IJAS Iranian Journal of Applied Animal Science

The Metabolic Effects of Conjugated Linoleic Acids (CLA) in Chickens: A Review

Review Article

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Received on: 27 Apr 2014
Revised on: 20 Jun 2014
Accepted on: 30 Jun 2014
Online Published on: Sep 2015

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Online version is available on: www.ijas.ir

ABSTRACT

Conjugated linoleic acids (CLA) are natural constituents of meat and dairy products from ruminants, originating from bacterial biohydrogenation in the rumen. CLA supplementation increases the health benefits of animal-derived foods. There are inconsistent reports of the effects of dietary CLA on chicken's performance; however, the majority of previous reports cite anti-lipogenic effects of CLA. Diets could be formulated to increase n-3 fatty acid (FA) concentration in chicken meat by feeding n-3 FAs as a replacement for n-6 FA rich ingredients. Off-flavors and the chances of oxidative deterioration during storage of meat have been attributed to highn-3 FA levels in poultry diets. An approach to increase the n-3 FA content and decrease the n-6/n-3 FAs ratio in meat, using conjugated linoleic acid in diets, has been suggested. This review deals with the main topics of CLA metabolic effects in chickens.

KEY WORDS chickens, conjugated linoleic acid, gene expression, lipid metabolism.

INTRODUCTION

In 1979, a research group from the University of Wisconsin-Madison detected a compound with anti-mutagenic activity in ground beef extract (Pariza and Hargraves, 1985); because of its structural similarity to linoleic acid, it was namedconjugated linoleic acid or CLA (Pariza *et al*. 1979; Ha *et al*. 1987). Later, it was found in many other food sources, especially in dairy products and in animals that have a ruminant-like, fermentative digestive process, alike wallabies and kangaroos (Chin *et al*. 1992; Joo *et al*. 2002). In the rumen, CLA is primarily synthesized by bacterial biohydrogenation of linoleic acid (18:2n-6) by a variety of bacterial species including *Butyrivibriofibrosolvens* (Kepler *et al*. 1966; Griinari *et al*. 2000). CLAs are derived mainly from the microbial activity of the rumen. Studies have found that cis-9, trans-11 CLA can also be endogenously synthesized via Δ9 desaturation of trans-vaccenic acid in the mammary glands of cows, and monogastrics such as mice and humans (Adolf *et al*. 2000; Santora *et al*. 2000; Turpeinen *et al*. 2002; Mosley *et al*. 2006). It wasbeen reported that the endogenous synthesis of CLA is the most important source of the cis-9, trans-11 CLA isomer in milk fat, representing up to 64-78% of the total CLA (Corl *et al*. 2001; Griinari and Bauman, 1999). Based on the high correlations between CLA and trans-vaccenic acid, it wassuggested that desaturation of vaccenic acid mightbethe main source of CLA in muscle lipids (Knight *et al*. 2003). In the liver of rats, delta 11-trans octadecenoicacidis enzymatically converted to the delta 9-cis, delta 11-trans octadecadienoic acid (Banni and Martin, 1998). It has been reported that the bacterial population of rodent intestine may also converted unsaturated fatty acids to CLA (Chin *et al*. 1994).

Differential effects of CLA isomers

CLAs have a variety of biological activities. CLA in chicken nutrition One reason for the diversity of biological effects of CLA is

that CLA is a blend of geometric and positional isomers, with double bonds located at the Δ [9,11], [10,12], [8,10], [7,9] and [11,13] positions. Although several CLA isomers exist in food (Kramer *et al*. 1998), most research is focused on the two major isomers, cis-9, trans-11 and trans-10, cis-12. Anti-cancer activity which has been shown for both major isomers of CLA can be additive (Ip *et al*. 2002; Masso-Welch *et al*. 2002; Masso-Welch *et al*. 2004). Moreover, it was observed that cis-9, trans-11 and trans-10, c12-CLA may affect lipid metabolism in diverse tissues, including liver, muscle and adipose tissue, in different ways (Evans *et al*. 2002).

These two isomers can work independently. The cis-9, trans-11 isomer improves growth performance in rodents. However, body fat reduction, inhibition of stearoyl-CoA desaturase and reduction of hepatic apolipoprotein B secretion resulted exclusively from trans-10, cis-12 CLA isomer activity (Chin *et al*. 1994; Cook *et al*. 1993; Park *et al*. 1999a; Pariza *et al*. 2000; Storkson *et al*. 2005; Valeille *et al*. 2004). These two isomers have antagonistic effects on each other. The cis-9, trans-11 isomer has a potential anti-diabetogenic effect and can correct insulin resistance while the trans-10, cis-12 isomer promotes insulin resistance (Song *et al*. 2004).

CLA and human health

The increased interest in the daily intake of CLA in humans results from evidences issued from animal models, which indicates the potential health benefits of CLA (Williams, 2000). CLA has been reported to act as afat-to-lean repartitioning agent (Park *et al*. 1997), as a growth modulator (Chin *et al*. 1994) and hypocholesterolemic and antiatherogenic agent (Lee *et al*. 1998). In animal models, CLA has been shown to reduce the incidence of skin, for estomach, colon, mammary, and liver cancers (Bhattacharya *et al*. 2006; Kelley *et al*. 2007; Lee *et al*. 2005). Milk fat, bein the richest natural source of CLA, hasa protective effect against the incidence of human breast cancer (Knekt *et al*. 1996).

Larsson *et al*. (2005) reported a negative correlation between CLA consumption and colorectal cancer incidence in a 15-year study. Furthermore, CLA have beneficial effects on lowering blood cholesterol (Bhattacharya *et al*. 2006; Kritchevsky, 2000; McLeod *et al*. 2004).

CLA in animal tissues

In animals, the highest CLA concentration was reported in the adipose tissue of kangaroos (38 mg/g fatty acids) (Engelke *et al*. 2004). In farm animal products, foods originating from ruminants are the main source of CLA, with the cis-9, trans-11 (rumenic acid) and trans-10, cis-12 isomers comprising 80-90% and 3-5% of the total CLA, respectively (Khanal and Dhiman, 2004).

Non-ruminant meats contain lower concentrations of CLA. For example, chicken and pork contain 0.9 and 0.6 mg/g, respectively (Chin *et al*. 1992). Level of CLA differs between fat meat and lean meat. Fat meat has a much higher concentration of CLA (cis-9, trans-11: 960-1310 mg/100 g) than lean meat (cis-9, trans-11: 6-43 mg/100 g) (Fogerty *et al*. 1988). CLA content in non-ruminants body fat is considerably lower than in the ruminants fat (Fogerty *et al*. 1988). Fritsche and Steinhart (1998) found no variation in the CLA levels or isomer distribution in bulls and bullocks fat samples and suggested that the CLA content of meat is independent of the hormonal status. Fish oils also contain low CLA concentrations (Chin *et al*. 1992). CLA is not found in any of the common plant oils. Small amounts of CLA maybe formed during their heating, bleaching and deodorisation in the refining process (Ip *et al*. 2002).

In vitro synthesis of CLA

The 50%-50% mixtures of CLA bioactive isomers are manufactured commercially through alkaline isomerisation or partial hydrogenation of either linoleic acid or, more frequently, sunflower or safflower oils that are rich in linoleic acid (Banni, 2002). Synthetically manufactured CLA includes equal levels of the cis-9, trans-11-CLA and trans-10, cis-12-CLA and significant levels of other CLA isomers (Pariza *et al*. 2001) in comparison to natural sources where the cis-9, trans-11-CLA isomer prevails.

CLA and fat metabolism

The trans-10, cis-12 isomer is responsible for fat reduction (Park and Pariza, 2007; Park *et al*. 1999a). The body fat-reducing effect of CLA is probably caused by CLA itself rather than its metabolites (Park and Pariza, 2007). The non-acidic derivatives of CLA were found not effective on fat metabolism, indicating that the carboxyl end is needed for CLAs activities (Cook *et al*. 2000; Park *et al*. 2004). *In vitro* experiments showed that CLAs stimulate modifications in the membrane of adipocytes and regulate its gene expression, resulting in a decrease in the activity of the delta-9 desaturase enzyme (Hur *et al*. 2007). The effects of CLA on body composition are mediated by the increase in energy expenditure, the lowering of body fat deposits, the stimulation of the apoptosis process in pre-adipocytes and an increase in lipolysis and β-oxidation in muscle tissue (Park and Pariza, 2007).

It was suggested that CLAs promote fatty acid β-oxidation in skeletal muscle (Pariza *et al*. 2001; Wahle *et al*. 2004). This effect is shown by an up-regulation of carnitine palmitoyltransferase I (CPT-I, the main enzyme for β-oxidation) in skeletal muscles (Bouthegourd *et al*. 2002; Degrace *et al*. 2004; Nagao *et al*. 2005; Park *et al*. 1997; Peters *et al*. 2001). CPT-I initiatesthe fatty acid transport into the mitochondrion for β-oxidation. In rat and mice adipose tissue, as well as in mice muscle, CLA up-regulate this enzyme (Park *et al*. 1997; Rahman *et al*. 2001). As a consequence, CLA increases the energy

supply from fat sources and helps to decrease body fat deposition. Higher peroxisomal β-oxidation activity has been reported in animals fed CLA (Moya-Camarena *et al*. 1999; Yamasaki *et al*. 2001; Choi *et al*. 2004; Degrace *et al*. 2004). A higher fatty acid β-oxidation may reduce the availablity of fatty acids for triglyceride synthesis, thus reducing fat deposition (Mersmann, 2002).

It has been reported that CLA might decrease leptin secretion (Inoue *et al*. 2004). This effect maybe due to the lower total adipose tissue in CLA-treated subjects, although the increased food intake in result of reduced levels of leptin was not been reported in such studies. CLA enhanced adiponectin and reduced TNF-α (Inoue *et al*. 2004; Nagao *et al*. 2003; Nagao *et al*. 2005; Pariza *et al*. 2000), which mayimprove insulin sensitivity; consequently, it can become an important mediator in several chronic disorders including obesity (Hotamisligil and Spiegelman, 1994). Alteration of interleukins by CLA was alsoreported (Bassaganya-Riera *et al*. 2003; Changhua *et al*. 2005). In the study of Royan *et al*. (2011a), dietary CLA effectively decreased fat deposition in broiler chicken meat, especially in breast tissue. Similarly, Kawahara *et al*. (2009) report that the total lipid and triglyceride content in breast meat tended to decrease in broilers fed 1-2% dietary CLA. Moreover, in the study by Kawahara *et al*. (2009), dietary CLA reduced the amount of thiobarbituric acid reactive substances (TBARS) in raw chicken meat during storage at 4 ˚C for 5 days. These results provided evidence that CLA feeding can be a practical strategy not only for adding nutritional benefits to chicken meat but also to improve meat quality, including oxidative stability.

CLA and energy expenditure

CLA may decrease body fat by promoting energy expenditure (Park and Pariza, 2007). West *et al*. (1998) demonstrated that dietary supplementation with CLA enhanced the energy expenditure in animals. This increase in energetic expenditure is sufficient to explain the lower fat accumulation in CLA-supplemented mice (Atkinson, 1999). The CLA mediated increase in energy expenditure is accompanied by higher oxygen consumption (Choi *et al*. 2004; Ohnuki *et al*. 2001; Terpstra *et al*. 2002; West *et al*. 2000) and the up-regulation of uncoupling proteins (UCPs) (Choi *et al*. 2004; Nagao *et al*. 2003; Peters *et al*. 2001), which are both indicators for energy expenditure.

It seems that uncoupling protein 2 (UCP2) acts to disconnect energy conservation from substrate oxidation, leading to higher heat production and lower energy conservation (Mersmann, 2002). Dietary CLA increased UCP2 in white and brown adipose tissue of mice (Tsuboyama-Kasaoka *et al*. 2000); nevertheless, in another report, CLA increased this protein only in brown, but not in white, adipose tissue (West *et al*. 2000). A lower respiratory quotient (RQ) is an indicator of increased fat oxidation. A reduced RQ was reported in mice fed a low-fat diet containing CLA, but not with inclusion of CLA in a high-fat diet (West *et al*. 1998). This effect of CLA might be species specific, because in pigs and sows fed CLA, the RQ was not affected (Muller *et al*. 1999; Muller *et al*. 2000).

CLA and adipose cells

CLA reduces adipose cell mass and number. This effect is attributed to inhibiting lipoprotein lipase (LPL) and stearoyl-CoA desaturase activities in adipose cells, increasing apoptosis of preadipocytes and adipocytes and modulating lipolysis. Lipoprotein lipase is the main enzyme for fat absorption and it's inhibition in adipocytes decreases fat uptake (Park *et al*. 1997; Park *et al*. 1999b; Park *et al*. 2004). This inhibitory effect has been linked to the trans-10, cis-12 isomer but not cis-9, trans-11 (Lin *et al*. 2001; Park *et al*. 1999b; Park *et al*. 2004). LPL is synthesized in adipose tissue and transfered to the endothelial cell surface, where it functions to remove fatty acids from blood lipoproteins. The released fatty acids can then move into the adipose tissue to be oxidized or to supply building blocks for the synthesis of complex lipids like triglycerides (Mersmann, 2002). A lower LPL activity would reduce the fatty acid available for triglyceride synthesis, and consequently would decrease lipid accumulation. Although it was been reported that very low levels of CLA increased LPL activity in 3T3-L1 cells, higher CLA levels inhibited the enzyme activity (Park *et al*. 1997; Park *et al*. 1999b; Lin *et al*. 2001).
The role of stearoly-CoA desaturase (known also as SCD-1 or Δ9-desaturase) is the Δ9-cis desaturation of many fatty acids including palmitoyl and stearoyl-CoA. Desaturation of these SFA is essential to generate mono unsaturated fatty acids (MUFA) necessary for inclusion into the sn-2 position of triglycerides (Cohen *et al*. 2002). It was shown that CLA consumption could down-regulate stearyl-CoA desaturase (Hur *et al*. 2007). Therefore, CLA mediated inhibition of stearoly-CoA desaturase activity may reduce body fat mass (Choi *et al*. 2002). The higher SFA / MUFA ratios in body fat following CLA feeding suggests that down-regulation of SCD-1 may be one explanation for CLA's lipid-lowering effects (Choi *et al*. 2000). Thus, CLA anti-lipogenic effects are mediated by inhibiting lipogenesis and triglyceride esterification by an interruption in the fatty acid desaturation process. Bretillon *et al*. (1999) reported that Δ6 desaturation of linoleic acid was reduced when the dietary ratio of cis-9, trans-11 CLA to linoleic acid increased, but the trans-10, cis-12 CLA isomer had only a small effect on the desaturation process and was only effective only at the highest levels. They also suggested that Δ9-desaturase activity is repressed only by thetrans-10, cis-12 CLA isomer. Another direct effect of CLA on lipid metabolism respect the decrease in the adipose tissue deposit byenhancing apoptosis of preadipocytes and adipocytes (Brodie *et al*. 1999; Brown *et al*. 2001; Brown *et al*. 2003; Cohen *et al*.

2002). Some reports indicate that the fat-reducing effects of the trans-10, cis-12 CLA isomer are not due to increased apoptosis (Xu *et al*. 2003) butare probably due to a reduction in preadipocyte differentiation into mature adipocytes (Brodie *et al*. 1999; Satory and Smith, 1999; Evans *et al*. 2000; Simon *et al*. 2005).

CLA and lipolysis

CLA`s effect on adipocytes may be mediated through the peroxisome proliferator-activated receptor-gamma (PPARγ). PPARγ is a member of the nuclear receptor superfamily and regulates the differentiation, proliferation and lipogenesis processes in adipocytes (Gregoire *et al*. 1998; Tontonoz *et al*. 1994). Down-regulation of PPARγ could lead to the effects cause by CLA. CLA has been reported to decrease PPARγ expression (Brown *et al*. 2001; Brown *et al*. 2003; Choi *et al*. 2000). It has also been shown that CLA strongly down-regulates PPARα, the predominant PPAR form in liver, with an antilipogenic effect (Moya-Camarena *et al*. 1999). Royan *et al*. (2011b) showed that the adipose PPARγ gene expression in palm oil-fed birds was significantly up-regulated; however, there were no significant differences in PPARγ gene expression in the adipose tissue between birds fed diets containing CLA, fish oil, soybean oil, or the mixture of these fats.

CLA and inhibition of adipocyte lipid synthesis

Accretion of triglycerides in adipocytes is the main indication for the growth of adipose tissue; therefore, a reduction in adipocyte lipid synthesis would reduce fat deposition (Mersmann, 2002). The effect of CLA on decreasing adipocyte hypertrophy was reported in the mouse (Tsuboyama-Kasaoka *et al*. 2000) and rat (Azain *et al*. 2000). Therefore, a combination of lower glucose and fatty acid uptake and reduced de novo fatty acid and triglyceride synthesis, is expected to be the reason for the lower fat accumulation in the adipocytes of animals fed CLA. Because CLA is not as effective for reducing hepatic lipogenesis (Choi *et al*. 2000), it was suggested that CLA lacks significant antilipogenic effects in birds (Du and Ahn, 2002).

Interaction of CLA with other fatty acids

Research examining the interaction between dietary CLA and fat level showed that CLA concentrations of 0.5-1% reduced fat mass equivalently in mice fed either a high-fat (45% of calories) or a low- fat (15% of calories) ratio (Delany *et al*. 1999). Therefore, it seems possible that the reduction of body fat through CLA supplementation is independent of dietary fat intake, at least in mice (Kennedy, 2007). It is obvious that the nature of dietary lipid used does affect the anti-adipogenic capability of CLA (Kennedy, 2007). Both the CLA and poly unsaturated fatty acids (PUFAs) are lipid metabolism modifiers; consequently, the use of CLA in combination with different PUFAs in the diet may improve the productive efficiency and reduce body fat deposition (Zanini *et al*. 2006). When CLA was combined with oleic, linoleic, or linolenic acids, the negative effects of higher saturated fatty acids were reduced (Kim, 2007).

Royan *et al*. (2013) reported that dietary CLA, either alone or in combination with soybean oil or fish oil significantly increased the CLA content of breast and thigh tissues as compared to CLA-free diets; however, the CLA content of tissues did not exhibit a dose-dependent response to CLA supplementation. In both the breast and thigh tissues, the combination of CLA and soybean oil resulted in more CLA deposition than the CLA or CLA combined with fish oil-containing diets. Herzallah (2013) demonstrated that lactic acid bacteria of animal origin (*L. reuteri*) significantly enhanced CLA synthesis in both eggs and broiler meat cuts.

Zanini *et al*. (2008) showed that adietary mixture of CLA and soybean oil increased total deposited fatty acids, including CLA, in meat, due to the higher lipid content in meat. The dietary CLA and canola oil mixture reduced SFA and MUFA and increased CLA content, with lower total lipid content in meat.

Zanini *et al*. (2006) reported that the fat-reducing effect of the mixture of CLA and canola oil was only observed in female birds, which normally have higher lipid accumulation than males. Moreover, they observed a lower total lipid content of the gizzard and a lower relative liver weight in females fed the CLA and canola oil mixture. However, when CLA in association with soybean oil was used, the total lipid content of the liver and gizzard increased linearly.

Aydin *et al*. (2001) reported that the use of CLA in association with oils rich in n-3 fatty acids optimized the CLA effect. In another study, the CLA in combination with soybean oil or coconut decreased body mass and epididymal fat mass in mice (Kennedy, 2007). However, CLA and fish oil in combination showed no effect on adiposity (Hargrave *et al*. 2005). Mixtures of CLA and n-3 PUFA accelerated recovery by activating PPARδ (i.e., induced UCP3) and upregulating the expression of KGF in the colon (Bassaganya-Riera and Hontecillas, 2006). Brown *et al*. (2001) have also shown that the CLA effect can be modified by the oil supplement; therefore, the association of CLA with other fats should be considered when interpreting the results of feeding studies using CLA in combination with other fats. The effect of CLA in reducing total serum cholesterol, liver weight and total lipid content of giblets of broiler chickens was also dependent on the oil source (Zanini *et al*. 2006). Shin *et al*. (2011) studied the effects of the combination of dietary CLA and n-3 fatty acids on the linoleic acid (C18:2n-6) and arachidonic acid (C20:4n-6) concentrations of broiler chicken breast and thigh muscles, and found that the combination of CLA and menhaden fish oil is effective to reduce the concentrations of linoleic and arachidonic acids in broiler chicken breast and thigh muscles.

Effects of CLA on PPARs expression

The anti-lipogenic effect of CLA is mediated through reducing uptake and transport of fatty acids as evidenced by lower lipoprotein lipase (LPL) and fatty acid binding protein (FABP) levels (Park *et al*. 1999a). It is suggested that for decreasing lipogenesis the trans-10, cis-12 isomer is a potent anti-lipogenic agent (Peterson *et al*. 2003). It seems that CLA applies the anti-lipogenic effects at the transcriptional level by regulating gene expression of important regulatory proteins and enzymes to some extent by PPARs (Berge *et al*. 2004).

Some studies showed that CLA could act as high-affinity ligands for PPARs isotypes, mainly PPARα and could alter the level and change the activation of different PPARs (Moya-Camarena *et al*. 1999). The majority of previous reports, refer that the trans-10, cis-12 and the cis-9, trans-11 isomers of CLA act as activator ligands of PPARα and δ, but activation of PPARγ was small (Houseknecht *et al*. 1998; Martin *et al*. 2000; Evans *et al*. 2002). The PPARγ mediated regulation of LPL and FABP support the idea that the antilipogenic effect of CLA are carried out through that transcription factor (Khan and Vanden-Heuvel, 2003), leading to increased β-oxidation and energy expenditure (Martin *et al*. 2000). In the study of Royan *et al*. (2011b), the PPARα gene expression in the liver tissue of broiler chickens fed CLA was lower than in tissue in birds fed fish oil.

CLA effects on the chicken immune system

The first reports showed that dietary CLA might prevent the growth depression induced by immune stimulation in mice and chickens (Cook *et al*. 1993). However, in the study by Long *et al*. (2011), the immunoregulatory actions of CLA of relevance to viral disease pathogenesis and immune responses were investigated. Their results indicated that dietary CLA enhanced immune function in chickens, particularly those of the IBDV-immunosuppressive status. Furthermore, at the molecular level, the immunoregulatory functions of CLA on chickens were attributable mainly to the antiinflammatory properties of CLA and were mediated, at least in part, by suppressing the IBDV-specific proinflammatory cytokine mRNA relative expression. Long *et al*. (2012) investigated the immunoregulatory actions of CLA and suggested that CLA alleviated the immunosuppression of T lymphocytes in broiler chickens exposed to cyclosporine A through increased peripheral blood T lymphocyte proliferation and interleukin-2 levels.

CLA effects on chicken performance

There is no consistency in previous reports concerning the effects of CLA on the chicken's weight gain. Thiel-Cooper *et al*. (2001) found a linear increase of daily weight gain associated with CLA supplementation. There are also reports on the moderate weight loss caused by thedietary CLA in chickens (Cook *et al*. 1993) and in mice (Miller *et al*. 1994) exposed to catabolic stress caused by endotoxin injections. In the study of Suksombat *et al*. (2007), the daily feed intake was not different in 3 week old broiler chickens fed up to 1.5% dietary CLA-compared to the control group; nevertheless, a lower daily weight gain was observed in CLA fed chicks. They also reported a decrease in abdominal fat and in drumstick and in boneless drumstick percents, along with higher liver weight in chicks fed CLA containing diets. Similar results on liver weight were also reported by Leaflet (2004).

Royan *et al*. (2011a) reported that broilers fed a high dietary CLA dose (4.2%), were found to have lower weight gains, but the chicks fed diets containing 2.1% CLA showed higher body weight gain than those fed 4.2% CLA in finisher diets. The adverse effect of CLA on body weight gain was reported by Szymczyk *et al*. (2001) and Suksombat *et al*. (2007) with dietary CLA levels up to 1.5%. Similar results were reported by Buccioni *et al*. (2009) using 1% dietary CLA; however, in their research, the weight gains of treated birds fluctuated and were lower at the initial and the final phases, but higher during the mid phase of the experiment. In the study of Royan *et al*. (2011a), the 4.2% CLA diet reduced feed intake during the grower phase, but birds recovered it later in the finisher phase. On the other hand the 2.1% dietary CLA level did not affect feed intake. It seems that the ability of chickens to use CLA increases with age, so that Suksombat *et al*. (2007) found that the feed intake of broiler chickens was significantly decreased by dietary CLA over the starter period, while no effects of CLA on this variable were noted over the grower-finisher period. In spite of that, the feed intake depression in the whole experimental period was statistically significant.

In most previous reports, feed intake was unaffected by the incorporation of CLA into broiler chickens diets. These observations were recorded using different dietary CLA levels: 0.4% (Denli *et al*. 2004), 1% (Takahashi *et al*. 2003; Zhang *et al*. 2005; Buccioni *et al*. 2009), 1.5% (Szymczyk *et al*. 2001; Suksombat *et al*. 2007) 1.8% (Simon *et al*. 2000), 2% (Bolukbasi, 2006) 3% (Du and Ahn, 2003) and 4% (Sirri *et al*. 2003). In other reports, Du and Ahn (2002) showed that up to 1% dietary CLA had no effect on feed consumption, but 2% and 3% levels reduced feed consumption of broilers, Also in the study by Javadi *et al*. (2007), the 1% dietary CLA was enough to reduce broiler chicken feed intake. The only report of a positive effect of CLA on feed intake is that of Bolukbasi (2006), who reported a higher feed intake in broiler chickens fed diets containing 1% CLA compared with the control group, but they found no difference between dietary 2 and 3% CLA levels and the control diet. In the study of Royan *et al*. (2011a) the diets containing CLA unfavorably increased FCR. The variations in FCR clearly resulted from differences in body weight gain, which is consistent with the report of Suksombat *et al*. (2007). However, most reports fail to demonstrate the existence of an effect of the dietary CLA on the feed

conversion ratio of broiler chickens (Simon *et al*. 2000; Javadi *et al*. 2007; Zhang *et al*. 2005; Szymczyk *et al*. 2001; Du and Ahn, 2002).

Moreover, a lower feed conversion ratio (which means higher efficiency) was observed in diets supplemented by CLA (Sell *et al*. 2001; Szymczyk *et al*. 2001; Bolukbasi, 2006), suggesting that the pattern of CLA effects in broiler chickens performance is highly variable among studies. However, it seems that the growth rate was more susceptible to CLA unfavorable effects than other performance traits. Apparently, CLA inclusion in diets at levels above 10 g/kg decreased the broilers growth rate (Szymczyk *et al*. 2001; Badinga *et al*. 2003). Royan *et al*. (2011a) found that the negative effects of CLA on growth rate are somewhat modified by dietary CLA dose, age of birds and the fat composition of the experimental diets, so that the chicks fed diets containing 2.1% CLA in the finisher phases showed an acceptable body weight gain compared to the 4.2% CLA level. The combination of CLA + soybean oil resulted in higher weight gain than the combination of CLA + fish oil.

Adverse results have also been reported on the effects of CLA on layer hen's performance. Szymczyk and Pisulewski (2003) reported that the feed intake and mass of eggs produced by hens fed with CLA-enriched diets were lower than those recorded for the control group. Thus, since the mass of eggs was more affected by dietary CLA, the resulting average feed conversion ratio, expressed as kg of feed required per kg of eggs produced, was higher in hens fed the CLA-enriched diets than in those fed the control diet. Cho *et al*. (2013) investigated the effect of CLA feeding on growth performance and fatty acid profiles in thigh meat of broiler chicken using meta-analysis on a total of 9 studies. They concluded that CLA was not beneficial for improving growth performance, although it might be estimated that CLA is effective in modulating n-6/n-3 fatty acids ratio in thigh meat. However a comparision of the loss from suppressed growth performance and increased saturated fatty acids with the benefit from enhanced n-6/n-3 ratio should be investigated in further studies in order to propose the appropriate use of dietary CLA in the broiler industry.

Effects of CLA on chicken carcass traits

The only report on the positive effects of CLA on carcass yield is that of Buccioni *et al*. (2009) where broiler chickens were fed diets with 1% CLA. Royan *et al*. (2011a) observed some differences in carcass yield (after feathers and gut had been removed) between CLA containing diets and other treatments. The combination of soybean oil with CLA (2.1% CLA+3.5% soybean oil) prevented the carcass yield depression caused by the diets containing 4.2% CLA. The breast percentage changed in the same manner as described above.

In the majority of the previous studies, carcass traits were not affected by dietary CLA. Bolukbasi (2006) did not found differences in the carcass yield and leg weight of chicks fed diets containing up to 3% CLA combined with sunflower oil, but they found an increase in the breast percentage in birds fed CLA. In the study of Szymczyk *et al*. (2001), the relative proportion of breast and leg muscles (% of carcass weight) responded differently to increasing levels of dietary CLA. The former variable was not affected by the treatment and the latter was significantly increased.

In other previous reports, the dietary inclusion of CLA at 0.4% (Denli *et al*. 2005), 1.5% (Suksombat *et al*. 2007) or 4% (Sirri *et al*. 2003) levels did not alter dressing percentage or thigh and breast yields. Buccioni *et al*. (2009) claimed that the increase in dressing percentage in CLA treated animals was related to a significant decrease in abdominal separable fat, and attributed such effect to the ability of CLA to reduce body fat accretion. In a comparable report, Suksombat *et al*. (2007) showed that the reduced abdominal fat pad in birds fed dietary CLA was not accompanied by an increase in carcass, breast or thigh percents. These controversies suggest that the observed CLA effect on carcass parameters of broiler chickens is not simply the result of abdominal fat pad alterations.

Effects of CLA on abdominal fat pad

There are some conflicting reports on the effects of CLA on the abdominal fat pad changes in chickens. Du and Ahn (2002) found that feeding a diet containing 0.5% CLA to broilers at 3 weeks of age, for a period of 3 weeks, resulted in an increase in abdominal fat content. Similar results have been reported by Denli *et al*. (2005) and Javadi *et al*. (2007). Nevertheless, there are also some conflicting reports concerning the effects of CLA on abdominal fat pad reduction in chickens (Simon *et al*. 2000; Szymczyk *et al*. 2001; Badinga *et al*. 2003). It was reported that CLA is effective in reducing body fat deposition only during the weight gain phase (at the earlier ages when a positive energy balance exists) (Atkinson, 1999; Kamphuis *et al*. 2003a; Kamphuis *et al*. 2003b; Larsen *et al*. 2006).

It seems that the CLA effect on fat accumulation can be modified by the associated of dietary fat supplements. Zanini *et al*. (2006) demonstrated a linear reduction in abdominal fat pad when canola oil was used with CLA, butnot when CLA was combined with soybean oil. In another report, it was shown that CLA, in combination with coconut oil or soybean oil (SFA and n-6 PUFA rich, respectively), could decrease body fat mass in mice (Kennedy, 2007); stil, mice fed CLA in combination with fish oil (n-3 PUFA rich) showed no effect on adiposity (Hargrave *et al*. 2005). According to the review of Park and Pariza (2007) the effects of CLA on body composition can be attributed to: the rise in energetic expenditure through the increased synthesis of uncoupling proteins; the decrease of body fat deposits

through a reduction in the number and size of adipocytes due to the inhibition of lipoprotein lipase enzyme, the stimulation of the apoptosis process of pre-adipocytes andthe increase in lipolysis and b-oxidation in muscle tissue as indicated by higher carnitine acyltransferase I and II.

Effects of CLA on the broiler's liver

An increase in liver weight of broiler chickens fed diets containing CLA has been reported (Du and Ahn, 2003; Leaflet, 2004; Suksombat *et al*. 2007; Royan *et al*. 2011a). In the report of Buccioni *et al*. (2009), the change in liver weight were dose-dependent, so that the 0.5% dietary CLA significantly increased liver weight but the 1% and 1.5% doses did not result in any difference compared with the control diet. Zanini *et al*. (2006) showed a reduction in the relative liver weight in female broiler chickens supplied with CLA and canola oil (n-3 rich). They found an interaction between oil source and CLA such that supplementation of CLA produced lower relative liver weight in birds fed canola oil compared to that of birds receiving CLA + soybean oil in their diet.

Based on animal studies, one main concern about CLA use, identified so far, is fatty liver (Pariza, 2004). In the study of Royan *et al.* (2013) the CLA containing diets increased liver fat accumulation and the diet with 4.2% CLA had a larger effect than diets containing 2.1% CLA. Fatty liver may be the result of CLA's pronounced effects on body fat mobilization, as well as increased fatty acid synthesis in the liver (Clement *et al*. 2002; Tsuboyama-Kasaoka *et al*. 2000; Yanagita *et al*. 2005). Zanini *et al*. (2006) reported an interaction between n-3 and n-6 fatty acids in relation to dietary CLA. In their research, adding CLA to the diets containing soybean oil resulted in an increase in the total lipid content of the liver, but the opposite was observed for the combination of canola oil and CLA. Nevertheless without CLA supplementation, liver fat content was higher for birds fed canola oil compared with those fed a diet containing soybean oil.

Effect of CLA on the chicken's serum parameters

The effect of dietary CLA on increasing serum TG concentration was reported by Du and Ahn (2003). However other studies report a decrease in serum or plasma TG following CLA administration (Bolukbasi, 2006). The reason for the higher serum TG concentration in birds fed dietary CLA is not clear, but it could be attributed to changes in the activities of enzymes involved in hepatic lipid metabolism. In birds, lipid synthesis occurs mainly in the liver. This effect may be a consequence of the inhibitory role of CLA on lipoprotein lipase together with stimulation of lipolysis in adipose tissue (Park *et al*. 1997). Consequently, reduced fat deposits and higher lipolysis in adipocytes could be the reason for the elevated serum TG levels, as observed in broiler chickens. In the study of Du and Ahn (2003) dietary CLA caused a significant increase in liver fatty acid synthase (FA synthase) activity and an increase (even though not significant) in acetyl-CoA carboxylase activity. FA synthase and acetyl-CoA carboxylase are the main enzymes regulating fatty acid synthesis. The higher FA synthase activity could be explained in part by the increased plasma TG levels. In the mammary glands of sows, dietary CLA caused a reduction in acetyl-CoA carboxylase and FA synthase (Piperova *et al*. 2000), but in cultured adipose cells, FA synthase gene expression was not decreased by dietary CLA (Choi *et al*. 2000). These results show that dietary CLA decreases lipogenesis in mammary glands and adipose tissues but not in liver. This could be the explanation for the ineffectiveness of CLA in decreasing fat deposition in birds (Du and Ahn 2002), because liver is the main site of lipogenesis. Fatty acid synthesis in mice, rats and pigs takes place mainly in adipose tissues, and the lipogenesis inhibitory effect of CLA in adipose tissue could considerably decrease fat accumulation in these species. The coordinated change of total cholesterol and LDL in birds fed CLA has been reported (Bhattacharya *et al*. 2006; Baddini Feitoza *et al*. 2009; Stangl *et al*. 1999), along with an increase in serum HDL level in broiler chickens fed CLA (Bolukbasi, 2006; Denli *et al*. 2005; Du and Ahn, 2003).

CONCLUSION

This review showed that CLA has the potential to modify metabolism and metabolic rate in broilers, but CLA feeding can also reduce productivity and increase SFA levels in chicken meat. There is no consistency in previous reports concerning the effects of different dietary dosages of CLA on chicken´s performance, but the levels above 1% are usually more risky. Therefore, CLA can be useful as an additive to produce meat rich in n-3 PUFA and to lower the n-6/n-3 ratio. A balance between improved meat quality and decreased growth performance is needed and the customer's preference should also be considered.

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