Asian-Aust. J. Anim. Sci.
Vol. 22, No. 3 : 448 - 458
March 2009

www.ajas.info

Conjugated Linoleic Acid as a Key Regulator of Performance, Lipid Metabolism, Development, Stress and Immune Functions, and Gene Expression in Chickens*

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ABSTRACT : It has been well documented from animal and human studies that conjugated linoleic acid (CLA) has numerous beneficial effects on health. In chickens, CLA exerts many effects on performance ranging from egg quality and yolk lipids to meat quality. Although there are several CLA isomers available, not all CLA isomers have the same incorporation rates into egg yolk: *cis*-9,*trans*-11 and *trans*-10,*cis*-12 CLA isomers are more favorably deposited into egg yolk than other isomers investigated, but of the two isomers, the former has a higher incorporation rate than the latter. CLA alters the amounts and profiles of lipids in plasma, muscles and liver. Furthermore, increased liver weight was reported in chickens fed dietary CLA. As observed in egg yolk, marked reduction in intramuscular lipids as well as increased protein content was observed in different studies, leading to elevation in protein-to-fat ratio. Inconsistency exists for parameters such as body weight gain, feed intake, feed conversion ratio, egg production rate and mortality, depending upon experimental conditions. One setback is that hard-cooked yolks from CLA-consuming hens have higher firmness as refrigeration time and CLA are increased, perhaps owing to alterations in physico-chemistry of yolk. Another is that CLA can be detrimental to hatchability when provided to breeders: eggs from these breeders have impaired development in embryonic and neonatal stages, and have increased and decreased amounts of saturated fatty acids and monounsaturated fatty acids (MUFAs), respectively. Thus, both problems can be fully resolved if dietary sources rich in MUFAs are provided together with CLA. Emerging evidence suggests that CLA exerts a critical impact on stress and immune functions as it can completely nullify some of the adverse effects produced by immune challenges and reduce mortality in a dose-dependent manner. Finally, CLA is a key regulator of genes that may be responsible for lipid metabolism in chickens. CLA down-regulates both expression of the gene encoding stearoyl-CoA desaturase-1 and its protein activity in the chicken liver while up-regulating mRNA of sterol regulatory element-binding protein-l. (**Key Words :** CLA, Egg, Meat, Quality, Lipid Composition, Embryos, Neonates, Development, Stress, Immunity, Gene Expression)

INTRODUCTION

Conjugated linoleic acid (CLA), derivatives of linoleic acid with anticancer properties found during cooking ground beef (Ha et al., 1987), is a collective term which indicates geometric (*cis*, *trans*) and positional isomers (7,9; 8,10; 9,11; 10,12; 11,13; 12,14) of *cis*-9,*cis*-12-octadecadienoic acid (C18:2n-6) (Figure 1). Since then, CLA has been shown to have a variety of beneficial effects including anti-atherogenic, anti-carcinogenic, anti-diabetic, and anti-obesogenic properties (Figure 2) (Bassaganya-Riera et al., 2002; Belury, 2002b; Evans et al., 2002; Tricon and Yaqoob, 2006). Whereas many studies on CLA have been performed in rodents and humans, recent research has been extended to food-producing animals including poultry. In poultry, most of the studies that have been so far done with CLA primarily focus on its effects on egg quality and yolk lipid compositions in laying hens and on meat quality in broilers. More recently, attempts have been made to investigate the effects on stress, immune functions, and gene expression. The present article reviews the effects of dietary CLA on egg and meat qualities and hatchability of fertile eggs, in which CLA greatly affects the composition of fatty acids in eggs and muscles, and then explores recent advances in CLA research that deals with about its effects

* Part of this paper was presented in the "International Symposium on Recent Advances in Animal Nutrition" as a Satellite Symposium of the 13th Animal Science Congress of the Asian-Australasian Association of Animal Production Societies (AAAP) on September 22-26, 2008, Hanoi, Vietnam.

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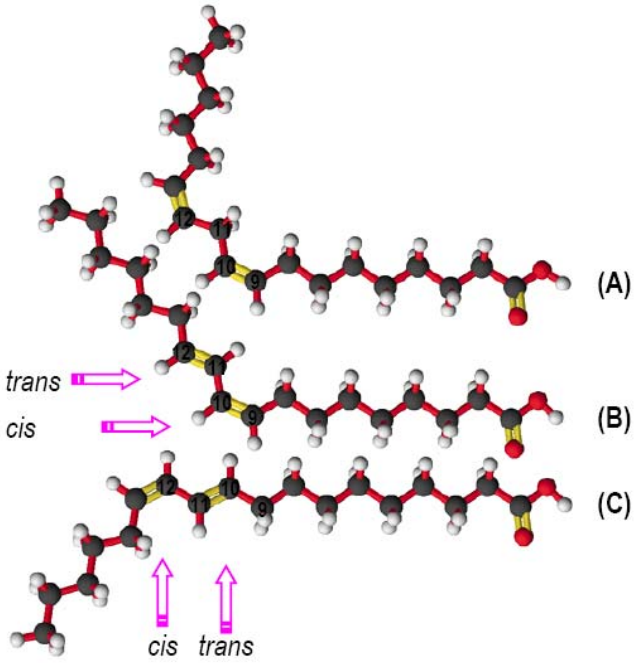

Figure 1. The structure of linoleic acid and its two CLA isomers. (A) Ordinary linoleic acid, cis-9, cis-12 octadecadienoic acid (C18:2n-6); (B) cis-9, trans-11 CLA; and (C) trans-10, cis-12 CLA. With modification of the diagram (Steinhart, 1996).

on stress, immune functions, and gene expression in chickens.

EFFECTS ON YOLK LIPIDS AND EGG QUALITY

As may be suggested by its name, the majority of the CLA studies having been made so far are related to testing the effects of dietary CLA on egg lipids and quality, and performance of laying hens. Perhaps the first study in this field was done by Ahn et al. (1999) who determined, using 79-week-old White Leghorn hens, the effects of dietary

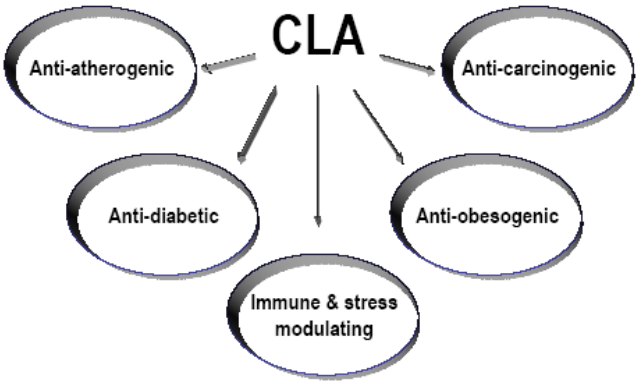

Figure 2. Physiological effects of CLA. CLA has been shown to possess beneficial effects against atherosclerosis, cancer, diabetes, and obesity and to modulate immune functions and stress in mammals (Belury, 2002a; Corl et al., 2008; House et al., 2005).

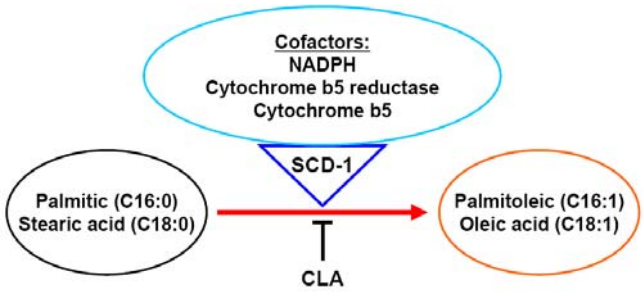

Figure 3. CLA inhibits stearoyl-CoA desaturase-1 (SCD-1) activity. SCD-1, together with the cofactors such as NADPH, cytochrome b5 reductase, and cytochrome b5, introduces a single double bond into its substrates (e.g., palmitic acid (C16:0) and stearic acid (C18:0)) producing palmitoleic (C16:1) and oleic acids (C18:1), respectively. The diagram is drawn based on the papers (Enoch et al., 1976; Miyazaki et al., 2001; Ntambi and Miyazaki, 2003).

CLA (0, 2.5, or 5.0%) on selected quality characteristics of fresh eggs and eggs refrigerated for different periods of time. As expected, dietary CLA substantially influenced the fatty acid composition of yolks as well as the quality of eggs. It was found that as dietary CLA was increased, the proportions of saturated fatty acids (SFAs) were increased in egg yolks while those of unsaturated fatty acids (UFAs) were decreased (Ahn et al., 1999) (for possible mechanisms, see Figure 3 and below). These results are supported by findings from different works (Du et al., 1999; Schafer et al., 2001; Watkins et al., 2001; Yin et al., 2008). On the other hand, Raes et al. (2002) observed that dietary CLA (1%), in combination with different fat sources and fat levels, resulted in decreased content in monounsaturated fatty acids (MUFAs) and increase in SFAs but failed to alter polyunsaturated fatty acids (PUFAs). The results were observed regardless of sources and amounts of fat in the diet (Raes et al., 2002). Comparable results have been recently observed irrespective of different genetic background within chickens or different avian species (Aydin and Cook, 2004; Yin et al., 2008).

CLA isomers provided to laying hens have been shown to have different incorporation rates into egg yolks. A higher transfer rate into egg yolks was observed for the *cis*-9,*trans*-11 isomer than for the *trans*-10,*cis*-12 isomer (Jones et al., 2000; Schafer et al., 2001; Raes et al., 2002), being consistent with the findings in broilers (Simon et al., 2000), Japanese quail (Aydin and Cook, 2004), rodents (Park et al., 1997), pigs (Bee, 2000), and cows (Chouinard et al., 1999).

Dietary CLA appears to alter the proportion of yolks in eggs. Ahn et al. (1999) initially reported that feeding CLA (0, 2.5, or 5.0%) led to an increase in the percentage of egg yolks. In laying hens fed diets containing 3% of either sunflower oil (control), fish oil (salmon oil) or CLA in triglyceride form (containing predominantly *cis*-9,*trans*-11

CLA and *trans*-10,*cis*-12 CLA) for 5 weeks, egg yolks from CLA-fed hens were heavier than those from either fish-oil-fed hens or control (Konig et al., 2008). In this study, however, it was not specified whether or not CLA altered egg size. It is thus not clear whether CLA modified the ratio of yolk weight to whole egg weight (percentage of yolks) in this study. By contrast, Yin et al. (2008) observed that, with the increased amounts of dietary CLA up to 5%, percentage of yolks was decreased while that for albumen was increased.

Dietary CLA has shown to affect the texture of egg yolks (Ahn et al., 1999). As both dietary CLA concentration and refrigeration time increased, the firmness of yolks from hard-cooked eggs increased with an interaction between dietary CLA and refrigeration time: the longer refrigeration time the higher yolk firmness as dietary CLA increased (Ahn et al., 1999). The sensory characteristics of yolks from hard-cooked eggs containing CLA were rubbery and elastic (Ahn et al., 1999), findings that were confirmed by others (Watkins et al., 2003; Alvarez et al., 2004; Shang et al., 2004). Water content in yolks was increased as dietary amount of CLA was increased (Ahn et al., 1999; Alvarez et al., 2004). Likewise, eggs produced from hens fed CLA had a higher yolk index compared with control (Schafer et al., 2001), which could be due to increased and decreased incorporation of SFAs and MUFAs into egg yolks, respectively (Raes et al., 2002). Shang et al. (2004) also noted that, with concurrent decreases in albumen pH, yolk firmness, yolk water content, and pH were increased with storage time and CLA content. For the content of Na, K, and Mg there was a linear increase in egg yolks with storage time but a decrease in Na content in albumen, which was unrelated to dietary CLA. These results suggest that the greater firmness of egg yolks from CLA-fed hens is likely to be due to changes in pH, water content, and/or ion concentrations during refrigerated storage and to increased content in SFAs (Shang et al., 2004). It is thus worthwhile to note that feeding CLA together with high-oleic acid sunflower oil attenuated the adverse effects of dietary CLA on sensory parameters such as aroma, taste, aftertaste, flavor, acceptability and firmness of yolks from hard-cooked eggs (Alvarez et al., 2005).

Feeding CLA also affects the percentage of albumen. The amount of albumen was reduced on day 1 as hens consumed more CLA, but was not different among dietary CLA treatments (0, 2.5, or 5.0%) (Ahn et al., 1999). However, it is noted that after 49 days of storage at 4°C, yolk content was increased while albumen decreased, regardless of dietary CLA (Ahn et al., 1999). Percentage of shell and yolk lipids, and yolk color were not affected by either CLA or storage duration (Ahn et al., 1999). Shang et al. (2004) found that weights of yolk, albumen and shell all decreased linearly with increasing dietary CLA (up to 6%) fed to 40 week-old hens for 56 days. On the other hand, dietary CLA (5%) did not affect albumen weight in older hens (62 week-old) (Chamruspollert and Sell, 1999), suggesting the possibility of age differences. Dietary CLA also modulated pH of yolk and albumen, with the former being increased with both CLA content and storage duration and the latter being unchanged by CLA but changed with storage time (Ahn et al., 1999).

Responses to dietary CLA by laying hens appear to be dependent upon age. For instance, feeding a 5.0% CLA diet to 26 week-old laying hens resulted in reduction in average weights of eggs and yolks but failed to affect egg production rate, body weight gain, and feed intake (Chamruspollert and Sell, 1999). In older hens (62 week-old), however, the diet did decrease feed intake but did not alter egg production rate, weights of eggs, albumens or yolks, or body weight gain (Chamruspollert and Sell, 1999). Age dependency was also seen in CLA accumulation into yolks. Maximum concentrations of CLA in egg yolks were observed to be 11.2% of the total fatty acids in egg yolks from younger laying hens (26 week-old) but to be 7.4% of those from older hens (62 week-old) after a 5% dietary CLA was provided (Chamruspollert and Sell, 1999), suggesting that transfer of dietary CLA into egg yolks is reduced with age. Ahn et al. (1999) also reported that there were negative relationships between dietary CLA (up to 5%) and feed consumption, egg production rate, feed efficiency, and body weight gain in 79 week-old laying hens, but no relation was detected with egg weight. These findings indicate that there are age differences in responses of laying hens to dietary CLA, as shown in elsewhere (Nielsen, 1998).

Incorporation rate of dietary CLA isomers into egg yolk lipids seems to be dependent upon both the composition of CLA isomers in the diet and the class of egg yolk lipids. In a study where 27 week-old laying hens were given diets containing 0, 1.25, 2.5 or 5% CLA, the source of which had 17.9% *cis*-9,*trans*-11, 20.3% *trans*-10,*cis*-12, 4.4% *cis*-8,*trans*-10, and 15.3% *cis*-11, *trans*-13 CLA isomers of the total dietary fat, CLA was to be proportionally incorporated into lipid, phosphatidylcholine (PC), phosphatidyl-ethanolamine (PE), and triglyceride (TG) of egg yolks, with the more favorable incorporation into TC than into the others (Du et al., 1999). Furthermore, the study also revealed that the incorporation rates of different CLA isomers into different classes of yolk lipids were also significantly different: the *cis*-9,*trans*-11 isomer was more favorably incorporated into yolk lipids, PC, PE and TG than was the *trans*-10,*cis*-12 isomer. Considering the amount of dietary *cis*-11,*trans*-13 CLA, the isomer was considerably less deposited into the four classes. The *cis*-8,*trans*-10 isomer was highly deposited into PE than into PC (Du et al., 1999).

In egg yolks from laying hens fed dietary yellow grease with or without fish oil, inclusion of CLA was shown to attenuate lipid oxidation during storage (Cherian et al., 2007). However, due to scarce data available, to draw conclusions about this area is premature.

It was shown that dietary CLA helps facilitate incorporation of docosahexaenoic acid (DHA) into egg yolks by an unknown mechanism when both at a small amount were provided together to laying hens (Watkins et al., 2001). These findings are interesting because either CLA or DHA alone at a higher amount resulted in much lower amounts of either of them in egg yolks. Thus, a proper ratio in the diet between CLA and DHA may be required to maximize the enrichment of both fatty acids into egg yolks. In a study where laying hens were fed diets with three levels of supplementation of CLA (1, 3 and 5 g/kg) and fish oil (0, 14 and 20 g/kg), Alvarez et al. (2004) also obtained similar, but not the same, results to those of Watkins et al. (2001).

Effects of dietary CLA on feed intake vary in laying hens depending upon experimental conditions such as the amounts of CLA in the diet, with no effect (Chamruspollert and Sell, 1999; Cherian and Goeger, 2004; Kim et al., 2007; Konig et al., 2008; Raes et al., 2002; Simon et al., 2000; Suksombat et al., 2007) or reduction (Ahn et al., 1999; Javadi et al., 2007; Yin et al., 2008). In an early study, dietary CLA was shown to stimulate feed intake in chicks (Cook et al., 1993).

The effects of CLA on egg production rates are not consistent among studies. Kim et al. (2007) reported approximately a 10% reduction in egg production rate from hens fed a 2% CLA diet. This reduction was fully recovered by supplementing the CLA diet with 2% linoleic acid. Shang et al. (2004) also found that egg production rate, in addition to feed intake, body weigh gain, egg weight, feed efficiency, and weights of yolk, albumen and shell, decreased linearly with increasing dietary CLA up to 6%. CLA diets, however, produced no changes in egg production rates across treatments (Konig et al., 2008).

EFFECTS IN BROILERS

Lipid content and fatty acid profile in muscle are important determinants to the quality of meat. Tenderness and flavor are two major aspects of meat quality to which the amount and type of fat in meat may contribute (Wood et al., 1999). Because CLA has marked impact on lipid metabolism, it is reasonable to link dietary CLA with meat quality and/or characteristics. As may have been expected, solid evidence has described the capability of dietary CLA to modulate fat content and profile in tissues. Zhang et al. (Zhang et al., 2007), for example, reported that the amounts of intramuscular fat in breast and thigh muscles and of abdominal fat were dose-dependently decreased as dietary amounts of CLA were elevated while the proportions of breast and thigh muscles were increased. In addition, shear force and yellowness for breast muscle were reduced by dietary CLA without changing pH in the muscles (Zhang et al., 2007). Studies by Simon et al. (2000) found that feeding CLA (1.8%), containing the isomers *cis*-9,*trans*-11 and *trans*-10,*cis*-12 at a proportion 1:1, significantly reduced fat content of breast and leg muscles, and liver in meat-type chickens but increased protein contents in leg muscles and liver, although body weight gain, feed intake and feed conversion ratio were not changed in this study. These findings are in line with those in rodents, in which feeding CLA at low levels (up to 1%) produced a rapid, marked decrease in fat tissue weights and body weight in a dose dependent manner while increasing protein content without any major effects on food intake (DeLany et al., 1999).

Not all studies show similar results to those aforementioned, however. In a study where male broiler chicks received for 21 days either control diet (1% sunflower oil) or diet containing CLA (1% of a 1:1 mixture of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 CLA isomers) (Javadi et al., 2007), CLA-fed group had higher fat content in body composition and higher loss of ingested energy through excreta, had significant reduction in feed and energy intake, apparent fat digestibility, energy metabolisability and heat expenditure, but did not show changes in the proportions of body water, ash, protein, and weight gain. Taken together, these studies suggest that the action of CLA on mass of fat and muscle may be influenced by the amount of CLA in the diet.

CLA affects the deposition and profile of lipids in chickens. Fat deposition in both drumstick muscle and abdominal fat pad was linearly reduced as dietary CLA was increased. Dieary CLA was positively and negatively related with the total amounts of SFAs and MUFAs, respectively, in breast muscle whereas those of PUFAs were not altered with the exception that C20:4 was gradually reduced (Zhang et al., 2007). With the exception of thigh muscle, in which MUFAs were reversely related with diet CLA, however both the amounts of SFAs and PUFAs were not changed in breast and drumstick muscles by CLA. The amount of SFAs was elevated but that of MUFAs was decreased, with no change in PUFAs (Badinga et al., 2003). Consistent are the findings that dietary CLA resulted in a higher percentage of SFAs and lower percentages of MUFAs and PUFAs in carcass fat of broilers (Javadi et al., 2007).

As is evident with egg yolks, CLA isomers seem to have different incorporation rates into muscles. *cis*-9,*trans*-11 CLA was favorably deposited into muscles compared with *trans*-10,*cis*-12 CLA (Simon et al., 2000). Compared with *trans*-10,*cis*-12 CLA isomer, *cis*-9,*trans*-11 CLA was

better deposited into breast muscle (Zhang et al., 2007) or into breast, drumstick, or thigh muscle (Suksombat et al., 2007), although both isomers were incorporated in a dose-dependent manner. The *cis*-9, *trans*-11 isomer was much favorably deposited in the broiler liver compared with *trans*-10, *cis*-12 isomer, although an equal amount (2.4%) of both isomers were supplemented into the diet (Badinga et al., 2003).

Interestingly, CLA treatments seem to alter liver weight. Du and Ahn (2003) previously reported that feeding CLA significantly increased liver weights of broilers without changing the amounts of crude fat in the liver and total free-fatty acids (FFAs) in plasma, and influenced the composition of individual FFAs (e.g., reduction in linoleic and arachidonic acids) in both plasma and liver (Du and Ahn, 2003). Percentage of liver weight was significantly increased ($p<0.01$) in broilers fed dietary CLA (0.5%) (Suksombat et al., 2007). Increased liver weight relative to body weight was also observed in laying hens although absolute body weight was similar between control and CLA groups (Schafer et al., 2001; An et al., 2008). These findings are in line with the results of an early study in mice by DeLany et al. (1999).

Effects of CLA on mortality show mixed results. Mortality was linearly reduced in slow-glowing chickens with the increase of dietary CLA (up to 2%) (Zhang et al., 2007). In contrast, dietary CLA (up to 4%) had little effect on mortality in Hisex Brown laying hens (Suksombat et al., 2006).

EFFECTS ON CIRCULATING LIPIDS AND EMBRYONIC AND NEONATAL DEVELOPMENT

Because of dietary CLA altering fatty acid compositions in egg yolks, it is not surprising to note that CLA treatment is likely to influence development during embryonic and/or neonatal stages and lipid metabolism during these periods. Indeed, maternal feeding of CLA (0.5%) resulted in changes in fatty acid compositions in the liver and yolk of their neonatal chicks, but failed to alter feed intake of laying hens, egg weight, fertility and hatchability of eggs, and mortality and body weight of their chicks (Latour et al., 2000a). In a subsequent study (Latour et al., 2000b), the same authors showed that feeding CLA to hens altered lipid metabolism in their chick embryos, with enhanced utilization of yolk as shown by relative yolk sac weight, and influenced the composition of circulating very low density lipoprotein particles. At this amount, however, neither chicks' body weight nor yolk-free body weight was altered.

In a sharp contrast, dietary CLA to breeder hens can be detrimental to hatchability of their eggs, depending on its amount supplemented to the diet. Previous studies have shown that feeding CLA at high levels to breeder hens and quails results in completely nullifying hatchability of their fertile eggs (Aydin et al., 2001; Aydin and Cook, 2004). Approximately 50% reduction in hatchability was observed in the eggs produced from breeder hens fed diet containing 2% (w/w) corn oil+1.0% CLA oil, compared with control containing 3% corn oil (Cherian et al., 2005). In these studies, failure of hatchability may have been linked to drastic alterations in fatty acid profile: increased, but decreased, content in SFAs (e.g., C16:0 and C18:0) and MUFAs (e.g., C16:1 n-7 and C18:1 n-9) in egg yolks, respectively (Aydin et al., 2001; Aydin and Cook, 2004). Therefore, the negative effect can be restored fully if those fatty acids could be replenished to normal levels via diets. In fact, this assumption proved true as the loss of chicken egg hatchability has been fully recovered by supplementing the CLA diet with either 10% olive oil (Aydin et al., 2001) or 6% soybean oil (Muma et al., 2006).

STRESS AND IMMUNE FUNCTIONS

A series of studies has shown that CLA alleviates stress responses caused by immune challenges in chickens (Table 1). Perhaps the first research paper of CLA in chickens was about prevention of the reduced body weight induced by lipopolysaccaride (LPS) (Cook et al., 1993). In that study, 1 day-old chicks were fed for 2 weeks a diet containing 0.5% CLA, the majority of which consisted of *cis*-9,*cis*-11, *cis*-10,*cis*-12, *trans*-9,*trans*-11, *trans*-10,*trans*-12, and *cis*-9,*cis*-12 linoleic acid isomers. At the age of 14 days chicks were injected with 0.5 ml of a 5% suspension of sheep red blood cells (SRBC), followed by blood collection at 3, 6 and 9 days thereafter to determine antibody response against SRBC. Seven days later, LPS derived from *Escherichia coli* was injected to chicks at a dose of 1 mg/kg, and changes in body weight were monitored before and 24 h after the injection. Chicks supplemented with 0.5% CLA gained more than those on basal diet 24 h after LPS treatment whereas increased body weight was observed for chicks fed either control or CLA diet but both injected with PBS. On the other hand, birds fed dietary CLA and treated with LPS gained significantly more while marked weight loss was monitored in those fed control diet but treated with LPS (Cook et al., 1993). Similarly, Zhang et al. (2008) have reported that dietary CLA prevented the loss of body weight gain produced by repeated treatments of LPS, with partial improvement of antioxidant capacity. Consistent are the findings that when challenged with LPS, chicks or mice consuming a diet containing CLA lost less and/or recovered more rapidly body weight than those consuming a diet containing fish oil (Miller et al., 1994). These results could be accounted for by attenuation in the reduction in food intake typically observed following immune challenge (Miller et al., 1994) and/or by changes in fractional protein

Table 1. Effects of CLA on immune functions in chickens

Parameters	Results[1]	Animals	Reference
Antibody production to BSA and/or SRBC	↑, ↔	Broiler chicks	Cook et al., 1993; Takahashi et al., 2003; Zhang et al., 2005a
Cyclooxygenases 1 and 2	↓	Broiler chicks	Zhang et al., 2006
Immunoglobulin-G	↑	Broiler chicks	Takahashi et al., 2003
iNOS and NO	↓	Broiler chicks	Zhang et al., 2006
LPS-induced alpha 1 acid glycoprotein	↓	Broiler chicks	Takahashi et al., 2002
LPS-induced body weight reduction	↓	Chicks	Cook et al., 1993; Miller et al., 1994; Takahashi et al., 2002; Zhang et al., 2008
LPS-induced ceruloplasmin	↓	Broiler chicks	Takahashi et al., 2002
LPS-induced H/L ratio	↓	Broiler chicks	Takahashi et al., 2002
Lysozyme activity	↑	Broiler chicks	Zhang et al., 2005a, b
PBL proliferation to concanavalin A	↑	Broiler chicks	Zhang et al., 2005b
PBMC proliferation	↑	Broiler chicks	Zhang et al., 2005a
Prostaglandin E_2	↓	Broiler chicks	Zhang et al., 2005a; Zhang et al., 2006

[1] ↑: enhanced; ↓: attenuated or blocked; ↔, not changed.
BSA = Bovine serum albumin; H/L = Heterophil-to-lymphocyte; LPS = Lipopolysaccharide; iNOS = Inducible nitric oxide; NO = Nitric oxide; PBL = Peripheral blood lymphocyte; PBMC = Peripheral blood mononuclear cells; PGE_2 = Prostaglandin E_2; SRBC = Sheep red blood cells.

synthesis rate (Klasing et al., 1987; Miller et al., 1994). Therefore it would be interesting to test if this effect could be observed in a long-term study with birds.

Dietary CLA has been shown to exert an anti-inflammatory effect by reducing superoxide production through activated macrophages and heterophils while enhancing the amount of urokinase plasminogen activator (u-PA) (Politis et al., 2003), a serine protease primarily involved in cell migration and tissue remodeling (e.g., chemotaxis, cell adhesion and apoptosis) (Crippa, 2007). Zhang et al. (2005a) have recently shown that CLA could enhance immune responses in chickens partly through enhancing lysozyme activity, stimulating T lymphocyte proliferation, elevating antibody production, and decreasing prostaglandin E2 (PGE_2) synthesis, but without affecting body weight (Zhang et al., 2005a; 2005b). These findings are supported by results that dietary CLA enhanced antibody production (Takahashi et al., 2003) and alleviated some undesirable metabolic and physiological changes, such as heterophil-to-lymphocyte ratio in plasma of immune-challenged broiler chickens (Takahashi et al., 2002).

Another line of evidence supporting roles of CLA in the regulation of stress and immune functions is presented by Zhang et al. (2006), who showed that CLA attenuated and LPS produced the activation of PGE_2, cyclooxygenases 1 and 2, and inducible nitric oxide in the spleen which are involved in inflammatory responses.

Dietary CLA has been shown to improve antioxidant capacity in broiler chicks by increasing total superoxide dismutase activities in the liver, serum and muscle and catalase activity in the liver, and decreasing malondialdehyde, a marker of lipid peroxidation in the liver, serum and muscle (Ko et al., 2004; Zhang et al., 2008). Therefore, CLA may help improve immune functions and reduce stress responses through enhancing antioxidant capacity thereby reducing superoxide production, stimulating lysozyme activity, antibody production, and T lymphocyte proliferation, and decreasing PGE_2 synthesis.

GENE EXPRESSION

One of the recent developments in CLA research is its effects on gene expression. In particular, genes responsible for lipid metabolism have been studied. Stearoyl-CoA desaturase-1 (SCD-1), for example, is the rate limiting enzyme in the biosynthesis of monounsaturated fats (Enoch et al., 1976; Miyazaki et al., 2001; Ntambi and Miyazaki, 2003). SCD-1, together with the cofactors (NADPH, cytochrome b5, and cytochrome b5 reductase), introduces a single double bond into its substrates palmitic (C16:0) and stearic acid (C18:0) and, as a result, produces the UFA products palmitoleic (C16:1) and oleic acid (C18:1) (Miyazaki et al., 2001; Ntambi and Miyazaki, 2003) (see Figure 3). The regulation of this enzyme has thus been considered as an important step responsible for being adiposity (Cohen et al., 2003; Cohen and Friedman 2004). Interestingly, CLA has been shown to downgrade either expression of the gene encoding SCD-1 or SCD-1's activity in several *in vitro* and *in vivo* experimental models (Choi et al., 2000; Choi et al., 2001; Choi et al., 2002; Purushotham et al., 2007). Likewise, treatment of dietary CLA resulted in a dose-dependent reduction in both SCD-1 gene expression and its protein activity in the chicken liver (Shang et al., 2005), providing a rationale for which elevation of SFAs and reduction of MUFAs are observed in yolks and muscles of CLA-fed chickens. It should be noteworthy that *trans*-10,*cis*-12 CLA directly inhibited SCD-1 activity of the

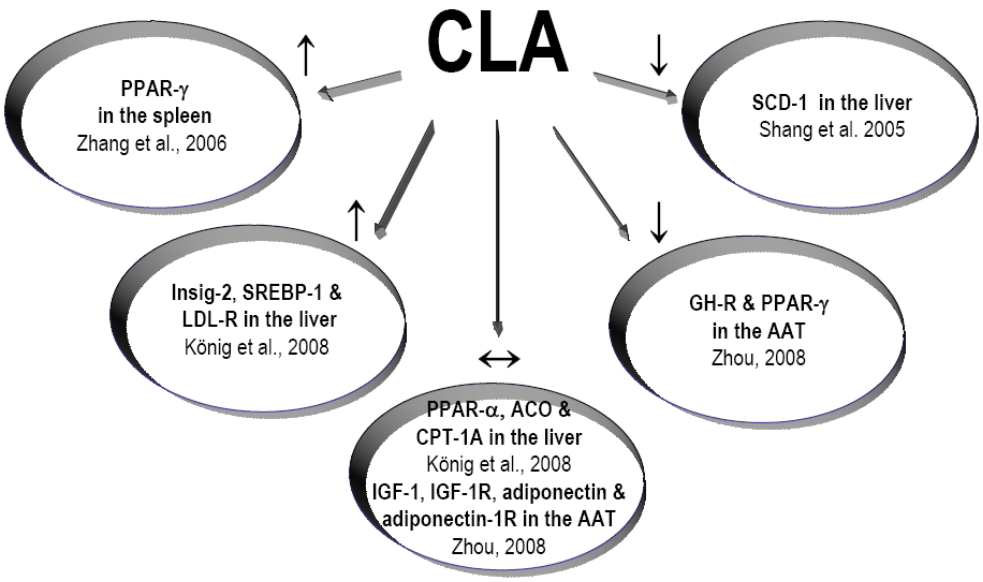

Figure 4. Effects of CLA on gene expression in chickens. Gene expression was primarily investigated in the adipose tissue, liver and spleen. Dietary CLA failed to alter expression of the genes for IGF-1, IGF-1R, adiponectin, and adiponectin-IR in the abdominal adipose tissue (Zhou, 2008) and ACC, FAS, HMG-CoA reductase, LDL receptor, and SREBP-2 in the liver (Shang et al., 2005). CLA may also be involved in the modulation of expression for other genes in other tissues (House et al., 2005; Corl et al., 2008). Abbreviations: AAT: abdominal adipose tissue; ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; adiponectin-1R: adiponectin-1receptor; CPT, carnitine palmitoyltransferase; FAS, fatty acid synthase; GH-R: growth hormone-receptor; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; IGF-1: insulin-like growth factor-1, IGF-1R: IGF-1 receptor; Insig, insulin-induced gene; LDL-R: low density lipoprotein-receptor; PPAR: peroxisome proliferator-activated receptor; SREBP, sterol regulatory element-binding protein. ↑: enhanced; ↓: blocked/attenuated; ↔, not changed.

murine liver whereas *cis*-9,*trans*-11 or *trans*-9,*trans*-11 CLA isomers did not (Park et al., 2000). Therefore, the effects of CLA isomers on SCD-1 activity might be different in chickens.

CLA has been shown to activate the genes encoding both peroxisome proliferator-activated receptor (PPAR)-α (Moya-Camarena et al., 1999) and PPAR-γ (Figure 4) (Houseknecht et al., 1998). Dietary CLA has been shown to enhance mRNA expression of PPAR-γ in the spleen of broiler chicks (Zhang et al., 2006). In this study, however, one bird per each dietary treatment (0, 5, or 10 g CLA/kg diet) was repeatedly challenged either with LPS or saline, and LPS treatment also resulted in enhanced expression in PPAR-γ mRNA. Because there was no interaction between dietary CLA and LPS, the actions of CLA and LPS to up-regulate mRNA expression of PPAR-γ appear to be independent. Another limit of the study is that body weight of chicks used was not reported (Zhang et al., 2006). Therefore, the overall significance of these findings is unclear.

In laying hens fed diets containing 3% CLA in triglyceride form (containing predominantly *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA) for 5 weeks, dietary CLA failed to alter expression of PPAR-α mRNA but rather increased triacylglycerol and cholesterol concentrations in the liver (Konig et al., 2008). In rodent cell lines, however, CLA has been also shown to regulate lipid metabolism through binding to and activating PPAR-α (Moya-Camarena et al., 1999), leading to reduction in triacylglycerol and cholesterol concentrations (Konig et al., 2007). In the abdominal adipose tissue of broiler chickens, on the other hand, dietary CLA was shown to down-regulate the gene expression of both growth hormone receptor and PPAR-γ but not of adiponectin and its 1 receptor (adiponectin-1R), and insulin-like growth factor 1 and its receptor (IGF-1R) (Zhou, 2008). Dietary CLA resulted in decreased abdominal fat weight and fat index concurrent with reduced serum leptin concentrations but failed to modulate mean daily body weight gain, feed intake, serum adiponetin concentrations. Of the two adiponectin receptors, which were ubiquitously expressed in chickens and responded differently to feeding conditions (Ramachandran et al., 2007), gene expression for adiponectin-1R was determined in that study (Zhou, 2008). Because there is likely to be strain difference in gene expression (Cassy et al., 2004), it is unclear whether or not the ineffectiveness of CLA on gene expression of adiponectin and its receptor stems from strain difference between layer and meat-type chickens. Whereas dietary CLA reduces fat mass, and adiponectin and leptin concentrations, and increases insulin concentrations in rodents (Ide, 2005; Poirier et al., 2005), circulating leptin

concentrations were decreased in response to dietary CLA without influencing adiponectin, adiponectin-1R, and their gene expression in chickens (Zhou, 2008), implying the possible species difference in CLA effects.

It is shown that dietary CLA enhanced nuclear concentrations of sterol regulatory element-binding proteins (SREBP)-l, insulin-induced gene-1, and LDL receptor in the liver of laying hens, but was largely ineffective in altering expression of several other genes (e.g., SREBP-2, acetyl-CoA carboxylase, fatty acid synthase, 3-hydroxy-3-methylglutaryl-CoA reductase) responsible for lipid metabolism (Konig et al., 2008) (Figure 4). Taken together, these data suggest existence of species differences in gene expression in response to CLA.

SUMMARY AND CONCLUDING REMARKS

As has been suggested from mammalian studies, CLA exerts a critical impact on lipid metabolism in laying hens and broilers. Because egg yolks contain a high amount of lipids, one of the research interests in laying hens is as to how dietary CLA can modulate the amounts and profiles of lipids in egg yolks. If so, how could the changes influence embryonic and neonatal developments in the eggs produced from breeders fed CLA? As there exist several isomers of CLA, another question is, which is more favorably deposited into egg yolks among CLA isomers available. Other questions include: is the quality of eggs and meat influenced by feeding CLA? Does feeding CLA modulate performance in broilers and laying hens?

Emerging evidence has shown that CLA alters the amounts and profiles of lipids in plasma, muscles and the liver. Eggs from hens fed CLA have increased and decreased concentrations in SFAs and MUFAs, respectively. Among CLA isomers investigated, *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers are more favorably deposited into egg yolks than others, but of the two isomers the former has a higher incorporation rate than the latter does. A marked reduction in intramuscular lipids as well as increased protein content is reported in different studies, leading to elevation in protein-to-fat ratio. Inconsistency exists for the effects of dietary CLA on body weight gain, feed intake, feed conversion ratio, egg production rate, or mortality in laying hens and broilers, which may depend upon experimental conditions such as age and strain of chickens, amount and composition of CLA, dietary composition of other lipids, and the duration of feeding CLA.

Two major problems in utilizing CLA are: i) yolks from CLA-fed hens have higher firmness when hard cooked, perhaps owing to alterations in physicochemistry; and ii) fertilized eggs from CLA-consuming breeders show impaired development in both embryonic and neonatal stages. Both problems can be completely prevented if dietary sources rich in UFAs are provided together with CLA.

CLA has been shown to alleviate stress responses and to enhance immune functions in chickens, being consistent results found in mammals (Miller et al., 1994), and to reduce mortality in a dose-dependent manner (Zhang et al., 2007). In addition, expression of several genes responsible for lipid metabolism is modulated by CLA.

Despite considerable progress in research on CLA in chickens, inconsistency still exists among the results available, making it difficult to conclude exclusively some of the effects of CLA in chickens. In particular, a few data are available on parameters involving broiler performance, necessitating more research in this field. It may be interesting to test whether or not the roles of CLA as a regulator of stress and immune functions are compromised in chickens when UFAs are provided together with CLA in order to prevent the negative effects of CLA. In this regard might expression of genes be modulated that may be responsible for lipid metabolism? As CLA was shown to increase liver weight but decrease others' relative to body weight (Schafer et al., 2001), what mechanisms underlie this paradox? Because eggs from CLA-fed hen also contain a high content of SFAs, long-term effects of consuming these eggs might be an interesting topic to explore.

ACKNOWLEDGMENTS

This work was supported in part by a Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

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