concanavalin (Con) A at a concentration of 10 μg/ml of splenocytes in chicks fed the CLA diet were greater than in chicks fed the SA diet, but not at 20 μg Con A/ml. Percentage of CD3-positive T cells in splenocytes did not differ between chicks fed the SA diet and CLA. Ratio of CD4-positive T cells to CD8- positive T cells was significantly affected by dietary fat source. In experiment 2, broiler chicks (1-d old) were fed the same diets as in experiment 1 for 14 d. Results of splenocyte proliferation to Con A were similar to those in experiment 1, but phytohemaggulutinin (PHA)- or pokeweed mitogen (PWM)- induced splenocyte proliferation did not differ between the CLA and SA fed groups. Supplementation with SA or CLA to the basal diet tended to have a depressive effect on the proliferation, with the greater effect being that of SA. In experiment 3, effect of an addition of CLA to splenocyte culture medium on splenocyte proliferation was determined. An addition of CLA to the culture medium resulted in reduction of the splenocyte proliferation to Con A, but an addition of linoleic acid. When PWM and PHA were used as mitogen, the inhibitory effect of CLA and linoleic acid on the proliferation did not differ. The results suggested that the effect of dietary CLA on splenocyte proliferation was similar to that of SA, although the effect of dietary CLA on sub-populations was slightly different from that of dietary SA. Further studies are needed to clarify whether use of CLA would be beneficial for maintaining or enhancing T cell immunity in chicks. (**Key Words :** CLA, Broiler Chicks, T Cell Population, Splenocyte Proliferation)

INTRODUCTION

Conjugated linoleic acids (CLA) are isomeric mixtures of 18:2 fatty acids that have conjugated double bonds. CLA protected catabolic responses against endotoxin in mice, rats and pigs (Cook et al., 1993; Miller et al., 1994; Bassaganya-Riera et al., 2001; Changhua et al., 2005). Dietary CLA enhanced immunoglobulin (Ig) production in immunocompetent organs and plasma IgG concentration in rats (Sugano et al., 1998), although Yamasaki et al. (2000) observed that CLA did not affect serum IgG level in rat. Some studies in rodents suggest that CLA affects certain aspects of the immune response such as lymphocyte proliferation (Chew et al., 1997; Wong et al., 1997) and interleukin (IL)-2 production in mice (Hayek et al., 1999). The CD8-positive T cells were higher in a group receiving CLA than in a control group in rats, and the ratio of CD4-positive T cells to CD8-positive T cells was reduced in a group receiving CLA (Yamasaki et al., 2003). In addition, the increase in CD8-positive T cells is consistent with the results of previous studies in a pig model of immunomodulation (Bassaganya-Riera et al., 2002, 2003). The results of studies on rodents and pigs suggest that CLA enhanced T cell proliferation and decreased inflammatory responses while ameliorating immune-mediated catabolism. On the other hands, CLA supplementation resulted in a dose-dependent reduction in the mitogen-induced activation of T lymphocytes (Tricon et al., 2004; Song et al., 2005) and had no effect (Kelley et al., 2000, 2001, 2002; Nugent

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Received September 26, 2006; Accepted January 18, 2007

Table 1. Composition of basal diet

Ingredient	Content (g/kg)
Maize	521.0
Sorghum	100.0
Soybean meal	288.0
Maize gluten meal	40.0
Soybean oil	10.0
Calcium carbonate	10.7
Calcium phosphate, dibasic	17.3
Sodium chloride	2.0
Vitamin mixture*	4.0
Mineral mixture*	4.0
DL-methionine	2.0
L-lysine	1.0
Calculated nutrient content	
Crude protein (g/kg)	210.0
Crude fat (g/kg)	30.0
Metabolisable energy (MJ/kg)	12.8

* See Akiba and Matsumoto (1978).

et al., 2005) in human although CLA decreased inflammatory responses and pro-inflammatory cytokine productions (Alber et al., 2003). Thus immunomodulatory effect of CLA appeared variable among animals. This immunomodulatory effect of CLA has been recently reviewed by O'Shea et al. (2004).

In chicken, there is a little information available on the effects of CLA on immunity although it has been noted that CLA is potential to use in poultry production under the production system without antibiotics (Cook, 1999). It has been only suggested that CLA protects the catabolic responses against endotoxin (Cook et al., 1993; Takahashi et al., 2002a, 2002b) and had an anti-inflammatory effect (Takahashi et al., 2002a, 2002b). Dietary CLA enhanced antibody production in broilers (Takahashi et al., 2003; Zhang et al., 2005). Recently, Zhang et al. (2005) showed that dietary CLA increased lysozyme activity in serum and spleen and the peritoneal blood mononuclear cell proliferation in response to Con A. Jang et al. (2004) indicated that ability of CLA to increase hepatic CAT activity suggest that dietary CLA may affect, at least in part, antioxidant defense system as well as lipid metabolism in the liver of broiler chickens. Thus, CLA may be a candidate nutrient to apply in chicks as an alternative to the use of feed antibiotics, or as a means of improving the response to vaccination and conferring disease resistance if CLA show the immunomodulatory effects observed as mentioned above. However, cell culture conditions especially type of mitogen and serum used affected the results of the lymphocytes proliferation (Calder, 1996). In the present experiment, we determined the effect of a mixture of CLA isomers on splenocytes responsiveness to several mitogen and splenic T cell population in chicks.

MATERIALS AND METHODS

Animals and diets

In experiment 1, unvaccinated male broiler chicks (one day old, Ross strain) were housed in a battery brooder with electronic heater and fed a commercial broiler starter diet (210 g crude protein content, 20 g crude fat and 12.8 MJ of metabolizable energy per kg diet) until 8 days of age. Twenty-four birds were selected from one and half larger population to obtain uniform body weight. Eight chicks in each group were reared in a wire-bottomed cage under a controlled temperature room (25°C). The birds were fed either a basal diet or a basal diet supplemented with 10 g safflower oil (SA) or 10 g CLA per kg diet for 14 days. In experiment 2, thirty unvaccinated male broiler chicks (one day old, Ross strain) were randomly assigned to 3 groups of 10 chicks and kept in a battery brooder with electronic heater until 14 days of age. They were fed either a basal diet or a basal diet supplemented with 10 g safflower oil (SA) or 10 g CLA per kg diet for 14 days. Feed and water were freely accessible over the experimental period in both experiments. Composition of the basal diet is shown in Table 1. Light was provided 24 h a day until the end of experiment. Major fatty acids of the basal diet consisted of 15.7 g palmitic acid, 4.5 g stearic acid, 30.5 g oleic acid, 46.5 g linoleic acid, 2.2 g α linolenic acid, 0.1 g arachidonic acid and 0.5 g eicosapentaenoic acid per 100 g of lipid fraction. Safflower oil consisted of 6.8 g palmitic acid, 2.5 g stearic acid, 12.6 g oleic acid, 77.4 g linoleic acid, 0.1 g α linolenic acid, 0.6 g other fatty acids per 100 g. CLA (obtained from Rinoru Oil Mills Co. Ltd., Tokyo, Japan) 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 per 100 g CLA mixtures (data from Rinoru Oil Mills Co. Ltd., Tokyo, Japan). Six chicks in each dietary group were randomly selected to perform measurements mentioned below in both experiments. For determination of *in vitro* splenocytes proliferation, six male chicks (one day old, Ross strain) were fed the commercial starter diet for 3 weeks to prepare splenocytes. The Animal Care and Use Committee of the Graduated School of Agriculture of Tohoku University approved all procedures.

Preparation of mononuclear (MNC) cell suspension

Mononuclear cells (MNC) were isolated from spleen by density-gradient centrifugation. Collected spleen was meshed and suspended in the RPMI-1640 medium (Invitrogen, Corp., Carlsbad, CA, USA) supplemented with a mixture of penicillin and streptomycin (Invitrogen, Corp., Carlsbad, CA, USA) at 100 unit/ml and 100 μg/ml, respectively. The suspension was gently added on

Histopaque-1077 (Sigma, St. Lois, MO, USA). Centrifugation was performed at 400×g for 50 min at 16°C and washing of splenic MNC were done three times with RPMI-1640.

Measurement of splenocyte proliferation

Splenocyte proliferative responses to mitogens were carried out the modified method reported by Takahashi & Akiba (1996). Briefly, splenocytes were cultured in 96 well microtiter plates at a concentration of 5×10^5 cells in a final volume of 0.1 ml of the RPMI-1640 medium supplemented with penicillin/streptomycin and 10% fetal bovine serum (FBS) in the presence or absence of Con A as a non-specific T cell mitogen, and were incubated at 39°C for 48 h in 5% CO_2 atmosphere. Cell proliferation was determined with a cell counting kit (Dojindo, Osaka, Japan) using sodium 2-(4-indophenenyl)-3-(4-nitrophenyl)-5-(2,4-disulfphenyl)-2H- tetrazolium (WST-1,) and 1-methoxy-5-methyl-phenazinium methylsulfate (1-methoxy PMS). The colour change was detected to subtract from absorbance at 650 nm to that at 450 nm with a microplate spectrophotometer (Bio-Rad model 450, Nippon Bio-Rad Laboratories, Tokyo, Japan). The assessment of cell proliferation using the cell counting kit (WST assay) is based on changes in lactate dehydrogenase activity in mitochondria. Proliferative response of splenocytes was assessed by increased rate of absorbance by addition of mitogens to subtract from absorbance at 650 nm to that at 450 nm. In experiment 1, Con A was used as mitogen to stimulate proliferation and its concentration in medium was prepared at 0, 10 and 20 μg/ml. In experiment 2, Con A, phytohemaggulutinin (PHA) as another non-specific T cell mitogen and pokeweed mitogen (PWM) as a non-specific T and B cell mitogen were used as mitogens to stimulate splenocytes proliferation in the culture medium with concentration of 10, 25 and 1 μg/ml, respectively. Medium without mitogen was served as an un-stimulated control.

Measurement of IL-2-like activity

IL-2 like activity assay was carried out by the method based on T cell proliferation as previously described by Myers et al. (1992) with slight modification. Splenic MNC prepared from chickens fed the each diets were cultured at a concentration of 2.0×10^6 cells in 1 ml of the RPMI-1640 medium containing 10% FBS with the absence or presence of ConA (20 μg/ml). After 24 h incubation at 39°C in 5% CO_2 atmosphere, the resulting supernatants (condition media) were collected and stored at -80°C until assayed for IL-2 like activity. The responder cells were prepared from splenic MNC of 5-week-old male chickens (Ross strain) which were fed a commercial broiler diet *ad libitum* and kept in a cage. MNC were cultured under the same condition of preparation of the condition media for 24 h incubation at 39°C in a humidified 5% CO_2 atmosphere, and the supernatants were discarded. The remained cells were re-suspended in the RPMI-1640 medium with 5% FBS and 0.05 M alpha methyl mannoside (αMM, Sigma, St. Lois, MO, USA). Following 30 min incubation at 39°C under 5% CO_2, the cells were then centrifuged for 10 min at 400×g 10°C, removed supernatant, and washed with RPMI-1640 twice. For measurement of IL-2 like activity of condition media, the responder cells were cultured in 96 well microtiter plates at a concentration of 5×10^5 cells in a final volume of 0.1 ml of RPMI-1640 in the presence or absence of Con A and the previously prepared-condition media at 39°C for 48 hours in 5% CO_2 atmosphere. Cell proliferation was measured using the Cell Counting Kit-8 (Dojindo, Osaka, Japan) according the manufacturers guidelines.

Flow cytometry

The expression of cell surface markers on the splenic MNC was investigated by immunofluorescenece. The cell surface expression of CD3 (as a T cell marker), CD4 and CD8 (as T cell subpopulation markers) of splenocytes was analyzed by flow cytometry. After the isolation of lymphocytes from the spleen, cells were washed with RPMI-1640 medium three times and treated with a phosphate buffer (PBS, pH 7.4) containing 10% bovine serum albumin for 1 h at 37°C. The prepared MNC were divided into two groups (5.0×10^5 cells each per 100 μl) One were incubated for 30 min at 4°C with fluorescein isothiocyanate-conjugated mouse anti-chicken monoclonal antibody to CD3 (0.025 μg in 10 μl, Southern Biotechnology Associates, Inc., Birmingham, AL, USA), and the other was double stained with fluorescein isothiocyanate-conjugated mouse anti-chicken monoclonal antibody to CD4 (0.25 μg in 5 μl, Southern Biotechnology Associates, Inc., Birmingham, AL, USA) and phycoerythrin (PE)-conjugated mouse anti-chicken monoclonal antibody to CD8 (0.25 μg in 5 μl, Southern Biotechnology Associates, Inc., Birmingham, AL, USA), respectively. All of the reactions were performed on ice for 1 h, and then cells were washed three times with the PBS buffer after the antibody treatment. Samples were subjected to flow cytometry (FACS Calibur, Becton Dickinson, Sunnyvale, CA) equipped with the software CellQuest™ Pro by first scatter-gating on the lymphocyte population. A total of 10^5 cells were analyzed to determine the percentage of CD3-, CD4- and CD8-positive lymphocytes.

Preparetion of CLA and linoleic acid

For determination of effects of CLA and linoleic acid on the proliferation in splenocytes, CLA and linoleic acid was dissolved in ethanol and added to the culture medium with

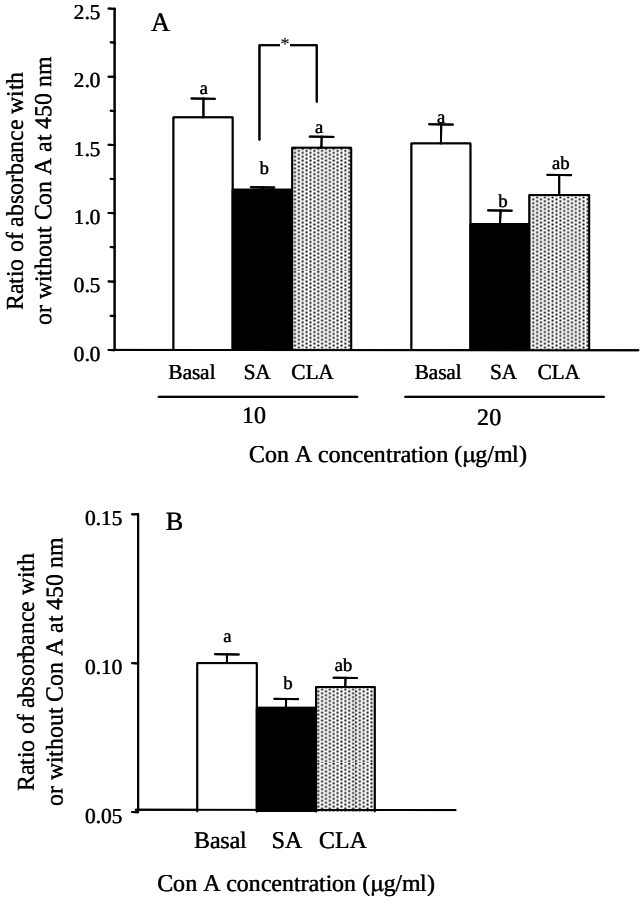

Figure 1. T cell proliferation of splenocytes (A) and interleukin (IL)-2-like activity in splenocytes (B) stimulated by concanavalin A (Con A) of chicks fed a basal diet, and diest supplemented with CLA or safflower oil (SA) for 14 days in experiment 1. Result was expressed as the basis of absorbance values (absorbance $_{A450\ nm-A655\ nm}$ of cells activity cultured with Con A/absorbance$_{A450\ nm-A655\ nm}$ of cells activity cultured without Con A. Each bar represents the mean and standard error from 6 individual chickens. For measurement of IL-2-like activity, splenocytes were stimulated by Con A for 24 h. IL-2 like activity in splenocytes of chicks fed a basal diet, diets supplemented with CLA or safflower oil for 14 d was expressed as the ratio of absorbance$_{A450\ nm-A655\ nm}$ of cells activity cultured with CM in presence of Con A/absorbance$_{A450\ nm-A655\ nm}$ of cells activity cultured with CM in absence from Con A. Each bar represents mean and standard error from 6 individual chickens. Different letters show significant differences within same Con A concentration of culture medium (A) and among the dietary treatments (B) ($p<0.05$). Asterisk shows significant differences between SA and CLA fed-groups within same Con A concentration of culture medium (A) and among the dietary treatments (B) ($p<0.05$).

concentration of 0, 10 and 30 mg/L. The same amount of ethanol was added to the culture medium to avoid any effect of proliferation of splenocytes. Con A concentration in culture medium was used at 10 μg/ml. In separated test, PHA (25 μg/ml) and PWM (1 μg/ml) were also served as mitogens to determine effect of CLA or linoleic acid at concentration of 10 mg/L on *in vitro* splenocyte proliferation. Determination of splenocyte proliferation was carried out mentioned above and fatty acid was added to culture medium at beginning of incubation of splenocytes.

Statistical analysis

Data was subjected to one-way analysis of variance (ANOVA) of SAS (SAS Institute, 1988. Cary, NC, USA), Mean value within the treatments were compared using Duncan's multiple range test when main effect was statistically significant ($p<0.05$). Because the two experimental diets were formulated by supplementing CLA or SA to the basal diet, it is appropriate to compare the data from CLA- and SA-fed birds. Thus, data from the fat supplemented groups was also subjected to Student's t-test to compare means. The threshold of significance was 0.05.

For determination of effects of CLA and linoleic acid when they were added to the culture medium on the proliferation in splenocytes, data in the same fatty acid concentration was applied by Student's t-test to compare means. The threshold of significance was 0.05.

RESULTS

Splenocyte proliferation and IL-2-like activity in experiment 1

Figure 1A shows proliferative responses of splenocytes in chicks fed basal, CLA or SA diet in experiment 1. Splenocytes proliferation induced by Con A was highest in chicks fed chicks fed the basal diet among the experimental groups at both Con A concentration. Splenocyte proliferation stimulated by Con A at concentration of 10 μg/ml was significantly greater in chicks fed the CLA diet than that in chicks fed the SA diet. The proliferation in chicks fed the CLA diet was slightly higher at 20 μg/ml than that in chicks fed the SA diet, but there was not significantly difference in the proliferation between both groups. Figure 1B shows IL-2-like activity of splenocytes in chicks fed the basal, CLA and SA diets. IL-2-like activity in splenocytes induced by Con A was highest in chicks fed chicks fed the basal diet among the experimental groups. There was no statistical difference in splenocyte proliferation between chicks fed the SA and CLA diets although the activity was slightly higher in chicks fed the CLA diet than in chicks fed the SA diet.

T cell subpopulation in experiment 1

Figure 2 shows CD3-, CD4- and CD8-positeve T cells in splenocytes of chicks fed the basal, CLA or SA diet. Feeding either fat supplemented diet resulted in a decrease in percentage of CD3-positive T cells of splenocytes as compared with the basal diet. Percentage of CD3-positive T

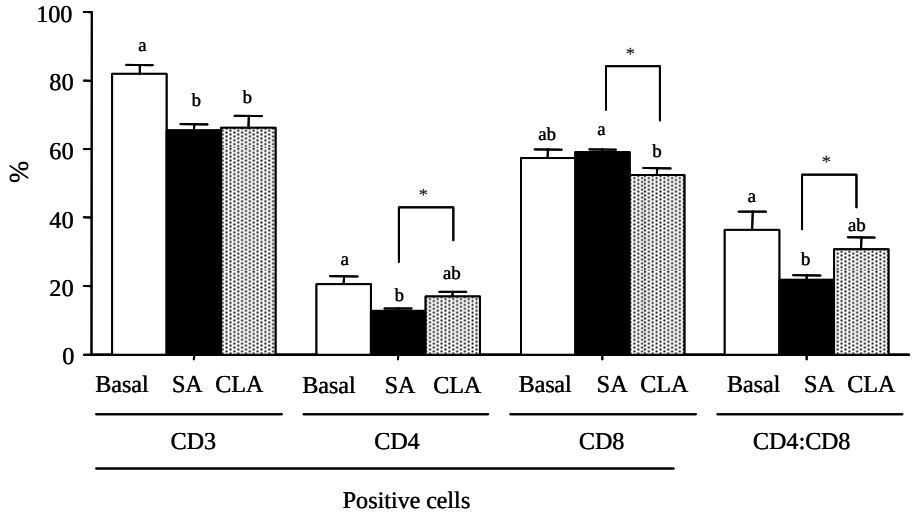

Figure 2. Percentage of CD3-, CD4- or CD8-positive cells in splenocytes of chicks fed a basal diet, diets supplemented with CLA or safflower oil (SA) for 14 days. Each bar represents mean and standard error from 6 individual chickens. Different letters within the same mitogen used show significant differences among the dietary treatments ($p<0.05$). Asterisk shows significant differences between SA and CLA fed groups within terms of CD3-, CD4-, CD8- positive T cells and ratio of CD4 to CD8 ($p<0.05$).

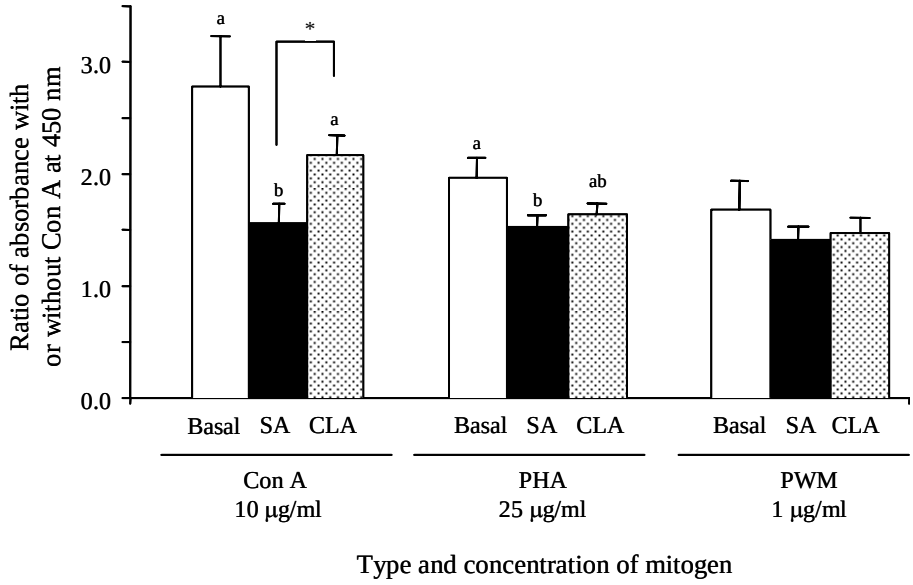

Figure 3. Changes in proliferative responses to Con A (10 μg/ml), PHA (25 μg/ml) and PWM (1 μg/ml) in splenocytes obtained from chicks fed the basal, CLA- or safflower oil (SA)-supplemented diet in experiment 2. Each bar represents the mean and standard error from 6 individual chickens. Result was expressed as the basis of absorbance values (absorbance $_{A450\ nm-A655\ nm}$ of cells activity cultured with Con A/absorbance$_{A450\ nm-A655\ nm}$ of cells activity cultured without Con A. Each bar represents mean and standard error from 6 individual chickens. Different letters within the same mitogen used show significant differences among the dietary treatments ($p<0.05$). Asterisk shows significant differences between SA and CLA fed groups within the same mitogen treatment ($p<0.05$).

cells in splenocytes did not differ between chicks fed the SA and CLA diet. Percentage of CD8-positive T cells was significantly higher in splenocytes in chicks fed the SA diet than in chicks fed the CLA diet. Percentage of CD4-positive T cells in splenocytes from chicks fed the SA diet was the lowest among the groups. Percentage of CD4-positive T cells in splenocytes and ratio of CD4-positive T cells to CD8-positive T cells in chicks fed the SA diet was significantly lower than in chicks fed the CLA diet.

Splenocyte proliferation in experiment 2

Figure 3 shows proliferative responses of splenocytes in chicks fed the basal, CLA or SA diet in experiment 2. Splenocytes proliferation was highest in chicks fed chicks fed the basal diet among the experimental groups regardless of mitogens. Splenocyte proliferation stimulated by Con A

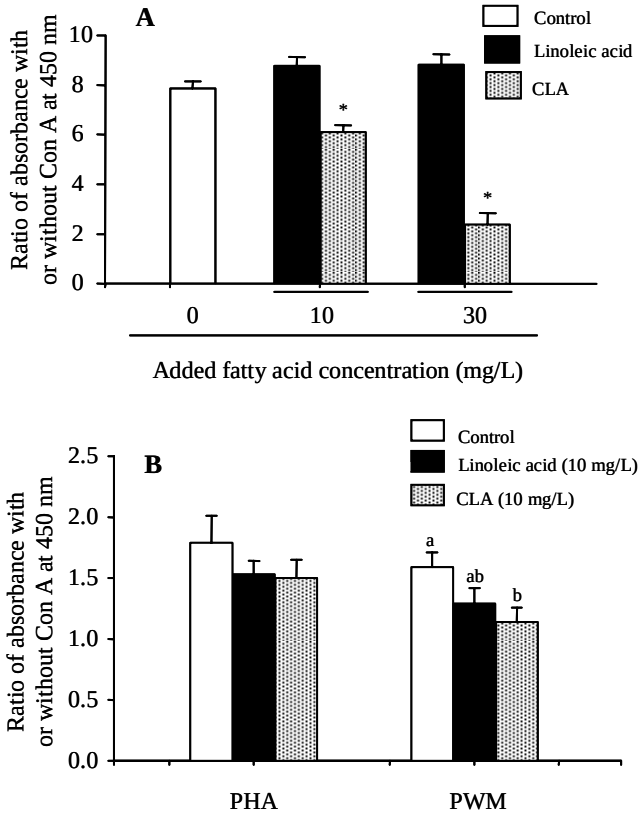

Figure 4. Changes in the proliferation of splenocytes by supplementation CLA or linoleic acid with culture medium when Con A was used as a mitogen (A) and when PHA and PWM were used (B). Result was expressed as the basis of absorbance values (absorbance $_{A450\ nm\text{-}A655\ nm}$ of cells activity cultured with mitogen/ absorbance$_{A450\ nm\text{-}A655\ nm}$ of cells activity cultured without mitogen). Each bar represents mean and standard error from 6 individual chickens. Different letters within the same mitogen used show significant differences among the treatments ($p<0.05$). Asterisk within same fatty acid concentration in culture medium (A) and within the same mitogen treatment (B) shows significant difference ($p<0.05$).

at concentration of 10 μg/ml was significantly greater in chicks fed the CLA diet than that in chicks fed the SA. On the other hands, PHA- or PWM-induced splenocytes proliferation did not differ between the CLA and SA fed groups.

Splenocyte proliferation *in vitro* by supplemental CLA and linoleic acid

Figure 4A shows effect of supplementation of CLA or linoleic acid to culture medium on splenocyte proliferation by Con A. An addition of CLA to splenocytes culture medium resulted in reduction in the proliferation to Con A with concentration dependent manner, while addition to linoleic acid up to 30 μg/ml did not affect the proliferation. Figure 4B shows effect of supplementation of CLA or linoleic acid to culture medium on splenocyte proliferation induced by PHA and PWM. There was no difference in splenocyte proliferation between supplementation of CLA or linoleic acid to the culture medium when splenocyte was stimulated by PHA and PWM. PHA and PWM induced splenocyte proliferation in the groups of addition of CLA or linoleic acid to culture medium were tended to be lower than that in that in the control group.

DISCUSSION

The finding that Con A induced-proliferation in splenocytes were higher in chicks fed the CLA diet than those in chicks fed the SA diet (Figure 1A) was accordance with the results of the experiment in chicks (Zang et al., 2005) and in mice (Chew et al., 1997; Wang et al., 1999). It has been reported that Con A mainly stimulates CD8-positive T cells which specifically express at membrane of cytotoxic T cells, while PHA stimulates CD4-positive T cells which specifically express at membrane of helper T cells in chickens (Chan et al., 1988). Therefore, it is presumed that dietary CLA mainly stimulated proliferation of cytotoxic T cells in chick spleen. However, an enhanced effect of dietary CLA on splenocytes proliferation compared with dietary SA was only observed at concentration of 10 μg/ml of Con A and there was no significantly difference in Con A induced IL-2 like activity between splenocytes in chicks fed the SA and CLA diet (Figure 1B). We also observed that effect of dietary CLA on splenocytes proliferation was similar to that of dietary SA when PHA and PWM were used as the mitogen (Figure 3). Thus effect of CLA on splenocyte proliferation in chicks is appeared to be almost similar to that of dietary SA. An addition of CLA to the proliferation assay medium reduced the proliferation by Con A in the CLA concentration dependent manner, but effect of linoleic acid on the proliferation was not observed (Figure 4A). In addition when PHA and PWM were used as the inducer of splenocytes proliferation, effect of supplementary linoleic acid and CLA to the culture medium on the proliferation did not differ (Figure 4B). Therefore, it is still questionable whether supplementation of CLA to poultry diet would be beneficial to enhance lymphocytes proliferation.

The present experiment showed that the percentage of CD3-positive T cells in chicks fed CLA in spleen was almost similar to that of dietary SA, although percentage of subpopulation of T cells of splenocytes such as CD8 and CD4 (Figure 2). The percentage of CD8-positive T cells was higher in mice fed diet containing a mixture of CLA than the control mice. The ratio of CD4- to CD8-positive T cells was lower in the mice fed diet containing a mixture of CLA than in the control mice (Yamasaki et al., 2003). The increase in CD8-positive T cells by feeding CLA is consistent with the results of previous studies in pig (Bassaganya-Riera et al., 2002, 2003). Conversely, ratio of

CD4-positive T cells to CD8-positive T cells, and percentage of CD4-positive T cells of splenocytes in chicks fed the CLA diet was higher than that in chicks fed the SA diet in this study (Figure 2). The results of the present experiment suggested that dietary CLA could modulate functional differentiation of T cells in chicks, but effect on T cells population by feeding CLA in chicks were different from that of rodents and pigs.

Dietary CLA prevented inflammatory responses and enhanced antibody response (Takahashi et al., 2003; Zhang et al., 2005). Feeding of diet supplemented with CLA up to 1% had no negative effects on performance in broiler chickens and laying hens (Du and Ahn 2001; Szymczyk et al., 2001; Takahashi et al., 2003; Zhang et al., 2005). These observations indicated that dietary CLA probably has beneficial effects on the control of the immunity of chicks. However the present study suggested that effect of CLA isomers on T cell subpopulation and responsiveness to mitogen of splenocytes in male broiler chicks was almost similar as that of SA or linoleic acid. Further studies need to clarify whether supplementation of CLA to poultry diet is beneficial for control of T cell immune system.

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