# Enrichment of egg yolk lipids with Conjugated Linoleic Acid (CLA) by feeding bitter melon (*Momordica charantia*) seed fat to layer chickens

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## 1. Introduction

Chicken egg is considered as a main dietary source of protein, fat, and other nutrients in human diet. It is made up of approximately two-thirds white and one-third yolk. The yolk contains lipids, vitamins, minerals and carotenoid pigments (Álvarez et al., 2004).

As consumer demand for health-promoting foods increases, a great interest has been created on production of value-added nutritious animal products such as eggs, milk and meat. Furthermore, scientists as well as industry are also keen to explore novel methods to incorporate health-promoting nutrients into the eggs. One of the successful examples is the creation of eggs enriched with omega-3 fatty acids, which are now available in Sri Lankan market. Thus, incorporation of fatty acids with health promoting effects into the egg yolk lipids has been proved as a feasible task. Conjugated Linoleic acid (CLA), a group of isomers of linoleic acid (18:2, 9c, 11t and 10t, 12c) reported with numerous health benefits such as anti-cancer, anti-obese and anti-diabetic actions by a minute level in our diet. Especially 9c, 11t isomer has been reported to exert anti-cancer properties and 10t, 12c isomer is more potent in anti-obese actions (Cherian et al., 2002).

Natural sources of CLA are found in milk and meat from ruminants. It has been reported that a conjugated trienoic fatty acid named alpha-eleostearic acid (ESA; 18:3: 9c, 11t, 13t), which is present in bitter melon (Karawila; *Momordica charantia*) seeds is metabolized into 9c, 11t isomer of CLA *invivo* and deposited in various tissue lipids in animals such as rats (Jayasooriya, 2000; Jayasooriya, 2017). Thus, this study was designed to determine whether the ESA derived from fat obtained from bitter melon seeds is incorporated into a poultry layer feed is converted in to CLA in bird's body and deposited in egg yolk lipids.

## 2. Materials and Methods

Twenty five weeks (25 weeks) old Hy-line strain white Leghorn laying hens reared on deep litter in a closed house system were selected randomly for the study. Ninety birds (90) (1.296 ± 0.478 kg) were allocated in a completely random design (CRD) with three groups (n=30 per group) and each group consists of replicates (n=10 per replicate). Thus, three experimental groups with three (03) replicates were used for the study. The regular layer ration, which was available commercially was used for the control group (C) without adding any extra fat whereas, 1.5% (w/w) desiccated coconut was added to the layer ration and used as treatment 2 (B). It was used to compare the effects of conventional feed fat source with bitter melon seed

fat on laying hens' production. Furthermore, 1.5 % (w/w) bitter melon seed powder was added to layer ration to incorporate bitter melon fat and was used as the treatment 1 (A). Water was given as *ad libitum* and specific experimental feeds were supplied according to average daily feed consumption (115 g/hen/day). All hens received 16 hours light per day throughout the experimental period. Room temperature was controlled close to $28^0$C. The experiment was conducted for 12 weeks.

Hen house production, egg yolk color, egg weight and the body weights of the birds were measured throughout the experimental period. Fatty acid profiles of lipids extracted from egg yolk using standard methods were analyzed by Gas chromatography. Individual fatty acid peaks including the CLA peak (9c, 11t) were identified using an authentic standard. Proximate analysis of the three feed samples was carried out using standard protocols. The ANOVA with repeated measures was utilized to compare the CLA levels in egg yolk lipids and $P < 0.05$ was considered for the determination of statistical significance. All data were analyzed using statistical software Graph Pad Prism 7.0.

## 3. Results and Discussion

The results revealed (Fig. 1) that at the end of the 12 weeks experimental feeding, there was a cumulative deposition of CLA (CLA 1: cis-9,trans-11) in the egg yolk of the treatment 1 group (A) compared with the control group (C) and the group fed with added desiccated coconut diet (treatment 2: B) . This was also evident in the 6$^{th}$ week and those effects were statistically significant ($P < 0.05$) compared with other dietary groups (B & C). The other CLA isomer (CLA 2: trans-10, cis-12) was not detected in any of the egg yolks of the other 2 groups throughout the experiment.

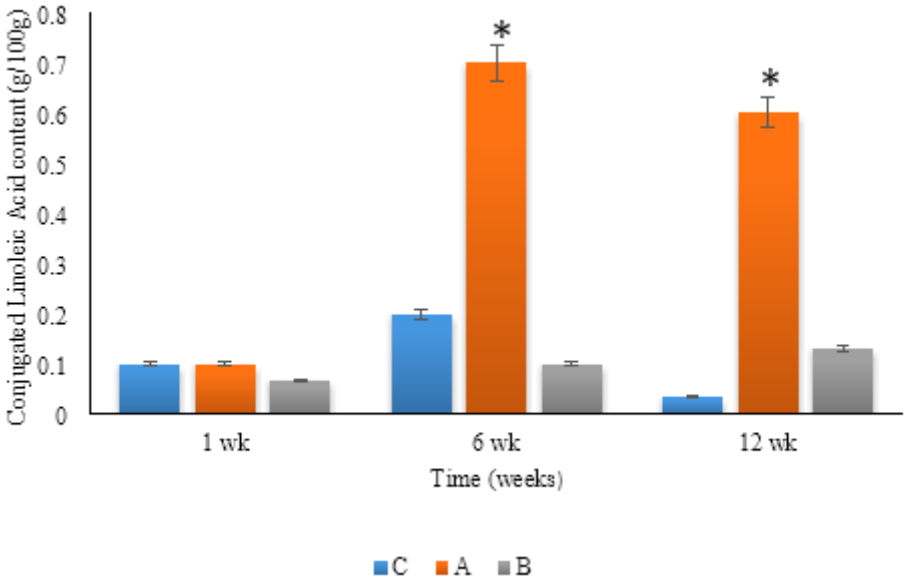


**Figure 1. The amount of CLA accumulated in the egg yolk of three experimental groups at different time points of the feeding trial (A: Treatment 1(Bitter melon seed powder), B: Treatment 2 (Desiccated coconut), C: Control), *P<0.05.**

The egg production and the body weight of the birds were not significantly different among experimental groups. However, the egg weights were reduced with the time and treatment 1 group showed the lowest egg weight at the 12$^{th}$ week, while control group showed the highest weight. Thus, the feed intake of treatment 1 group was reduced and feed conversion ratio was

lower than the control group. The analysis of fatty acid profiles of three feed types showed that neither CLA1 nor CLA2 was present in the experimental feeds.

A previous research conducted has shown that feeding Tung oil that contains 80% ESA at 1% dietary level has resulted in a deposition of CLA in egg yolk lipid and adipose tissue (Lee et al., 2002). These results also clearly indicated the fact that dietary ESA is effectively converted in to CLA and readily deposited in body lipids. Though the ESA in Tung oil readily converted to CLA and deposited in egg yolk lipids, however it could exert toxic effects on human and animals (Lee et al., 2002). In the current study the ESA source is bitter melon seed fat, which contains relatively low level of ESA and nontoxic to animals and human subjects. Thus, it can be considered as a safe ESA source.

It has been reported that the hens fed with 1.6% CLA, egg-laying and feed conversion ratio were lower than the control group (Shang et al., 2004; Xuelan et al., 2017). Furthermore, some other factors such as oestrogen has been shown to promote follicular growth, while cortisol reduces egg production (Zhu 2003). Thus, the negative effect of cortisol which was significantly higher in the 1.6% CLA group might have hindered the positives effect of thyroid hormone and oestrogen (Xuelan et al., 2017). However, Cherian et al (2002) has reported that CLA-supplementation did not affect feed consumption, daily egg production, feed efficiency, or egg weight. The reason for the reduction of egg weight in group A (Treatment 1) could be due to a hormonal change that might have occurred in those birds due to feeding of ESA rich bitter melon seeds. This might be due to the negative effect of cortisol which was significantly higher in the 1.6% group, which offsets the positive effect of thyroid hormone and estradiol (Xuelan et al., 2017).

Overall, the results of this current study reflects the fact that dietary ESA is effectively converted in to CLA and readily deposited in egg yolk lipids in the treatment group. This concept can be effectively used for a value addition to the eggs.

## 4. Conclusions

The alpha-eleostearic acid (ESA) present in locally available bitter melon seed fat added to layer ration (1.5% w/w seed powder is added to the diet) is converted to CLA *in vivo* in poultry layer birds and deposited in egg yolk lipids at a significant level. The current study suggested a method of enrichment of natural CLA into a food item (chicken egg) using a fat source from a locally available vegetable seed (Bitter melon /Karawila; *Momordica charantia*).

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