# Poultry Foods as Alternative Sources of Conjugated Linoleic Acids

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## SUMMARY

Consumers are highly interested in foods that prevent or ameliorate disease progression processes. Several different strategies have been adopted by the poultry food industry to enhance the nutritional value of eggs and meat. Poultry products such as eggs and meat, due to their high content of nutrients, low cost, and versatility in food preparation are popular food items for all cultures and incorporation of health-enhancing fatty acids could lead to alternate sources of these nutrients to humans. Conjugated linoleic acid (CLA) is usually contributed by ruminant foods. However, as Americans are opting for low fat dairy and beef products and choosing more poultry foods, availability of CLA in Western diets may be increased by access to CLA-enriched poultry foods. Adding CLA oils to layer or broiler diets can enrich chicken egg or meat with CLA. The effect of feeding CLA oils to layer and broiler birds and its effect on eggs and meat fatty acids, lipid stability and organoleptic characteristics are reported. The effect of feeding CLA-rich egg yolk powder to a rat model on tissue fatty acid composition is also reported.

## INTRODUCTION

Conjugated linoleic acid (CLA) is the generic name for a group of polyunsaturated fatty acids (PUFA) that exists as positional and geometric conjugated dienomic isomers of linoleic acid (18:2 n-6). The conjugated double bonds are typically located at the ninth and eleventh or the tenth and twelfth carbon atoms and all cis and trans isomers are possible. The dietary CLA is contributed by fats of ruminant animals such as beef, goat and sheep as well as dairy foods derived from these ruminant sources. The major CLA isomer that occurs in ruminant products such as milk and beef is cis-9, trans 11 (c9, t11) (Bauman et al., 2000). It is produced by the bacterium *Butyrivibrio fibrosolvents* in the rumen or by desaturation of C18:1 trans 11 at the ninth carbon in muscle, liver or mammary gland (Griinari et al. 2000).

## HEALTH ENHANCING PROPERTIES OF CLA

CLA has received considerable attention for its neutraceutical properties such as anticarcinogenic, antiatherogenic, antiadipogenic, and antiatherosclerotic effects (Belury, 2002a). In addition, CLA modulates immunity, bone formation, lipid metabolism and eicosanoid formation, gene expression in the liver muscle and adipose tissue (Scimeca,

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1999; Belury 2002b). CLA has been reported to reduce the chemically induced tumorigenesis of mammary, skin and colon in experimental animals. Feeding CLA-rich butter, Ip et al., 1999 reported reduction in terminal end bud (TEB) population of mammary gland, reduced TEB cell proliferation, inhibited mammary tumor yield and increased retention of CLA in tissues when compared with commercial CLA preparations.

## CLA INTAKE AND THE AMERICAN DIET

Current intake of CLA is estimated to be several hundred milligrams/day (Fritsche et al., 1999). However, considering the variation in CLA content of food products, these estimates are questionable. Based on animal data, it is estimated that approximately 3 g/day of CLA would be required to produce beneficial effects in humans (Ha et al., 1989). Humans do not have the ability to synthesize CLA and so it has to be supplied through foods of ruminant origin. However, as Americans are opting for low fat dairy products and choosing more vegetable oil-based spreads and poultry foods rather than beef, it is likely that dietary contribution of CLA will further be reduced in a typical US diet. An alternate way to provide CLA is by incorporating them to poultry foods through dietary manipulation. In this respect, development of CLA-enriched chicken eggs and meat may be an alternative vehicle for delivering health-promoting fatty acids to consumers

## INCORPORATION OF CLA IN POULTRY-BASED FOODS

### CLA-Enrichment and Associated Nutrient Changes in Eggs

The poultry industry has been applying new technology for developing eggs as a delivery system for functional nutrients (Cherian, 2002). The production and marketing of n-3 PUFA enriched eggs has been a success story in this aspect. Enhancement of other minor nutrients like fat soluble vitamins, antioxidants, pigments and folic acid in eggs through diet manipulation has also been reported. Poultry products contain relatively low CLA concentrations (0.9 and 0.6 mg CLA/g of fat, respectively (Chin et al., 1992). Increasing the concentration of CLA in poultry foods is a possible way for humans to increase their CLA intake and obtain potential health-benefits of CLA. A significant incorporation of CLA in eggs by dietary manipulation has been reported (Champruspoller and Sell, 1999; Jones et al., 2000). The diet-induced incorporation of CLA in egg is dose-dependent. Diets containing 5% CLA resulted in approximately a 15% CLA in egg lipids (Du et al., 1999). From the research reported, consumption of an average chicken egg weighing 60 g could provide over one-third of the daily CLA recommendation (3 g) for an adult human (Du et al., 1999). Table 1 depicts the reported studies and the amount of CLA supplied by one serving of CLA-modified chicken eggs. Cherian et al. (2002a) used menhaden fish oil (as source of long chain n-3 PUFA) along with CLA in the diet of laying hens to produce n-3 PUFA-CLA rich eggs. Such eggs from hens fed a diet containing 1.0% fish oil and 2.0% CLA oil could provide over 300 mg of CLA along with 230 mg of n-3 fatty acids to human diet. Feeding CLA along with fish oil did not alter the total fat content of eggs and the total lipids varied from 28 to 30%. However, a significant increase was observed in the content of saturated fatty acids (16:0, 18:0) with a concomitant reduction in monounsaturated fatty acids (MUFA) especially 18:1 n-9. These results corroborate other reported research on CLA feeding in poultry (Du et al., 1999; Jones et al., 2000) and other mammals (Belury and Kempa-Stezko,1997). Among the different isomers of CLA, c9,t11 isomer is preferentially incorporated in egg yolk lipids compared with t10,c12 isomer in all the studies reported. The incorporation of CLA isomers into egg yolk lipid classes were also different with phosphatidyl choline fraction incorporating more c9,t11 and t10,c12 than triacylglycerol which may suggest that it is the most active form. Feeding CLA has been reported to reduce egg triacylgycerol, phospholipids and total lipids. However, no difference was observed in the egg cholesterol with dietary CLA. Crude protein content and the concentrations of phosphorus and zinc have been reported to be high in CLA-rich eggs (Schafer et al. 2001). No difference was observed in the total egg edible portion, egg weight or albumen quality by feeding 2.5% CLA to hens (Cherian et al. 2002a).

### CLA-Enrichment:Egg Quality and Sensory Aspects

The appearance and texture of CLA-enriched egg is an important factor due to consumer acceptance. The inclusion of CLA altered the yolk shape, albumen height and yolk index (Schafer et al. 2001). The changes in fatty acid composition associated with CLA enrichment also lead to significant changes in texture. Feeding 2.5 or 5.0% CLA to hens resulted in eggs that are rubbery, elastic and firm when hard-cooked (Ahn et al. 1999). A similar effect was also observed by the author when diet contained 1.0% CLA. Egg quality aspects of CLA-rich eggs were also affected by storage. Ahn et al., 1999 reported an increase in egg yolk and albumen pH and darker colored yolk with white spots. The increase in trans fatty acids may increase the permeability of yolk membranes affecting ionic movements and yolk texture. Long term storage over 3 months in our laboratory resulted in an apricot-like color egg yolk and a whitish milk-like tint to egg white.

## EFFECT OF COOKING OR IRRADIATION ON CLA-ENRICHED EGGS

The effect of two types of cooking (scrambling vs. hard cooking) on the fatty acid changes in CLA-rich eggs were investigated. A significant decrease in CLA isomers (c9, t11, t10, c12), arachidonic acid, MUFA and PUFA content due to scrambling was observed compared with hard boiled eggs (Cherian et al., unpublished). Irradiation of foods, including eggs, is an effective tool for assuring food safety and controlling bacteria such as salmonella. Irradiation also leads to increased production of lipid peroxides and may reduce consumer acceptability. Production of volatiles is closely related to oxidative changes in eggs. We tested the total volatile content of irradiated CLA-enriched eggs. Dietary CLA at 1.0 and 2.0% reduced the total volatiles in raw and hard cooked eggs upon irradiation suggesting a protective effect of CLA on the degradation of lipids and sulfur-containing amino acids in eggs (Cherian et al., 2002b)

## CLA ENRICHED EGGS AS A DELIVERY SYSTEM FOR FUNCTIONAL LIPIDS: ANIMAL STUDIES

Although CLA modification of egg yolk has been documented, the health effects of CLA-rich yolk on animal models have not been reported. CLA when associated with food has been reported to have higher tissue retention and better anticancer effects than commercially available supplements (Ip et al.,1999). We determined whether CLA, when associated with a non-ruminant food such as chicken egg yolk, has tissue enriching and immuno-modulatory properties. Chicken eggs with no (N-CLA), low (L-CLA) or high CLA (H-CLA) were produced by feeding hens different levels of CLA. The CLA content of N-CLA, L-CLA and H-CLA eggs constituted 0, 1.4 and 5.4%, respectively. Sprague-Dawley rats (n=21) were distributed randomly to three treatments and were fed a basal diet with added 15% egg yolk powder containing either N-CLA, L-CLA or H-CLA for a period of 28 days. No difference was observed in body weight gain, feed consumption, carcass total lipids, protein or carcass ash content ($P>0.05$). Liver total lipids and weight were not affected by the dietary treatments ($P>0.05$). Rats consuming L-CLA and H-CLA diets accumulated more CLA in the splenocytes, liver and plasma ($P<0.05$) than those consuming the N-CLA diet (Figure 1). The increase in CLA was associated with a reduction in monounsaturated fatty acids in plasma, liver and splenocytes ($P<0.05$). A reduction in serum immunoglobulin content was observed in rats fed L-CLA and H-CLA diets ($P<0.05$). The current study shows that CLA in a naturally occurring non-ruminant food form could produce biological activity. The concept of CLA-enriched chicken egg yolk could be appealing to health-conscious consumers interested in functional foods and a diet-based approach for disease prevention. The success of such CLA-modified eggs will depend upon acceptable sensory characteristics and stability during cooking or storage or processing. These factors are yet to be investigated in detail.

## FEEDING CLA TO BROILER CHICKENS

Compared to investigations to increase CLA content of eggs, relatively few efforts have been directed toward CLA enrichment of poultry meat since the product is inherently low in fat. However, westerners are consuming more chicken than red meat and increasing the CLA content of poultry meat may be an easier way to incorporate CLA into human diet.

### Effects on Body Weight, Carcass Yield, and Abdominal Fat

The effect of dietary CLA on final body weight, carcass cut-up-parts, carcass fat and abdominal fat pads are important economic and environmental factors to be considered when incorporating CLA to broiler diets. As more and more poultry is sold as further processed or as cut-up portions, the information is important in developing further processed value-added poultry meat. Addition of CLA oil to broiler diets as a means of increasing the CLA content of broiler meat has been a focus in some recent studies. A list of broiler studies and the reported responses of broiler birds are shown in Table 2. Du et al. (2002a) fed diets containing 0, 0.25, 0.5 and 1.0% or 2.0 and 3.0% CLA to broilers in two different experiments. Feeding CLA did not alter the final body weight in the two experiments. The whole body fat content decreased from 14.2 % to 11.9 and 12.2% for 2 and 3% CLA-fed birds. CLA feeding did not alter the abdominal fat weight in these studies. From the studies conducted at Oregon State University, feeding CLA at 1.5 and 3.5% did not alter the total carcass fat, crude protein, dry matter, or ash. CLA feeding also resulted in lower thigh meat yield and final body weight (Cherian et al., 2003; Selvaraj and Cherian (in press). Szymczyk et al. (2001) fed CLA at 1.5% and found no significant effect on carcass yield and relative proportion of breast and leg. However, abdominal fat deposition was reduced from 2.7% for control (0% CLA) compared to 1.9 %for birds fed 2.0% CLA diet. Similarly, Takahasi et al. (2002) reported no effect on body weight gain in broilers fed CLA at 3.0% or 10 g/kg. Feeding 5% CLA, Badinga et al. (2003) reported a significant reduction in body weight. Up to 1.0% dietary CLA had no effect on feed consumption. In general, higher levels (>2%) reduced feed consumption in birds.

### Effects on Breast, Thigh and Carcass Fatty Acids

Effect of feeding CLA on meat and carcass fatty acid composition are similar to the results reported in eggs. The incorporation of CLA in edible tissues occurs in a dose–dependant manner. Research in our laboratory showed that feeding broilers 3.5% CLA resulted in a total CLA enrichment of 7.4 and 7.2% in the breast and thigh muscle, respectively (Cherian, in progress research). No difference was found in the total fat content of breast and thigh meat. Feeding 1.5% CLA, Szymczyk et al. (2001) reported a total of 9.35 and 10.3% CLA in breast and leg meat. Du and Ahn (2002a) reported 17.7% total CLA in the breast fillets from birds fed 3.0% CLA. Both isomers were deposited in meat with c9, t11 being the major isomer. Reported fatty acid composition of breast and thigh muscle is shown in Table 3. Considering the total fat content of breast and thigh meat (1.1 and 2.4% for breast and thigh meat, respectively), dietary contribution of CLA through meat is marginal and could vary from 0.17 to 0.20 g for a 100-g skinless thigh piece from hens fed 3.5% CLA. Feeding diets containing 0, 1.5 or 3.0% CLA to broiler birds resulted in total carcass CLA concentrations of 0, 3.3 and 8.3% (Cherian et al., 2003). In all studies reported, feeding CLA to chickens resulted in a significant increase in saturated fat (over 20%) with a concomitant reduction in monounsaturated fatty acids (over 25%) and arachidonic acid (20:4 n-6). In view of the dietary recommendation to decrease saturated fatty acids, consumption of CLA-enriched food products with a high level of saturated fatty acids also has to be considered.

## CLA-RICH BROILER MEAT: ORGANOLEPTIC CHARACTERISTICS

If the health benefits associated with the consumption of CLA-customized poultry foods are to be realized, however, these products must meet sensory expectations of the consumer. Very few studies have reported the sensory effects of CLA-enriched poultry meat. Up to 1% dietary CLA did not produce any effect on the hardness, color, pH and sensory characteristics of broiler meat. However, when dietary levels of CLA were increased to 2 or 3%, there was a significant increase in hardness and the meat was darker in color (Du and Ahn, 2002a). Dietary CLA has been reported to have an antioxidant effect when fed to layer birds (Cherian et al., 2002b). Poultry meat is high in PUFA and low in natural antioxidants. Therefore, poultry meat is susceptible to quality deterioration by lipid oxidation during storage. This results in loss of color, flavor, texture, nutritive value and may lead to the accumulation of lipid oxidation products which are measured through determination of thiobarbituric acid reactive substances (TBARS) and through the volatiles generated during cooking. Du et al. (2002b) observed no difference in volatile profiles of breast fillets harvested from broilers fed 0, 0.25, 0.5, or 1.0% CLA. A reduction in TBARS of breast fillets was also observed by these authors. Du et al. (2002b) investigated the oxidative stability and color of ready to eat turkey fillets prepared from turkeys fed 2% CLA. CLA treatment improved the oxidative stability as measured by TBARS. Feeding CLA lowered the redness and increased the lightness of turkey breast fillets during the entire storage period. Sensory evaluation revealed that feeding CLA did not influence the texture and juiciness of ready to eat turkey fillets. A similar improved storage stability of cooked meat patties from laying hens fed 2.5 or 5% CLA was reported by the same authors (Du et al., 2001). Lipid oxidation is an important problem facing the further-processed poultry muscle food industry in the U.S. A great variety of substances and conditions have been studied to control lipid oxidation. The use of CLA in this aspect warrants further investigation.

## CONCLUSIONS

Functional foods have been introduced as a new concept in enhancing health as consumer's perception of the effect of diet on human health has increased markedly over the last 20 years. Ruminant foods are the primary sources of CLA. However, as Americans are opting for low fat dairy and beef products and choosing more poultry foods, availability of CLA in Western diets may be increased by access to CLA-enriched shell eggs and a variety of other foods such as mayonnaise, egg-based pasta, salad dressings and poultry meat products. Successful marketing of such CLA-modified products however will depend upon further research elucidating the optimum level of CLA in diet for egg/muscle incorporation without causing dramatic changes in saturated and monounsaturated fatty acids as well as sensory characteristics. As consumer preference for animal products are likely to continue, it would be important to modify poultry-based foods including eggs in such a way that the dietary risks are minimized. Further research is needed in stability, aesthetic appeal, sensory and labeling issues to develop specialty poultry-based CLA-modified functional foods.

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Table 1. Reported Values on Conjugated Linoleic Acid Content of CLA-Enriched eggs

| Reference | Total Egg CLA | Calculated CLA/ Average Egg (g)[1] | Percent of CLA supplied/per serving to meet the suggested requirements[2,3] |
|---|---|---|---|
| Du et al. (1999) | 14.8%* | 0.88 | 58 |
| Champruspoller and Sell (1999) | 11.2%* | 0.66 | 44 |
| Ahn et al. (1999) | 8.6%* | 0.51 | 34 |
| Jones et al. (2000) | 12 umol/g fat | 0.15 | 10 |
| Schafer et al. (2001) | 7.75 g/100g** | 0.39 | 26 |
| Cherian et al. (2002) | 5.4%* | 0.32 | 21 |
| Raes et al. (2002) | 5.43 g/100g** | 0.27 | 18 |

* percent of total fatty acids .** g CLA per 100 g total fatty acids

[1]Calculated for an egg weighing 60 g, with a 18 g yolk and 6 g yolk fat.

[2]One serving is two average eggs.

[3] Percent CLA is calculated from 3 g of CLA needed for optimum health from reported animal studies.

Table 2. Reported effects on feeding conjugated linoleic acid to broiler birds

| Reference | Highest Level Fed[1] | Reported bird performance or other responses |
|---|---|---|
| Szymczyk et al. (2001) | 1.5 | Reduced weight gain, feed intake and abdominal fat deposition. Increase in thigh meat yield |
| Du and Ahn (2002) | 3.0 | Reduction in carcass fat. No effect on body weight or abdominal fat |
| Cherian et al. (2003) | 3.5 | No effect on carcass fat, or proximate. Reduced final body weight |
| Takahashi et al. (2003) | 1.0 | No effect on final body weight, feed intake or feed efficiency |
| Badinga et al. (2003) | 5.0 | Slow growth, reduced feed intake and final body weight |
| Du and Ahn (2003) | 3.0 | Increase in liver weight, plasma triglycerides and cholesterol |
| Selvaraj and Cherian (in press) | 1.5 | Reduced final body weight and feed consumption |

[1]Percent diet

Table 3. Reported Values on Conjugated Linoleic Acid Content of Skinless Breast or Thigh meat from Broiler Birds

| Reference | Highest Level Fed[1] (%) | Total Breast Meat CLA[2] (%) | Total Thigh Meat CLA[2] (%) |
|---|---|---|---|
| Szymczyk et al. (2001) | 1.5 | 9.3 | 10.3 |
| Du and Ahn (2002 ) | 3.0 | 17.75 | NR |
| Selvaraj (2002) | 1.5 | 1.0 | 1.1 |
| Cherian et al. (2003) | 3.0 | 7.4 | 7.2 |

[1]Percent diet.
[2]Percent of total fat.
NR= not reported.

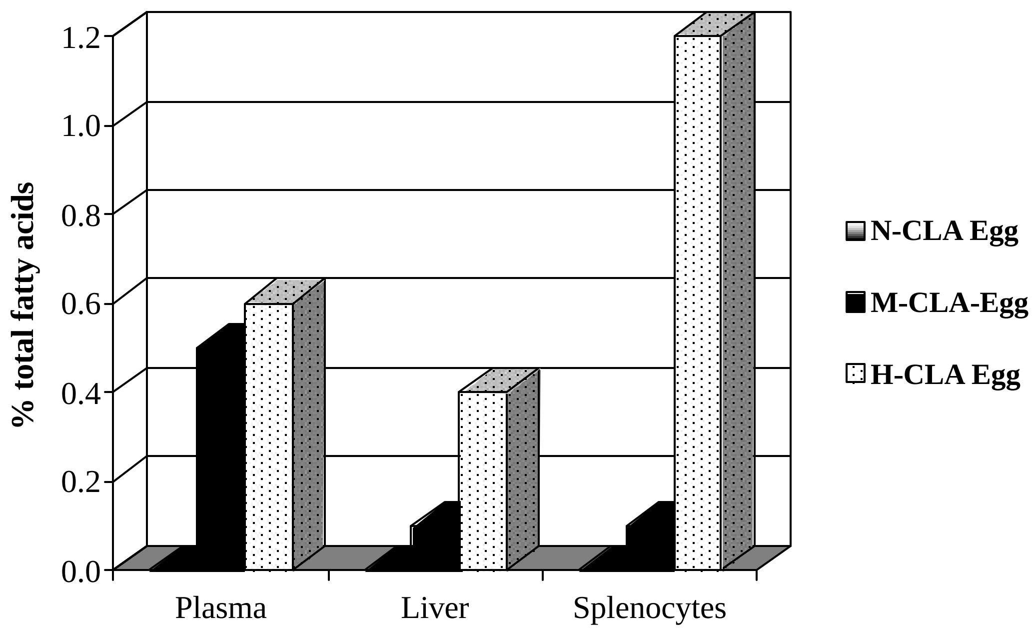


Figure 1. Feeding Conjugated Linoleic Acid (CLA)-Rich Egg Yolk Powder to Rats and Changes in Tissue CLA Concentration. [a-c]Means significantly different ($P<0.05$). Fatty acids reported as percent. N-CLA, L-CLA and H-CLA represents diets containing no, low or high CLA egg yolk powder.