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# Comparison between conjugated linoleic acid and essential fatty acids in preventing oxidative stress in bovine mammary epithelial cells

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## ABSTRACT

Some in vitro and in vivo studies have demonstrated protective effects of conjugated linoleic acid (CLA) isomers against oxidative stress and lipid peroxidation. However, only a few and conflicting studies have been conducted showing the antioxidant potential of essential fatty acids. The objectives of the study were to compare the effects of CLA to other essential fatty acids on the thiol redox status of bovine mammary epithelia cells (BME-UV1) and their protective role against oxidative damage on the mammary gland by an in vitro study. The BME-UV1 cells were treated with complete medium containing 50 μ*M* of *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, α-linolenic acid, γ-linolenic acid, and linoleic acid. To assess the cellular antioxidant response, glutathione, NADPH, and γ-glutamyl-cysteine ligase activity were measured 48 h after addition of fatty acids (FA). Intracellular reactive oxygen species and malondialdehyde production were also assessed in cells supplemented with FA. Reactive oxygen species production after 3 h of $H_2O_2$ exposure was assessed to evaluate and to compare the potential protection of different FA against $H_2O_2$-induced oxidative stress. All FA treatments induced an intracellular GSH increase, matched by high concentrations of NADPH and an increase of γ-glutamyl-cysteine ligase activity. Cells supplemented with FA showed a reduction in intracellular malondialdehyde levels. In particular, CLA isomers and linoleic acid supplementation showed a better antioxidant cellular response against oxidative damage induced by $H_2O_2$ compared with other FA.
**Key words:** conjugated linoleic acid, essential fatty acids, oxidative status, bovine mammary cells

## INTRODUCTION

Lipids contained in dietary fat are known to be an excellent source of energy, and studies undertaken in the first quarter of the 20th century demonstrated that they were necessary for growth and normal physiological function (Spector and Kim, 2015). In recent years, interest has grown in the health properties of functional fatty acids, such as long-chain n-3 and n-6 fatty acids and CLA, because of their biological roles in cells (Bauman and Lock, 2006; Lunn and Theobald, 2006). Omega-6 and n-3 fatty acids are regarded as essential fatty acids (**EFA**) because mammals cannot synthesize them and they must be obtained from diet. Essential fatty acids form an important constituent of all cell membranes and confer to membranes properties of fluidity, thus determining and influencing the behavior of membrane-bound enzymes and receptors (Das, 2006). Among n-6 fatty acids, linoleic acid (**LnA**; 18:2n-6) is the most common n-6 PUFA, whereas α-linoleic acid (**aLnA**), belonging to *cis* n-3 PUFA, is the most prevalent n-3 fatty acid. Linoleic acid is often found in nature and is present in the seeds of most plants, except for coconut, cocoa, and palm. α-Linoleic acid, on the other hand, is found in the chloroplasts of green leafy vegetables and in the seeds of flax, rape, chia, perilla, and in walnuts (Lunn and Theobald, 2006; Simopoulos, 2008). It is from these 2 parent EFA that the n-3 and n-6 fatty acid families are derived through a series of enzyme-catalyzed desaturation and elongation reactions, which generally take place in the cell cytosol or in the mitochondria. α-Linoleic acid is metabolized to docosahexaenoic acid (22:6n-3) via eicosapentaenoic acid (**EPA**; 20:5n-3) and docosapentaenoic acid (22:5n-3), whereas LnA is metabolized to arachidonic acid (**ArA**; 20:4n-6) via γ-linolenic acid (**gLnA**; 18:3n-6) or eicosadienoic acid (20:2n-6), as 2 pathways are active (Lunn and Theobald, 2006). Arachidonic acid, the derivative of LnA, can be converted into the 2-series of thromboxanes and the 4-series of leukotrienes. These are very important, active, and short-lived hormones termed eicosanoids, which are involved in various patho-physiological processes concerning inflammatory conditions such as atherosclerosis, obesity, and inflammatory bowel disease. In contrast, the aLnA derivate, such as EPA, gives rise to an entirely different set of eicosanoids. These are the 3-series prostaglandins and

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thromboxanes and the 5-series leukotrienes, which are considered to be less inflammatory or even anti-inflammatory in comparison to the eicosanoid family derived from ArA (Patterson et al., 2012).

In dairy cows, it has been observed that dietary LnA and aLnA supplementation can be a nutritional strategy to improve reproductive performance and increase the percentage of pregnancies in lactating cows (Dirandeh et al., 2013). This is because EFA can stimulate the follicular growth and steroid production (Leroy et al., 2013). Furthermore, LnA and aLnA are able to modulate the inflammation and the immune response in dairy cows (Lessard et al., 2003; Caldari-Torres et al., 2011).

Conjugated linoleic acids are a group of constitution and conformation isomers of LnA that are formed in the course of biohydrogenation in the rumen and later endogenous conversion from vaccenic acid (Schmid et al., 2006; Bauman et al., 2008). The isomers that contain a double bond in the *trans* configuration are biologically active. The most studied bioactive CLA are the *cis*-9,*trans*-11 isomer and the *trans*-10,*cis*-12 isomer. The role of CLA on health has been investigated in animal and human studies (Pires and Grummer 2008; Mele et al., 2013). It was observed that CLA might play an important protective role in cancer, cardiovascular diseases, obesity, osteoporosis, and in immune and inflammatory responses (Benjamin and Spener, 2009; Moraes et al., 2012; Oliveira et al., 2012; Du et al., 2014).

Ruminants have evolved to feed on fresh grass and leaves. According to their feed preferences, Hofmann and Stewart (1972) classified them as concentrate selectors, intermediate-mixed feeders, or grass and roughage eaters. The dairy cow belongs to the group of grazer or roughage eaters, such as *Bos primigenius* (aurochs), relying on grasses and roughage. Modern TMR diets differ significantly from the natural diet of a cow (e.g., in its ruminal production of CLA). Elgersma et al. (2004) showed that milk from cows feeding on fresh grass quickly decreases in CLA when the diet was changed to the more modern silage or concentrate TMR type. In a review, Elgersma et al. (2006) concluded, in general, that cows on fresh grass (e.g., pasture) produce milk with a significant higher level of CLA. This observed difference in CLA content in milk triggered extensive research into increasing CLA levels in milk from TMR-fed cows, with the focus being on human health. However, these studies (Elgersma et al., 2004, 2006) triggered only limited interest considering CLA as a nutrient and the wellbeing of the cow in the first place. Studies on farm animals showed that some CLA isomers decrease milk fat synthesis, and may thus improve energy balance and alleviate energy demands in lactating dairy cattle. It has been routinely observed that CLA reduce milk fat percentage, increase milk yield, improve reproduction in early lactation, reduce incidence of metabolic disorders during early lactation (ketosis), and reduce negative effects of inflammatory processes during the periparturient period (Bauman et al., 2008; Galamb et al., 2016). Some studies have shown the protective effects of CLA isomers against oxidative stress and lipid peroxidation in animal models (Andreoli et al., 2010; Chinnadurai et al., 2013). Gessner et al. (2015) in cows, and in dairy ewes, Zeitz et al. (2015) demonstrated significantly higher vitamin E and A concentrations in milk from CLA-supplemented animals. Changes in the oxidative metabolism occur in the transition period of dairy cows, and several studies have suggested that oxidative stress increases the susceptibility of dairy cattle to diseases (Bernabucci et al., 2005; Castillo et al., 2005; Sordillo and Aitken, 2009). Nevertheless, results regarding the effects of supplementing antioxidants (vitamins and trace elements) on dairy cow health and performance have been inconsistent, because, in most cases, the antioxidant potential of the animals was not assessed beforehand and the nutritional strategies were not planned accordingly (Abuelo et al., 2015). Therefore, reviewing the redox balance in dairy cattle could help establish new nutritional strategies that could improve transition cow health.

Our recent study (Basiricò et al., 2015) showed that CLA isomers have an antioxidant role by developing a significantly high redox status in bovine mammary cells. Only a few and contradictory reports have been published regarding the protective potential of aLnA, LnA, and gLnA (Arab et al., 2006b). Fagali and Catalá (2008) demonstrated in an in vitro study that CLA exhibited greater free radical quenching activity than LnA or aLnA. Therefore, the aim of the current study was to compare the effects of CLA with other EFA on the thiol redox status of cells and their protective activity against oxidative damage on mammary gland by an in vitro model based on bovine mammary epithelial cells (BME-UV1).

## MATERIALS AND METHODS

### *BME-UV1 Culture Conditions*

The BME-UV1 cell line was created at the University of Vermont from primary bovine mammary epithelial cells in culture by stable transfection with SV40 large T-antigen; BME-UV1 cells were provided by Antonella Baldi (Department FENS, University of Milan, Italy). Cells were routinely cultivated into 75-cm$^2$ tissue culture flasks (Costar, Corning, NY), in a mixture of 50% DMEM-F12, 30% RPMI-1640, and 20% NCTC-135