Conjugated Linoleic Acid (CLA) Enriched Beef Production

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SUMMARY

The identification of the anti-carcinogenic effects of beef extracts due to the presence of conjugated linoleic acid (CLA) has heightened interest in increasing the amount of CLA deposited in beef. Beef cattle can produce CLA and deposit these compounds in the meat thus beef consumers can receive bio-formed CLA. Beef contains both the bio-active CLA isomers, namely, CLA *cis 9, trans 11* and CLA *trans 10, cis12* unlike milk. The relative content of these CLA isomers in beef depends upon the feeds consumed by the animals during production. Feeding cattle linoleic acid rich-oils for extended periods of time increases the CLA content of beef. Depending upon the type and relative maturity of the pasture, beef from pastured, rather than from grain or silage fed cattle had higher CLA content. In feedlot animals fed high grain diets, inclusion of dietary oil along with hay during, both the growth and finishing phases led to an increase in CLA content from 2.8 to 14 mg g^{-1} of beef fat, which would provide 90 mg CLA per 100 g serving of beef. The CLA appear to be concentrated in intra-muscular and subcutaneous fat of beef cattle, with the CLA *trans 10, cis 12* being greater in the subcutaneous fat and is at greater concentrations in cattle fed higher proportions of grain than cattle fed lower proportions of grain. Culture of bovine adipo-fibroblasts with media containing the CLA isomers separately indicated that the CLA *cis 9, trans 11* was anti-proliferative especially when included at levels of 70 mg/L. Can it be an energy partitioning agent?

INTRODUCTION

The formation of conjugated dienes in the rumen during bio-hydrogenation of lipids in feed had been observed previously (Kepler et al., 1966). However, the anti-carcinogenic effect of beef extracts was first demonstrated by Pariza et al. (1979) and Pariza and Hargraves (1985). It was later identified that the anti-carcinogenic effect was due to the presence of conjugated linoleic acid (CLA) in the beef extracts. It is now known that many such conjugated dienes are formed in the rumen, depending upon the fat content of the diet and the fatty acid composition of the fat in the feed. These bio-

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converted fatty acids are deposited in the tissues and can be available to consumers in ruminant products such as milk and beef. These CLA appear to have the ability to influence the metabolism in animal models which can be beneficial in humans to control a wide range of metabolic disorders if similar effects are observed in humans and thus provide health benefits to consumers. In animal models dietary CLAs have induced many positive effects (Azain, 2003) such as anti-carcinogenesis in mice (Vanden Heuvel 1999), enhancement of immunity in mice (Hayek et al., 1999), alleviation of allergies and asthma (Cook et al., 2000), decrease in blood cholesterol in hamsters (Nicolosi et al., 1997), thus it is an anti-atherosclerotic in rabbits (Kritchevsky et al., 2000), decrease obesity in mice (Delany et al., 1999) and enhance insulin sensitivity in Zucker obese rats (Houseknecht et al., 1998). These effects appear to be mediated by two isomers, the CLA *cis 9, trans 11* and the CLA *trans 10, cis 12,* which are now recognized as having biological activity.

CLA IN MILK

Research has indicated that the CLA *cis 9, trans 11* is found in milk and beef in larger amounts than the CLA *trans 10; cis 12* (Bauman et al., 2000; Dhiman et al., 2000; AbuGhazela et al. 2003). The CLA *cis 9,trans 11* is more abundant because there appear to be two routes of formation of this fatty acid, one a ruminal route and a second route, where the precursor fatty acid - trans vaccenic acid (C*18:1 trans 11*) is converted to CLA *cis 9, trans 11* by oxidation (removal of two hydrogens) at the 9^{th} carbon of the fatty acid by the enzyme $?^{9}$ desaturase, present in the mammary gland and perhaps in the muscle (Santora et al., 2000; Corl et al., 2001). As a result, under appropriate feeding management the yield of CLA from milk can be as high as 16 g/d because concentration of CLA can be elevated to an average of 20 mg/g of fat in milk (Dhiman et al. 2000) by feeding appropriate oil supplements in the diet.

CLA IN BEEF

Unlike milk, beef contains both the biologically active isomers of CLA. Although very small amounts of CLA *trans 10, cis 12* occurs in beef there is a greater opportunity for this compound to be found in beef than in milk, because it is a rumen product (Kucuk et al., 2001). Fattening cattle fed a high proportion of grain with soybean oil (Beaulieu et al., 2002) or high oil corn (Duckett et al., 2002) had higher levels of CLA *trans10, cis 12* than animals not provided dietary oil. A linear increase in CLA *trans10, cis 12* was observed with increasing dietary soybean oil (Beaulieu et al., 2002). However, the amount of the CLAs found in milk and meat is small and the current daily consumption in humans ranges between 300 mg (Riserus et al., 2001) to 1g per day (Ha et al., 1989). The current CLA consumption is substantially lower than the recommended daily intake for appreciation of health benefits in humans, which is 3500 mg/d (Ha et al., 1989). The potential to increase the CLA content of ruminant foods is not unlimited and the ability to achieve the recommended intakes from foods may be challenging. However, the recommended daily intake has been revised to range between 2.5 to 5 g/d (Azain, 2003). Moloney et al. (2001) have summarized the CLA concentration in beef fat observed by different researchers and the values range from 1.2 to 12.9 mg/g of fat (Table 1). Some

other observed values have been added to the list provided by Moloney et al. (2001). This variation in concentration of CLA is dependent on the system employed for beef production and the diet employed to finish the animals. The factors that can affect the CLA concentration in beef are related to whether animals are pasture or feedlot finished, oil or oilseed in diet, the fatty acid composition of the oil and the other dietary components in the feed such as proportion of grain, silage and hay.

Rule et al. (2002) compared CLA concentrations in three different muscles of pasture or feedlot finished cattle and found that CLA concentrations of both isomers in fat extracted from muscle was greater in range finished cattle in the longissimus and supraspinatus muscles, but not in the semitendinosis muscle. The CLA concentrations differed among the muscles investigated. However, total fatty acid concentration of range finished cattle was generally lower than that of feedlot finished cattle. Therefore, when CLA content of the beef as consumed by the consumer was calculated the differences were less pronounced between pasture and feedlot finished cattle; indicating the importance of considering the net CLA content of beef than merely the concentration per unit weight of fat.

Duynisveld et al. (2002) found that the CLA content of beef (mg/100 mg meat) from pasture finished cattle was greater than from those fed preserved silage/grain (60:40) based diets, which concurs with earlier observations (French et al., 2000; Rule et al., 2002). However, Duynisveld et al. (2002) found that the provision of a supplement of crushed soybean for the pasture finished cattle further increased the CLA and the total fat content of the meat. Provision of oil supplements has not always resulted in substantial increase in CLA concentration in muscle (Beaulieu et al., 2002; Madron et al., 2002) especially that of CLA *cis 9, trans 11*. Griswold et al. (2003) reported a decrease in CLA *cis 9, trans 11* in lean beef from cattle fed 4% soybean oil through the finishing phase in 72% corn diets. Even when cattle were finished on a diet with a forage to concentrate ratio of 60:40, no change in CLA *cis 9 trans 11* concentration in fat from meat was observed when soybean oil was provided at 4 or 8% of diet DM. However the forage used was corn silage. The absence of a response in CLA concentrations appears to be related to the ruminal response of the animal to the feed provided to the animal. Using *in vitro* batch and continuous fermentation techniques, Martin and Jenkins (2002) found that concentrations of C18:1 *trans 11* and CLA *cis 9, trans 11* were higher when the continuous cultures contained at least 1.0 g/L soluble carbohydrates, an out flow rate of 0.1/h, and an extra cellular pH greater than 5.5. Decreasing the pH to 5.0 eliminated the production of C18:1 *trans 11* and CLA *cis 9, trans 11*. Indicating that at low pH and at low ruminal passage rates, as can be expected in feedlot cattle fed high grain diets, the production of CLA can be limited or curtailed. Thus only marginal increases in beef CLA content have been reported in cattle fed oil-containing diets.

Provision of dietary sunflower oil at 0, 3 or 6% in beef cattle finishing diets (80% barley, 20% barley silage) increased the CLA concentration in beef fat by 75% (Table 2) when cattle received the 6% sunflower oil diet. But the content of CLA in beef was increased by 100% from 10.5 to 19.5 mg for the animals fed the 0 and 6% sunflower oil (Mir et al., 2003a) respectively, because a marginal increase in fat content also occurred

concomitantly. Increases of similar magnitude in CLA concentration in fat from muscle have been reported in lambs fed barley grain and barley silage based diets (Ivan et al., 2001). In contrast, when weaned lambs were fed a 1:1 barley grain: alfalfa pellet with safflower oil at 6% of diet DM the CLA deposited in diaphragm, leg muscle, adipose and liver was increased by two to four fold (Table 3, Mir et al., 2000).

Although supplementation with oil or oilseed increases CLA content in muscle, the inclusion of oil or oil seeds rich in linoleic acid, such as safflower or sunflower, in the diet of ruminants, appears to be most effective (Casutt et al., 2000). In cattle, the impact of sunflower oil supplementation at 6% of diet DM on CLA concentration in muscle fat was determined in steers fed diets containing barley grain and pea hay (Mir et al., 2002). The progressive deposition of CLA was monitored in biopsies from the semitendinosis muscle of these steers through the growth period. These biopsies were obtained a month after initiation of feeding of the weaned steers and prior to initiation of the finishing diet where the grain component of the diet was increased substantially (from 35 to 78% of total diet DM). Finally upon slaughter a sample of the muscle was procured for determination of the CLA concentration. The provision of dietary oil increased the CLA concentration in the muscle and the values were 4.5 and 10.4 mg/g fat in the first biopsy and 4.1 and 16.3 mg/g of fat in the second biopsy in control and oil fed steers, respectively (Mir et al., 2001). The CLA concentration in the muscle at slaughter was 2.1 and 14.8 mg/g fat for control and sunflower oil fed steers, respectively.

As indicated earlier, aside from the concentration of CLA the total fat content of the muscle will affect the net available CLA for the consumer. The study described previously was conducted at Washington State University with steers from three breed types namely: the Wagyu (Japanese - high marbling beef breed), Wagyu x Limousin crossbred and Limousin breeds. As expected the fat content of the muscle of the Wagyu steers was greater than that of the crossbred steers, which was greater than that of the Limousin steers (Mir et al., 2002). Since the CLA concentration was increased to the same extent in all breeds the animals that had higher fat content had higher levels of CLA per 100 g of meat (Table 4).

Further experiments have been initiated at the Lethbridge and Lacombe Research Centres in Alberta with funding from the members of Alberta Agriculture Research Institute, the Matching Investment Initiative of Agriculture and Agri-Food Canada and Pioneer Hi-Bred. The first experiment is to determine the effect of sunflower seed provided at 15% of diet DM during the finishing phase on CLA concentrations when other aspects of the feed were altered. The CLA concentrations for muscle samples obtained from the Chuck of the carcasses are provided (Table 5). The CLA concentrations are higher than commonly observed in barley fed cattle because a polar solvent system (hexane: isopropanol; 10:14) was used to extract the fat. Thus phospholipids are successfully extracted by this solvent system and it can be expected that CLA being an unsaturated fatty acid could be included in membrane phospholipids to a greater extent than into storage lipids. However, despite the alterations in the feed components the increase in CLA concentrations were small and total available CLA was

increased by 56% only, quite unlike what was observed previously (Mir et al., 2002) and probable reasons for the differences have been itemized in Table 6.

The rib primal cut from these animals was saved for determination of meat quality and beef shelf life in retail display. Further experiments have been planned to determine the effect of provision of sunflower seeds to pasturing cattle on CLA concentration if processed straight from pasture or after a 100-d finishing period with and without sunflower seed supplements.

CLA AND MEAT QUALITY AND POSSIBLE EFFECT IN CONSUMERS

There have been concerns that the feeding oil with unsaturated fatty acids to meat producing animals can affect meat quality. It has been suggested that CLA may have anti-oxidant properties (Ha et al., 1990). Although this claim is disputed, it is recognized that CLA does inhibit the production of lipid peroxidation products. Inclusion of synthetic CLA at 0, 0.25 and 0.5% of diet DM in rabbits for 49 d enhanced the oxidative stability of the meat linearly, as indicated by diminished amounts of thibarituric acid reactive substances (Corino et al., 2002), which concurs with the observation that dietary CLA treatment reduced lipid oxidation in raw chicken during storage (Du and Ahn, 2002). The impact of increasing CLA via inclusion of 6% oil in the diet on the shelf life of beef was examined. It was found that even after four days of exposure in the retail case the deepening of the color was restricted (Mir et al., 2003a) and meat from steers fed 6% sunflower oil maintained better retail acceptability scores than beef from control or steers fed only 3% sunflower oil. Dietary oil did not appear to affect the tenderness or palatability scores of the beef (Mir et al., 2003a). Duynisveld et al. (2002) also failed to find any differences in acceptability of beef from pasture fed versus silage and grain fed steers and they suggest that the absence of differences is due to the elevated CLA content of the meat.

It was hypothesized that fatty acids esterified to the sn-2 position of the triglyceride would be retained preferentially and perhaps participate in providing protection against lipid peroxidation. Investigation into the location at which the CLA occurred on the triglyceride seemed to indicate that they were esterified at the sn-2 position (Mir et al., 2003a). The digestion, absorption and ultimate metabolic fate of fatty acids in this position has been suggested to be at variance from that of fatty acids in the sn 1/3 position. Provision of meat from animals that had relatively higher concentration of CLA to weaned rats caused the adipocyte number to be decreased in both the retroperitoneal and inguinal fat pads relative to that in rats fed meat with low CLA concentrations (Mir et al 2003b). Differences in adipocyte number between rats fed the CLA enriched meat diets or a diet with synthetic CLA included at 1.1% of diet DM were not significant (Table 7). But only inclusion of synthetic CLA led to decreases in retroperitoneal fat pad weight and concurs with published reports (Azain et al., 2000; Poulous et al., 2001). The effectiveness of low concentrations of bio-formed CLA in decreasing adipocyte number in comparison to the synthetic CLA is intriguing and needs further investigation.

The effect of incorporation of CLA isomers separately into culture media of 3T3L1 mouse adipocytes at concentrations of 10 or 70 mg/L at various stages of development was investigated. The inclusion of CLA *cis 9, trans 11* during the proliferation stage decreased cell numbers, but when the same isomer was included during the lipid accumulation stage only, lipid accumulation rate as C16:0 increase per 10,000 cells was increased (Figure 1; He et al., 2003a,b). While inclusion of the CLA *trans 10, cis 12* was found not to affect proliferation, but strongly decreased lipid accumulation rate (Table 8). These results suggest that the CLA enriched beef provided the young weanling rats adequate amounts of CLA *cis 9, trans 11* to limit the adipocyte hyperplasia but not sufficient CLA *trans 10, cis 12* to arrest lipid accumulation. Similarly when the treatments used in cell culture of the 3T3L1 cells were applied to bovine visceral or peri-muscular adipofibroblasts the CLA *cis 9, trans 11* was found to be strongly anti-proliferative relative to CLA *trans 10, cis 12* (Unpublished data). The effectiveness of these compounds on adipocyte development appears to be related to stage of development of the adipo-fibroblasts. If the CLA are provided at the appropriate developmental stage there may be advantages to be appreciated in terms of redirecting energy pathways in the animal.

In general, CLA concentrations in beef can be increased, yet the extent of increase may not be sufficient to meet current recommended dietary intakes. The suggested intake of CLA is based on extrapolation of intakes of the synthetic free fatty acid compounds in animal models to observe particular effects. It is possible that these suggestions have to be revised after taking into account consumption over a lifetime as opposed to set, short duration experiments. One has to consider the extent of benefit that would be sacrificed by consuming CLA at levels lower than the current recommended intakes. If modest CLA intake imparts benefits greater than no CLA consumption at all, then there are many advantages to producing foods such as milk and beef with enhanced levels of bio-formed CLA because the consumption of these foods is high and expanding in the world.

INCREASING CLA IN BEEF

In order to increase the CLA yield in beef, it is essential to provide cattle an appropriate substrate for formation of CLA. The provision of a source of dietary linoleic acid appears to increase the CLA concentration to the greatest extent. Dietary forage as grass or legume hay appears to facilitate the establishment of the microflora that enhances the formation and deposition of CLA in the tissues. The provision of modest amounts of grain is more conducive to CLA synthesis rather than high levels of grain. Certain breeds of cattle that have a tendency to deposit high amounts of fat in muscle will deliver a greater amount of CLA to the consumer.

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Table 1. CLA concentrations in uncooked beef

Diet	Country	CLA concentration mg/g fat	Reference
Unknown	Canada	1.2-3.0	Ma et al. (1999)
Barley(800g/kg diet)	Canada	1.7-1.8	Mir et al. (2000)
Grass silage & conc.	UK	3.2-8.0	Enser et al. (1999)
Maize (820g/kg diet)	USA	3.9-4.9	McGuire et al. (1998)
Unknown	USA	2.9-4.3	Chin et al. (1992)
Unknown	USA	1.7-5.5	Shantha, et al.(1994)
Grain	USA	5.1	Shantha et al. (1997)
Concentrate	Japan	3.4	Tsuneishi et al. (1999)
Grass	USA	7.4	Shantha et al. (1997)
Grass (?)	Australia	2.3-12.5	Fogerty et al. (1988)
Grass	Ireland	3.7-10.8	French et al. (2000)
Unknown	Germany	1.2-12.0	Fritsche et al. (1998)
Corn + extruded soybeans	USA	6.6-7.8	Madron et al.(2002)
Range	USA	3.5-5.6	Rule et al. (2002)
Feedlot	USA	2.9 - 3.2	Rule et al.(2002)
Feedlot + soybeans	USA	3.2 - 3.6	Beaulieu et al. (2002)
Feedlot + soybean oil	USA	2.5 - 3.1	Griswold et al. (2003)
Feedlot + sunflower oil	USA	2.7-12.9	Mir et al. (2002)
Feedlot + sunflower oil	Canada	2.0-3.5	Mir et al. (2003)

Table 2. Effect of feeding sunflower oil during fattening phase of beef CLA content.

	Sunflower oil (% of diet DM)			
Item	Control	3%	6%	SEM
Fat (% of DM)	15.0	16.8	15.9	0.72
CLA (mg/g fat)	2.0[a]	2.6[b]	3.5[c]	0.17
CLA mg/100g beef (As is - 65% moisture)[1]	10.5	15.3	19.5	-

Abstracted from Mir et al. (2003a)

[a,b,c]Means without the same superscript in a row differ significantly ($P < 0.05$).

[1]calculated from mean values

Animals were not implanted, and not provided ionophores or antibiotics.

Table 3. Effect of dietary supplementation with safflower oil (SAFF) on CLA content (mg/g fat) in various lamb tissues.

Tissue	Control	SAFF	SEM
Muscle			
Diaphragm	0.64[a]	2.60[b]	0.173
Leg	1.78[a]	4.41[b]	0.432
Adipose	2.77[a]	7.33[b]	0.232
Liver	1.72[a]	3.53[b]	0.349

[a,b]Means without the same superscript in a row differ significantly ($P < 0.05$).
Mir et al. (2000).

Table 4. Breed effects on fat (%) and CLA content (mg/g fat) of beef longissimus muscle of steers fed control or oil-containing (6% sunflower oil of DM).

	Wagyu		Wagyu x Limousin		Limousin		
	Control	Oil	Control	Oil	Control	Oil	SEM
Fat content (% DM)	26.3	29.8	18.7	18.4	7.3	9.3	1.25
Fat content (as is 65% moisture)[1]	9.2	10.4	6.5	6.4	4.2	4.8	-
CLA content							
mg CLA/100 g	2.7	12.9	2.8	11.9	2.9	12.2	0.13
beef (as is)[1]	25	134	18	76	12	59	-

[1]calculated from mean values.
Mir et al. (2002).
Animals were not implanted, and not provided ionophores or antibiotics.

Table 5. Lipid (%) and CLA concentration (mg/g fat) in samples from Chuck of carcasses from animals provided with 15% sunflower seeds (SS) in various finishing diets.

	Diet					
Item	84% barley +15% silage	69% barley +30% SS & alfalfa hay pellet	69% barley +15% SS +15% silage	69% barley + 15% SS + 15% silage and hay	69% barley + 15%SS + 15% alfalfa hay	84% barley + 15% SS
Initial weight, kg	429	426	423	417	426	435
Final weight, kg	606	567	589	582	577	573
DMI, kg	9.3	8.9	8.7	9.2	9.1	7.9
ADG, kg	1.14	0.91	1.07	1.06	0.97	0.90
FE (kg feed/ kg gain)	8.2	9.8	8.1	8.7	9.4	8.8
Carcass weight kg	354	335	344	336	336	326
Fat in chuck (%)	1.55	1.63	1.64	1.36	1.85	1.93
CLA c 9, t 11 (% fat)	0.47	0.48	0.62	0.34	0.44	0.56
CLA t 10,c 12(% fat)	-	-	0.02	0.01	0.05	0.03
Total mg CLA/ 100g beef	7.3	7.8	10.2	4.8	9.1	11.4

c - cis, t – trans
n=8
Animals were not implanted, and not provided ionophores or antibiotics.

Table 6. Factors affecting CLA concentration

Factors	Mir et al. (2002)	Current Study
1. Cattle	Weaned steer calves	Yearling steers
2. Duration of study	259days	155 days
3. Oil source	Sunflower oil	Sunflower seed
4. Forage	Pea hay	Alfalfa hay
5. Muscle fat analyzed	Longissimus and Semitendinosis	From chuck in carcass
6. Grain proportion in diet	29% in backgrounding diet and 74% in finishing diet	69 and 84% in finishing diets

Table 7. Adipocyte number in retroperitoneal (P) and inguinal (I) fat pads of rats fed synthetic CLA or meat containing diets.

Treatment	P – pad weight (g)	P – cell number (10^6/mg)	I pad weight (g)	I-pad cell number (10^6/mg)
Casein	5.7[a]	0.98[b]	6.7	0.95[c]
Control meat	7.1[a]	2.30[a]	7.9	2.51[a]
CLA meat	6.9[a]	1.19[b]	7.7	1.46[b]
Casein+SCLA	3.5[b]	1.06[b]	6.0	1.04[bc]
SEM	0.48	0.16	0.58	0.16

SCLA- synthetic CLA incorporated into rat diets at 1.1% of dit DM
CLA -meat - produced by feeding steers sunflower oil at 6% of diet DM. The rat diet contained substantially lower concentration (0.05%) of CLA than the Casein +SCLA diet
[a,b,c]Means without the same superscript in a column differ significantly ($P < 0.05$).
Mir et al. (2003b)

Table 8. Effect of CLA $_{c9,\ t11}$ and CLA $_{c10,\ t12}$ isomers on proliferation of 3T3-L1 preadipocytes – absorbance at 420 nm and cell number counted on a hemocytometer (Experiment 1).

Group	LA or CLA (mg L^{-1})	Treatment	Absorbance	Cell number ($\times 10^4 well^{-1}$)
1	0	Control (-EtOH)	0.054[c]	2.78[d]
2	0	Control (+EtOH)	0.064[d]	2.68[d]
3	10	LA	0.050[c]	2.43[cd]
4	10	CLA $_{c9,t11}$	0.051[c]	2.21[c]
5	10	CLA $_{t10,c12}$	0.066[d]	2.15[c]
6	70	LA	0.059[cd]	2.01[c]
7	70	CLA $_{c9,t11}$	0.0001[a]	0.16[a]
8	70	CLA $_{t10,c12}$	0.017[b]	0.83[b]
SEM			0.003	0.12
P-value			0.001	0.001

[a-d]Means without the same superscript in a column differ significantly ($P < 0.05$).
For absorbance n=8, for cell number n=6.

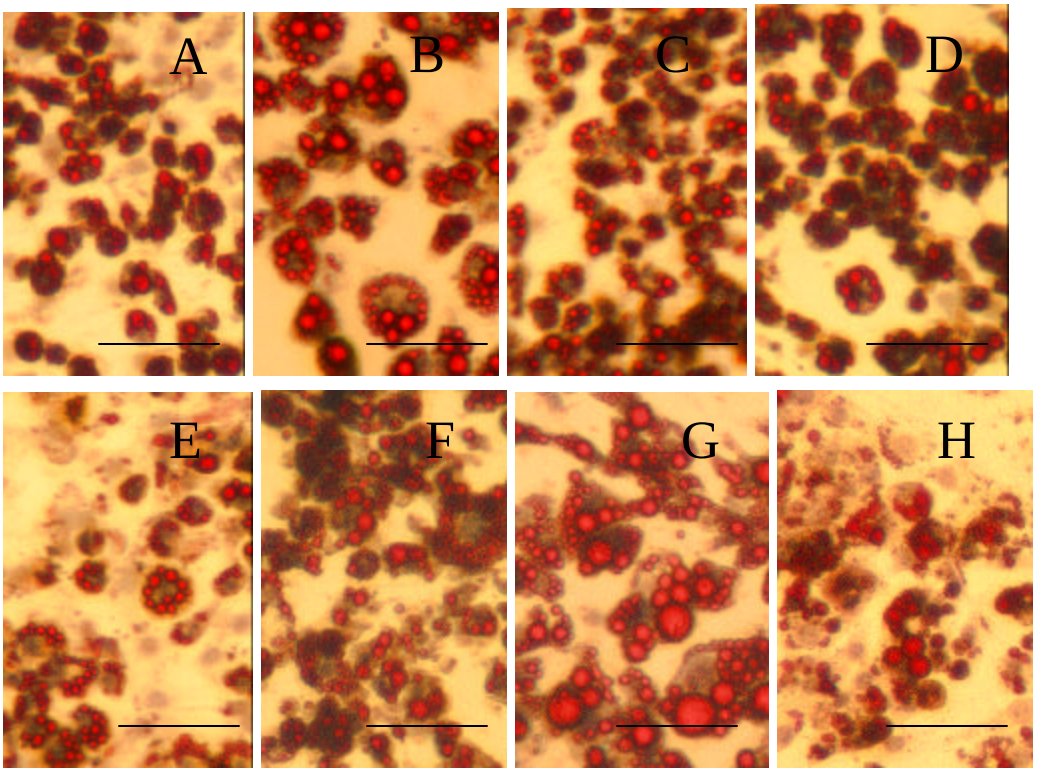

Figure 1. 3T3-L1 adipocytes cultured with media supplemented with or without CLA during an overall proliferation, differentiation and lipid accumulation period for 12 d in Experiment 3: A. Con (-EtOH), B. Con (+EtOH), C. 10 mg L^{-1} LA, D. 10 mg L^{-1} CLA $_{c9, t11}$, E. 10 mg L^{-1} CLA $_{t10, c12}$, F. 70 mg L^{-1} LA, G. 70 mg L^{-1} CLA $_{c9, t11}$ and H. 70 mg L^{-1} CLA t10, c12. Bar = 50 µm.