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Anti-inflammatory effects of conjugated linoleic acid isomers and essential fatty acids in bovine mammary epithelial cells

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Fatty acids are important modulators of inflammatory responses, in particular, n-3 and n-6 essential fatty acids and CLA have received particular attention for their ability to modulate inflammation. The objectives of this study were to compare the effects of CLA and essential fatty acids on the expression of pro and anti- inflammatory cytokines and their protective efficacy against inflammatory status in mammary gland by an in vitro *model based on bovine mammary epithelial cells (BME-UV1). Bovine mammary epithelial cells were treated with complete medium containing either 50 μM of* cis-*9,* trans-*11 CLA (*c*9,*t*11 CLA) or* trans-10, cis-*12 CLA (*t*10,*c*12 CLA) or (α)-linolenic acid (aLnA) or (γ)-linolenic acid (gLnA) or linoleic acid (LA). After 48 h by fatty acids administration the cells were treated for 3 h with 20 μM of lipopolysaccharide (LPS) to induce inflammatory stimulus. Reactive oxygen species (ROS) production after treatments was assessed to verify and to compare the potential protection of different fatty acids against LPS-induced oxidative stress. The messenger RNA abundance of bovine pro and anti-inflammatory cytokines (tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and interleukine-10 (IL-10)) and peroxisome proliferator receptor-α/γ (PPARγ/α) were determined in BME-UV1 by real-time PCR. The results showed that cells treated with fatty acids and LPS increased ROS production compared with control cells. Among treatments, cells treated with* c*9,*t*11 CLA and* t*10,*c*12 CLA isomers revealed significant lower levels of ROS production compared with other fatty acids. All fatty acids reduced the gene expression of pro- and anti-inflammatory cytokines. Among fatty acids,* t*10,*c*12 CLA, LA and gLnA showed an homogeneous reduction of the three pro-inflammatory cytokines and this may correspond to more balanced and efficient physiological activity and may trigger a better protective effect. The PPARγ gene expression was significantly greater in cells treated with* t*10,*c*12 CLA, aLnA and LA, whereas the PPARα gene expression levels were significantly lower in cells treated with all different fatty acids, compared with the control. These results suggest that fatty acids inhibited the transcription of pro-inflammatory cytokines by the upregulation of PPARγ expression.*

Keywords: CLA *cis*-9, *trans*-11, CLA *trans*-10, *cis*-12, essential fatty acids, bovine mammary gland cells, inflammatory status

Implications

The supplementation of CLA and essential fatty acids (EFAs) in bovine mammary epithelial cell line exposed to severe inflammatory conditions, reduced the gene expression of pro-inflammatory cytokines. This study suggests and strengthen the utilization of these fatty acids as a useful management tool for dairy cattle, their use on high-yielding dairy cow at the time of calving, could act by reducing the development of inflammatory processes related to parturition.

Introduction

The chronic inflammatory reactions are important in the pathogenesis of several cow disorder, particularly during the transition period of dairy cow (Bertoni *et al.*, 2008). During this period dairy cow cannot adapt to growing demand of energy, which is necessary for the fetal growth, calving and onset of lactation (Abuelo *et al.*, 2015). As a consequence, non-esterified fatty acids (NEFA) increase in bloodstream and impact both inflammatory responses and immune function of transition cows (Scalia *et al.*, 2006; Lacetera *et al.*, 2007; Sordillo and Raphael, 2013). Moreover, plasma NEFA composition is modified: saturated (palmitate and stearate) and monounsaturated fatty acids (oleic acid) are increased, in contrast polyunsaturated fatty acids (especially n-3 fatty acids) are reduced (Sordillo and Raphael, 2013). Lipid changes can be responsible for the changes of the composition of the immune and endothelial cells membranes, and so may affect their functions.

Some studies have observed that T-cells treated with fatty acids modulated the secretion of various cytokines, such as tumor

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necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8, interleukin-2, interleukin-10 (IL-10) and interferon-γ (de Jong *et al.*, 2014), inducing a pro-inflammatory cascade by activating toll-like receptors and modulating the production of adipokines (de Heredia *et al.*, 2012).

Inflammatory state of the transition cow is also induced by oxidative stress which frequently occurs in this physiological phase (Bernabucci *et al.*, 2005) due to the excessive lipolysis that accentuates the production of reactive oxygen species (ROS) through the process of β-oxidation in overconditioned cows (Bernabucci *et al.*, 2005). The excessive amount of ROS would act by activating the nuclear factor κ-light-chain-enhancer of activated B cells (NF-Kβ) transcription factor that increases the expression of pro-inflammatory mediators, including cytokines (Sordillo and Raphael, 2013).

Fatty acids are important modulators of inflammatory responses; in particular, n-3 and n-6 EFAs and CLA isomers have received particular attention. Important active and short-lived hormones termed 'eicosanoids' are produced from EFAs. The eicosanoids originated from n-3 are known to have anti-inflammatory properties, whereas those derived from n-6 have pro-inflammatory properties (Teng *et al.*, 2014). Also CLA play an important protective role in the immune and inflammatory responses (Reynolds and Roche 2010; Hammond *et al.*, 2014). Hontecillas *et al.* (2002) observed that dietary CLA supplementation suppressed colon inflammation in pigs with bacterial-induced colitis.

Some authors (Boudjellab *et al.*, 2000; McClenahan *et al.*, 2005; Medina-Estrada *et al.*, 2016) observed that cultured bovine mammary gland epithelial cells could secrete IL-1α, IL-6 and TNF-α in response to inflammation induced by lipopolysaccharides (LPS).

Therefore, the aim of the current study was to evaluate and to compare the protective efficacy of CLA isomers (*c*9, *t*11 and *t*10,*c*12 CLA) and EFAs (aLnA, gLnA and LA) on inflammatory status of mammary gland after LPS stimulation by an *in vitro* model on bovine mammary epithelial cells (BME-UV1).

Material and methods

Cell 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 SV-40 large T-antigen. Bovine mammary epithelial cells were kindly provided by Prof. Antonella Baldi (Department of Health, Animal Science and Food Safety, University of Milan, Italy). Cells were routinely cultivated into 75 cm^2 tissue culture flasks (Costar, Corning, NY, USA), in a mixture of 50% DMEM-F12, 30% RPMI-1640 and 20% NCTC-135 (Sigma-Aldrich, St. Louis, MO, USA), supplemented with 10% fetal bovine serum, 0.1% lactose, 0.1% lactalbumin hydrolysate, 1.2 Mm glutathione, 1 μg/ml insulin, 5 μg/ml transferrin, 1 μg/ml hydrocortisone, 0.5 μg/ml progesterone, 10 μg/ml L-ascorbic acid and antibiotics (penicillin 100 IU/ml; streptomycin 100 μg/ml). All medium supplements were from Sigma-Aldrich (Milano, Italy). The cells were maintained at 37°C in a humidified 5% CO_2 incubator until confluence. Cells used in the present work were at passage number between 39 and 41.

Experimental design

In order to test the effects of fatty acids on inflammatory status of bovine mammary gland induced by LPS stimulation (from Escherichia coli 055:B5, Sigma-Aldrich, Milano, Italy), Bovine mammary epithelial cells were re-suspended in complete culture medium to a concentration of 5×10^5 cells/ml and dispensed into cells seeded in culture flasks and 96-wells tissue culture plates. After 24 h of incubation at 37°C in an atmosphere of 5% CO_2, medium was removed and cells were treated for 48 h with complete medium containing either 50 μM of *cis*-9,*trans*-11 (*c*9,*t*11) CLA, or *trans*-10,*cis*-12 (*t*10,*c*12) CLA, or (α)-linolenic acid, (aLnA; 18:3n-3), or (γ)-linolenic acid (gLnA; 18:3n-6) or linoleic acid (LA; 18:2n-6). After this period cells were incubated at 37°C for 3 h with LPS. Control cells were treated for 48 h with complete medium and then treated with LPS at 37°C for 3 h. Gene expression of pro- and anti-inflammatory cytokines, peroxisome proliferator-activated receptor-γ and α (PPARγ/α) was determined.

Also cell viability and ROS production after LPS addition was measured. CLA isomers and EFAs were pure (CLA isomers >98%; EFA >99%), free molecules and purchased from Larodan (Malmo, Sweden). The fatty acids were first dissolved in ethanol 95%, and the dilutions of the stock solution were made into aqueous buffers (culture medium) before performing biological experiments. To ensure that the residual amount of organic solvent was insignificant, as organic solvents may have physiological effects at low concentrations, a control test of cell viability was performed and no cytotoxic effects or biological differences were observed (data not shown). The experiments included at least three replicates per treatment and were repeated at least twice.

Cell viability

Bovine mammary epithelial cells were seeded into 96-wells microplates at an optimal density (5×10^5 cells/ml) and were grown with different fatty acids for 48 h. Then cells were incubated at 37°C for 3 h with LPS (20 μM). Cell viability after incubation with LPS assay was determined using Cell Proliferation kit II (XTT: sodium 30-[1-(phenylaminocarbonyl) 4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate; Roche Applied Science, Indianapolis, IN, USA) according to the manufacturer's instructions. For each treatment, after 3 h of exposure to LPS, 50 μl of XTT labeling mixture was added to each well. After 24 h incubation at 37°C, absorbance was measured at 450 nm. Background absorbance was subtracted from each value. Results were expressed as optical density.

Measurement of reactive oxygen species production

To determine ROS concentration, cells were washed twice with PBS (Lonza, Swiss) and incubated with 20 μM 2', 7'-dichlorodihydrofluorescin diacetate probe (Sigma-Aldrich) in PBS at 37°C for 40 m. Fluorescence was measured at 485 nm (excitation) and 535 nm (emission) wavelengths on a

microplate reader (Multimode Detector DTX 880; Beckman Coulter Inc., Indianapolis, IN, USA).

RNA isolation and real-time polymerase chain reaction

The messenger RNA (mRNA) abundance of bovine pro and anti-inflammatory interleukins (TNF-α, IL-1β, IL-6 and IL-10) and PPARγ/α were assayed in BME-UV1 cultured under the conditions described above and were carried out by real-time PCR. All primers and probes sequences used, were previously reported by Bionaz *et al.* (2008), Gonzalez *et al.* (2013) and Liu *et al.* (2012).

In order to isolate total RNA, BME-UV1 were seeded in cell culture flasks at the concentration of 5×10^5 cells/ml in complete medium containing 10% FBS (Thermo Fisher Scientific, Waltham, MA, USA) and treated as described above. Total RNA was extracted by QIAzol Lysis Reagent (Qiagen, Hilden, Germany), according to the manufacturer's instructions and stored at −80°C. RNA was quantified using Quant-iT RNA assay Kit (Invitrogen, Carlsbad, CA, USA) and fluorescence was measured at excitation/emission of 644/673 nm. Total RNA of 1 μg was reverse transcribed using Quantitect reverse transcription kit (Qiagen) in a total volume of 20 μl on a PCR Express thermal cycler (Hybaid, Ashford, UK). Quantitative SYBR Green and probes real-time PCR were performed following the manufacturer's recommendations using LightCycler® 2.0 (Roche; Roche Applied Science, Indianapolis, IN, USA). To account for possible variation related to complementary DNA input or the presence of PCR inhibitors, the endogenous reference gene glyceraldehyde 3-phosphate dehydrogenase was simultaneously quantified for each sample, and data were normalized accordingly. In Table 1 are shown the specific characteristics of primers used for the real-time PCR.

Table 1 *DNA sequences of bovine sense and antisense primers and probes used for real-time PCR-analysis*

Gene	Primers and probes	Temperature of annealing (°C)
PPARγ F	AATAACGCGATTCGTTTTGG	60
PPARγ R	TCCATGTCGTGGATGAGAAA	
PPARα F	CGGTGTCCACGCATGTGA	60
PPARα R	TCAGCCGAATCGTTCTCCTAAA	
TNF-α F	TCTTCTCAAGCCTCAAGTAACAAGT	60
TNF-α R	CCATGAGGGCATTGGCATAC	
TNF-α P	FAM-AGCCCACGTTGTAGCCGACATCAACTCC-TAMRA	
IL-1β F	TCCACCTCCTCTCACAGGAAA	58
IL-1β R	CTCTCCTTGCACAAAGCTCATG	
IL-1β P	FAM-CACCACTTCTCGGTTCA-MGB	
IL-6 F	GGGCTCCCATGATTGTGGTA	60
IL-6 R	GTGTGCCCAGTGGACAGGTT	
IL-6 P	FAM-TTCCTGGGCATTCCCTCCTCTGGT-TAMRA	
IL-10 F	CTTGTCGGAAATGATCCAGTTTT	60
IL-10 R	TTCACGTGCTCCTTGATGTCA	
IL-10 P	FAM-CCACAGGCTGAGAACCACGGGC-TAMRA	
GAPDH F	GCATCGTGGAGGAGGGACTTATGA	60
GAPDH R	GGGCCATCCACAGTCTTCTG	
GAPDH P	FAM-CACTGTCCACGCCATCACTGCCA-TAMRA	

PPARγ = peroxisome proliferator-activated receptorα-γ/α; TNF-α = tumor necrosis factor-α; IL-1β = interleukin-1β; IL-6 = interleukin-6; IL-10 = interleukin-10; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; F = Forward; R = reverse; *P* = probe.

PCR products were subjected to a melting curve analysis on the LightCycler. To allow relative quantification after PCR, standard curves were constructed from the standard reactions for each target and housekeeping genes by plotting crossing point values, that is, the cycle number at which the fluorescence signal exceeds background *v.* log complementary DNA dilution. The crossing point readings for each of the unknown samples where then used to calculate the amount of either the target or housekeeping relative to the standard, using the second derivative maximum method with the LyghtCycler analysis software 3.5 (Roche Applied Science).

Statistical analysis

All data of the experiment are presented as least-squares means and SEM. The data were analyzed by ANOVA using Statistica-7 Software package (Stat Soft Inc., USA). The model included fatty acid as fixed effect, replicates as random effect and the error term. The significance of the differences was assessed by the Fisher's LSD test. Significance was declared at $P < 0.05$.

Results

Viability of bovine mammary epithelial cells

Cell viability (Figure 1) was carried out using the Cell Proliferation kit II and was evaluated after 3 h of LPS exposure. As showed in Figure 1, LPS treatments did not modify cell viability. These results indicate that exposure to LPS, does not affect the metabolic activity of BME-UV1 through a decrease or an increase in cells viability and that the treatment did not have a cytotoxic effect.

Quantification of reactive oxygen species production

Intracellular ROS production by 2',7'-dichlorodihydrofluorescin diacetate probe fluorescence measurement was assessed in cells supplemented with fatty acids and

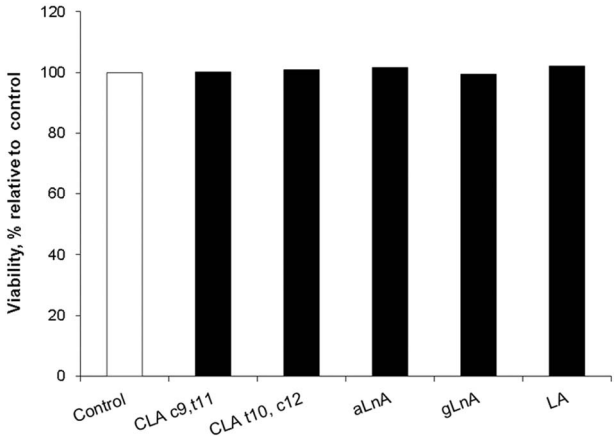

Figure 1 Cell viability of bovine mammary epithelial cells (BME-UV1) observed, after 48 h of fatty acids supplementation, and after 3 h of lipopolysaccharide treatment (20 μM). Absorbance was measured at 450 nm, and data are reported as least square means ± SEM ($n = 6$). CLA c9,t11 = cis9, trans 11 conjugated linoleic acid; CLA t10, c12 = conjugated linoleic acid; aLnA = α-linolenic acid; gLnA = γ-linolenic acid; LA = linoleic acid.

stimulated with LPS (Figure 2). Cells treated with fatty acids showed an increased ROS production ($P < 0.05$) compared with the control. Among treatments, cells treated with *c*9,*t*11 CLA and *t*10,*c*12 CLA revealed significant lower levels of ROS production compared with other fatty acids.

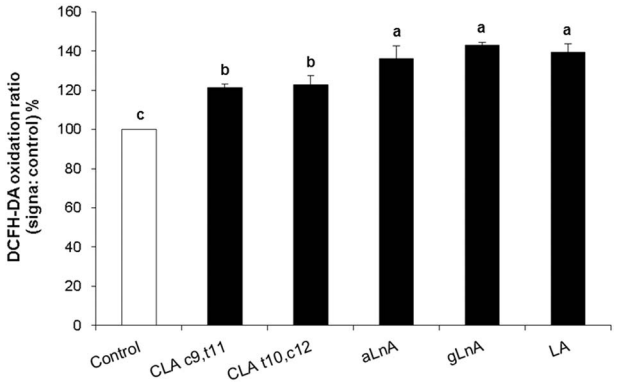

Figure 2 Intracellular production of reacting oxygen species by 2', 7'-dichlorodihydrofluorescin diacetate probe (DCFH-DA) in bovine mammary epithelial cells (BME-UV1) after 48 h of fatty acids supplementation, and after 3 h of lipopolysaccharide treatment (20 μM). Data are reported as least square means ± SEM ($n = 6$). Significant differences among control and treatments are represented by different letters ($P < 0.05$). CLA c9,t11 = cis9, trans 11 conjugated linoleic acid; CLA t10,c12 = conjugated linoleic acid; aLnA = α-linolenic acid; gLnA = γ-linolenic acid; LA = linoleic acid.

Quantification of messenger RNA expression of pro and anti-inflammatory cytokines

The mRNA expression of TNF-α, IL-1β, IL-6 and IL-10 are shown in Figure 3a, b, c and d, respectively. The gene expression of pro-inflammatory cytokines was lower ($P < 0.05$) in cells treated with different fatty acids compared with the control. However, some differences were observed between the different fatty acids.

The lowest level of TNF-α gene expression (Figure 3a) was observed in cells treated with gLnA, compared with the other fatty acids ($P < 0.05$). TNF-α gene expression was not different between cells treated with aLnA and *c*9,*t*11 CLA and between *t*10,*c*12 CLA and LA. aLnA and *c*9,*t*11 CLA showed greater ($P < 0.05$) TNF-α mRNA when compared with *t*10, *c*12 CLA and LA.

The lowest level ($P < 0.05$) of IL-1β gene expression (Figure 3b) was observed in cells treated with *c*9,*t*11 CLA compared with other FA. LA treatment showed greater ($P < 0.05$) IL-1β gene expression compared with *c*9,*t*11 CLA and lower when compared with *t*10,*c*12 CLA, aLnA and gLnA. No differences were observed between *t*10,*c*12 CLA, aLnA and gLnA.

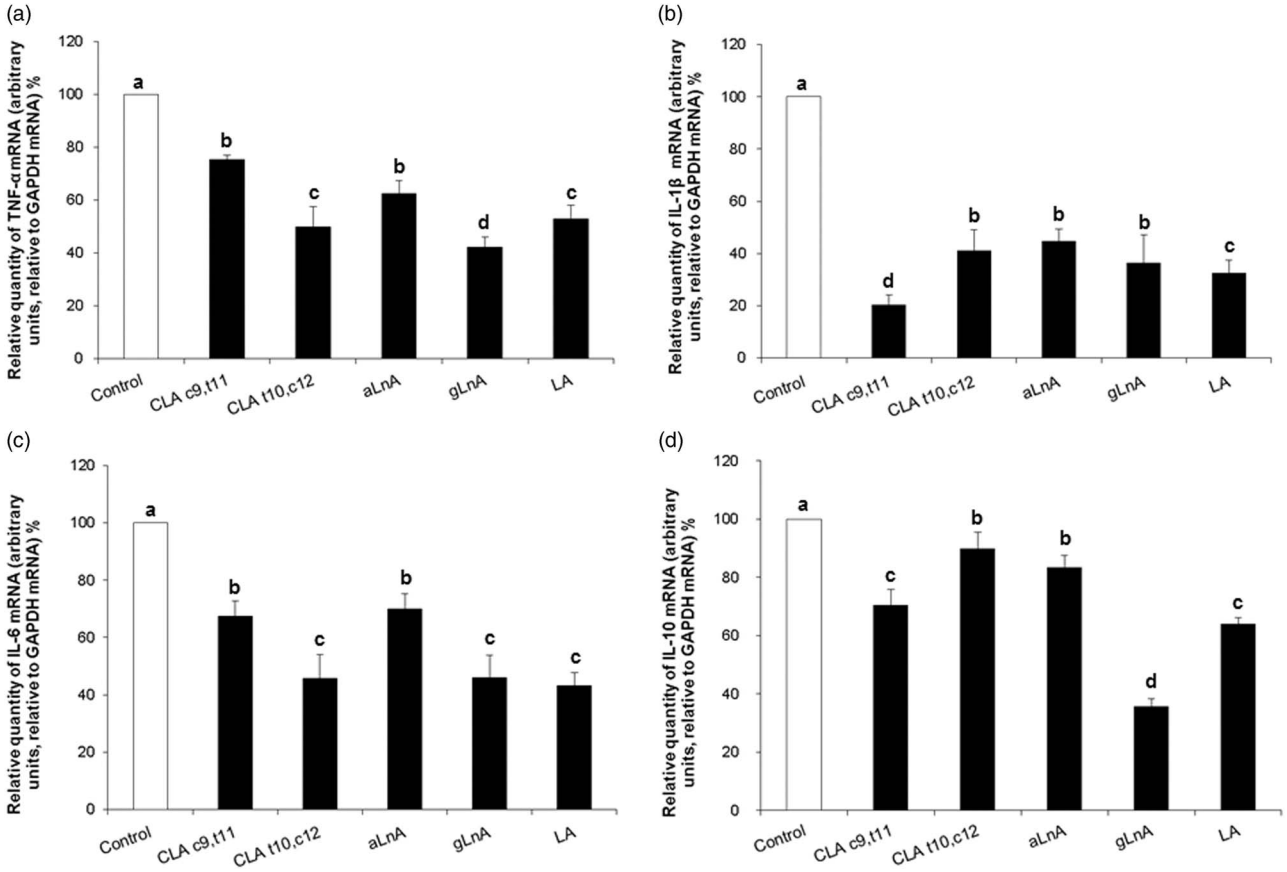

Figure 3 Messenger RNA abundance of tumor necrosis factor-α (TNF-α) (a), interleukin-1β (IL-1β) (b), interleukin-6 (IL-6) (c), and interleukin-10 (IL-10) (d) in bovine mammary epithelial cells (BME-UV1) after 48 h of fatty acids supplementation, and after 3 h of lipopolysaccharide treatment (20 μM). Data are reported as least square means ± SEM ($n = 6$). Significant differences between control and treatments and treatments between them are represented by different letters ($P < 0.05$). CLA c9,t11 = cis9, trans 11 conjugated linoleic acid; CLA t10,c12 = conjugated linoleic acid; aLnA = α-linolenic acid; gLnA = γ-linolenic acid; LA = linoleic acid.

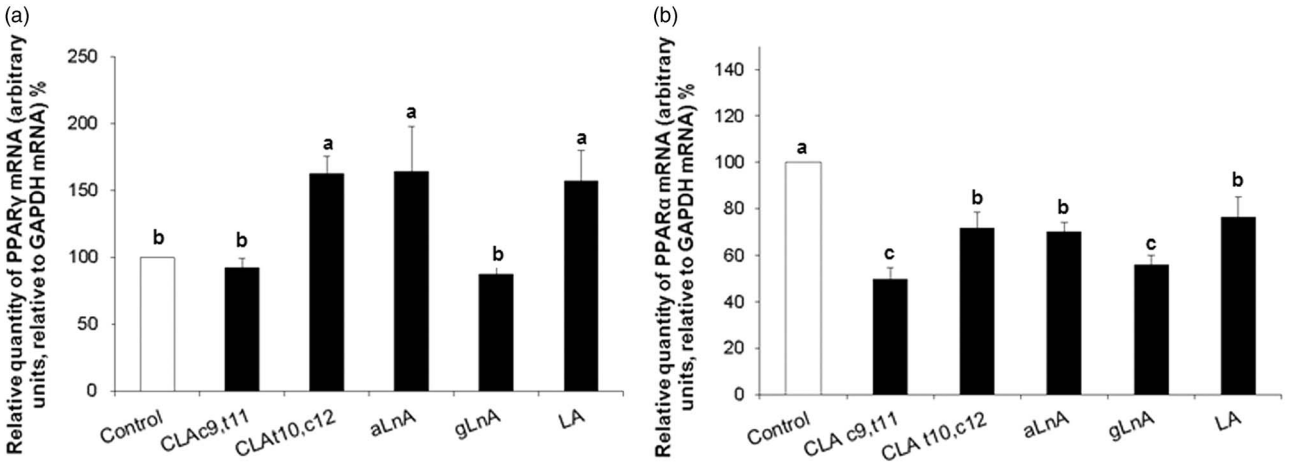

Figure 4 Messenger RNA abundance of peroxisome proliferator-activated receptorα- γ/α [PPARγ (a) and PPARα (b), respectively] in bovine mammary epithelial cells (BME-UV1) after 48 h of fatty acids supplementation, and after 3 h of lipopolysaccharide treatment (20 μM). Data are reported as least square means ± SEM ($n = 6$). Significant differences between control and treatments and treatments between them are represented by different letters ($P < 0.05$). CLA c9,t11 = cis9, trans 11 conjugated linoleic acid; CLA t10,c12 = conjugated linoleic acid; aLnA = α-linolenic acid; gLnA = γ-linolenic acid; LA = linoleic acid.

The lowest ($P < 0.05$) level of IL-6 gene expression (Figure 3c) was observed in cells treated with *t*10,*c*12 CLA, gLnA and LA, compared with *c*9,*t*11 CLA and aLnA. No differences were observed between *t*10,*c*12 CLA, gLnA and LA, and between *c*9,*t*11 CLA and aLnA.

The gene expression of IL-10 (Figure 3d) was lower ($P < 0.05$) in cells treated with different fatty acids compared with the control. Among fatty acids, the lowest ($P < 0.05$) level of IL-10 gene expression was observed in cells treated with gLnA compared with other FA. IL-10 gene expression was not different between cells treated with *t*10,*c*12 CLA and aLnA, and between *c*9,*t*11 CLA and LA. *t*10,*c*12 CLA and aLnA showed greater ($P < 0.05$) IL-10 mRNA when compared with *c*9,*t*11 CLA and LA.

Quantification of messenger RNA expression of peroxisome proliferator-activated receptor-γ and -α

The quantification of PPARγ and PPARα mRNA expression is showed in Figure 4a and b, respectively. PPARγ gene expression was greater ($P < 0.05$) in cells treated with *t*10, *c*12 CLA, aLnA and LA compared with control and with *c*9, *t*11 CLA and gLnA. No differences were observed between cells treated with *t*10,*c*12 CLA, aLnA and LA, and between control, *c*9,*t*11 CLA and gLnA.

The gene expression of PPARα was lower ($P < 0.05$) in cells treated with different FA when compared with control cells. Between FA treatments, PPARα gene expression was lower ($P < 0.05$) in cells treated with *c*9,*t*11 CLA and gLnA compared with LA, aLnA and *t*10,*c*12 CLA. No differences were observed between *c*9,*t*11 CLA and gLnA and between LA, aLnA and *t*10,*c*12 CLA.

Discussion

In the present study, for the first time, the effect of several fatty acids (*c*9,*t*11 CLA, *t*10,*c*12 CLA, aLnA, gLnA and LA) on the inflammatory response of BME-UV1 cells was investigated. The results showed that bovine cell line had different susceptibility to the different EFAs and CLA.

Under the experimental conditions, the exposure to 50 μM of fatty acids for 48 h has protected the cells limiting the triggering of the inflammatory process induced by LPS, known as a potent endotoxin responsible to stimulate the inflammation response and inflammatory cytokine synthesis. The results of the present study showed that cell supplemented with fatty acids has reduced the gene expression of the main pro-inflammatory cytokines and of the anti-inflammatory cytokine IL-10, matched by increased gene expression of PPARγ. The data presented here are consistent with other findings indicating an improvement of the inflammatory status in cells treated with CLA and EFAs (Changhua *et al*., 2005; Zhao *et al*., 2005; Erdinest *et al*., 2012). Erdinest *et al*. (2012), on human corneal epithelial cells culture incubated for 2 h with different concentrations of aLnA, gLna and LA, showed a reduced gene expression of TNF-α, IL-1β and IL-6 in cells treated with aLnA. Those authors stated that inhibitory effect of aLnA was mediated through NF-Kβ signal transduction. Also Zhao *et al*. (2005) in a study on monocytic THP-1 cells pre-incubated with aLnA, LA and DHA observed that fatty acids decreased TNF-α, IL-1β and IL-6 gene expression and the NF-Kβ DNA-binding activity, whereas PPARγ activity was increased and the anti-inflammatory properties of fatty acids were attributed, at least in part, to PPARγ-dependent mechanism. Changhua *et al*. (2005) in an *in vitro* study on the anti-inflammatory effect of *c*9,*t*11 CLA and *t*10,*c*12 CLA on cultured peripheral blood mononuclear cells, noted that CLA isomers, in particular *t*10,*c*12 CLA, suppressed the production and expression of TNF-α, IL-1 and IL-6. A recent study (Cao *et al*., 2016) on primary goat mammary gland epithelial cells treated with gLnA and LA showed that gLnA but not LA reduced inflammation induced by LPS, through the inhibition of the activity of NF-Kβ transcription factor and therefore the transcription

of IL-1, IL-6 and TNF-α. Also *in vivo* studies revealed similar results (Lin *et al*., 2013). Song *et al*. (2005), in humans, observed that dietary CLA supplementation reduced levels of TNF-α and IL-1β while increased the anti-inflammatory cytokine IL-10. Lin *et al*. (2013) showed that rats, supplemented with diet rich in n-3 (fish oil to 5%), had lower mammary mRNA abundance of xanthine oxidoreductase, protein level of TNF-α and greater levels of IL-10 and PPAR-γ. Renner *et al*. (2013) showed that there were no differences in IL-10 expression in bovine peripheral blood mononuclear cells treated with *c*9,*t*11, *t*10,*c*12 CLA isomers and LA. In contrast, Verlengia *et al*. (2004) reported that the B-lymphocyte cell line treated with eicosapentanoic acid and docosahexaenoic acid reduced the production of IL-10. This suppression of cytokine production was also observed in spleen lymphocytes from mice fed fish oil-rich diets (Wallace *et al*., 2001). The effects of fatty acids on the gene expression of the anti-inflammatory IL-10 are currently ambiguous and contradictory. On the other hand, Tamayo *et al*. (2011) in a human study on the relationship between the responses of cytokines in septic shock, observed that the secretion of pro- and anti-inflammatory cytokines occurs in a simultaneous manner by the moment which begins the immuno-inflammatory response. Results of the present study and findings from others (Wojdasiewicz *et al*., 2014) suggest the hypothesis that the reduced anti-inflammatory cytokines gene transcription could be positively correlated with reduced transcription levels of pro-inflammatory cytokines in view of an harmonized inflammatory response.

The PPARγ transcription factor, through its activation or gene up-regulation, plays an important role in the control of inflammation (Mandard and Patsouris, 2013). PPARγ inhibits the production of inflammatory mediators such as TNF-α, IL-1 and IL-6 interacting physically with NF-Kβ and preventing NF-Kβ translocation into the nucleus (Tak and Firestein, 2001). NF-Kβ is the main transcription factor that activate the expression of pro-inflammatory cytokines (Lawrence, 2009).

In this study it was observed an up-regulation of PPARγ in cells treated with *t*10,*c*12 CLA, aLnA and LA, but no differences on the expression of PPARγ were observed in cells treated with *c*9,*t*11 CLA and gLna. Our results suggest that *t*10,*c*12 CLA, aLna and LA inhibited the transcription of pro-inflammatory cytokines through the upregulation of PPARγ expression, whereas *c*9,*t*11 CLA and gLnA probably acted stimulating the activity of the constitutive PPARγ, as also observed and reported by Cao *et al*. (2016).

The level of expression of the PPARα gene, contrarily to PPARγ, was lowest in all cells treated with the different fatty acids and stimulated with LPS. Probably this phenomenon is linked to higher production of ROS observed in BME-UV1 cells. Cabrero *et al*. (2002) observed that skeletal muscle cells treated with low concentration of etomoxir, a substance which contains a fatty acid-derived structure, showed a downregulation of PPARα mRNA expression, through increased production of ROS and NF-Kβ activation. It is important to remark that generation of ROS have not solely negative effects but within certain concentrations ROS are useful signaling molecules regulating physiological processes. The beneficial or detrimental effects depends on many variables such as the site of ROS production, the persistence of ROS flow or the antioxidant status of target cells (Barbieri and Sestili, 2012).

Conclusions

All fatty acids reduced the gene expression of pro-inflammatory cytokines, among fatty acids, *t*10,*c*12 CLA, LA and gLna showed an homogeneous reduction of the three cytokines and this may correspond to more balanced and efficient physiological activity and may trigger a better protective effect. Our results suggest that fatty acids inhibited the transcription of pro-inflammatory cytokines by the upregulation of PPARγ expression.

Acknowledgements

None.

Declaration of interest

There are no conflict of interests.

Ethics statement

None.

Software and data repository resources

Data is not deposited in an official repository.

References

Abuelo A, Hernández J, Benedito JL and Castillo C 2015. The importance of the oxidative status of dairy cattle in the periparturient period: revisiting antioxidant supplementation. Journal of Animal Physiology and Animal Nutrition 99, 1003–1016.

Barbieri E and Sestili P 2012. Reactive oxygen species in skeletal muscle signaling. Journal of Signal Transduction 2012, 1–17.

Bernabucci U, Ronchi B, Lacetera N and Nardone A 2005. Influence of body condition score on relationships between metabolic status and oxidative stress in periparturient dairy cows. Journal of Dairy Science 88, 2017–2026.

Bertoni G, Trevisi E, Han X and Bionaz M 2008. Effects of inflammatory conditions on liver activity in puerperium period and consequences for performance in dairy cows. Journal of Dairy Science 91, 3300–3310.

Bionaz M, Baumrucker CR, Shirk E, Vanden Heuvel JP, Block E and Varga GA 2008. Characterization of Madin-Darby bovine kidney cell line for peroxisome proliferator-activated receptors: temporal response and sensitivity to fatty acids. Journal of Dairy Science 91, 2808–2813.

Boudjellab N, Chan-Tang HS and Zhao X 2000. Bovine interleukin-1 expression by cultured mammary epithelial cells (MAC-T) and its involvement in the release of MAC-T derived interleukin-8. Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology 127, 191–199.

Cabrero A, Alegret M, Sanchez RM, Adzet T, Laguna JC and Carrera MV 2002. Increased reactive oxygen species production down-regulates peroxisome proliferator-activated alpha pathway in C2C12 skeletal muscle cells. Journal of Biological Chemistry 277, 10100–10107.

Cao D, Luo J, Zang W, Chen D, Xu H, Shi H and Jing X 2016. Gamma-linolenic acid suppresses NF-κB signaling via CD36 in the lipopolysaccharide-induced inflammatory response in primary goat mammary gland epithelial cells. Inflammation 39, 1225–1237.

Changhua L, Jindong Y, Defa L, Lidan Z, Shiyan Q and Jianjun X 2005. Conjugated linoleic acid attenuates the production and gene expression of proinflammatory cytokines in weaned pigs challenged with lipopolysaccharide. Journal of Nutrition 135, 239–244.

de Heredia FP, Gómez-Martínez S and Marcos A 2012. Obesity, inflammation and the immune system. Proceedings of the Nutrition Society 71, 332–338.

de Jong AJ, Kloppenburg M, Toes REM and Ioan-Facsinay A 2014. Fatty acids, lipid mediators, and T-cell function. Frontiers in Immunology 5, 1–7.

Erdinest N, Shmueli O, Grossman Y, Ovadia H and Solomon A 2012. Anti-inflammatory effects of alpha linolenic acid on human corneal epithelial cells. Investigative Ophthalmology & Visual Science 53, 4396–4406.

Gonzalez DD, Rimondi A, Perez Aguirreburualde MS, Mozgovoj M, Bellido D, Wigdorovitz A and Dus Santos MJ 2013. Quantitation of cytokine gene expression by real time PCR in bovine milk and colostrum cells from cows immunized with a bovine rotavirus VP6 experimental vaccine. Research in Veterinary Science 95, 703–708.

Hammond S, Regna N, Chafin C and Reilly C 2014. Conjugated linoleic acid reduces inflammation via PPARγ in murine autoimmune glomerulonephritis (THER6P.861). Journal of Immunology 192, 201–217.

Hontecillas R, Wannemeulher MJ, Zimmerman DR, Hutto DL, Wilson JH, Ahn DU and Bassaganya-Riera J 2002. Nutritional regulation of porcine bacterial-induced colitis by conjugated linoleic acid 1,2. Journal of Nutrition 132, 2019–2027.

Lacetera N, Scalia D, Mashek D, Bernabucci U and Grummer R 2007. Effects of intravenous triacylglycerol emulsions on lymphocyte responses to mitogens in fasted dairy cows undergoing intense lipomobilization. Journal of Dairy Research 74, 323–328.

Lawrence T 2009. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harbor Perspectives in Biology 1, 1–10.

Lin S, Hou J, Xiang F, Zhang X, Che L, Lin Y, Xu S, Tian G, Zeng Q, Yu B, Zhang K, Chen D, Wu D and Fang Z 2013. Mammary inflammation around parturition appeared to be attenuated by consumption of fish oil rich in n-3 polyunsaturated fatty acids. Lipids in Health and Disease 12, 1–13.

Liu Y, Biarnés Costa M and Gerhardinger C 2012. IL-1β is upregulated in the diabetic retina and retinal vessels: cell-specific effect of high glucose and IL-1β autostimulation. PLoS One 7, e36949.

Mandard S and Patsouris D 2013. Nuclear control of the inflammatory response in mammals by peroxisome proliferator-activated receptors. PPAR Research 2013, 1–23.

McClenahan DJ, Sotos JP and Czuprynski CJ 2005. Cytokine response of bovine mammary gland epithelial cells to *Escherichia coli*, coliform culture filtrate, or lipopolysaccharide. American Journal of Veterinary Research 66, 1590–1597.

Medina-Estrada I, López-Meza JE, Ochoa-Zarzosa A and Ochoa-Zarzosa A 2016. Anti-inflammatory and antimicrobial effects of estradiol in bovine mammary epithelial cells during *Staphylococcus aureus* internalization. Mediators of Inflammation 2016, 1–16.

Reynolds CM and Roche HM 2010. Conjugated linoleic acid and inflammatory cell signaling. Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA) 82, 199–204.

Renner L, Kersten S, Duevel A, Schuberth HJ and Dänicke S 2013. Effects of cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid, linoleic acid, phytanic acid and the combination of various fatty acids on proliferation and cytokine expression of bovine peripheral blood mononuclear cells. Nutrients 5, 2667–2683.

Scalia D, Lacetera N, Bernabucci U, Demeyere K, Duchateau L, Burvenich C, Aoki T, Etoh T, Hashimoto T, Naruse M, Sano H, Utsumi H and Nawata H 2006. In vitro effects of nonesterified fatty acids on bovine neutrophils oxidative burst and viability. Journal of Dairy Science 89, 147–154.

Song H-J, Grant I, Rotondo D, Mohede I, Sattar N, Heys SD and Wahle KWJ 2005. Effect of CLA supplementation on immune function in young healthy volunteers. European Journal of Clinical Nutrition 59, 508–517.

Sordillo LM and Raphael W 2013. Significance of metabolic stress, lipid mobilization, and inflammation on transition cow disorders. Veterinary Clinics of North America: Food Animal Practice 29, 267–278.

Tak PP and Firestein GS 2001. NF-κB: a key role in inflammatory diseases. Journal of Clinical Investigation 107, 7–11.

Tamayo E, Fernández A, Almansa R, Carrasco E, Heredia M, Lajo C, Goncalves L, Gómez-Herreras JI, de Lejarazu RO and Bermejo-Martin JF 2011. Pro- and anti-inflammatory responses are regulated simultaneously from the first moments of septic shock. European Cytokine Network 22, 82–87.

Teng K-T, Chang C-Y, Chang LF and Nesaretnam K 2014. Modulation of obesity-induced inflammation by dietary fats: mechanisms and clinical evidence. Nutrition Journal 13, 1–15.

Verlengia R, Gorjão R, Kanunfre CC, Verlengia SB, Gorjão R, Kanunfre CC, Bordin S, de Lima T M, Martins EF, Newsholme P and Curi R 2004. Effects of EPA and DHA on proliferation, cytokine production, and gene expression in Raji cells. Lipids 39, 857–864.

Wallace FA, Miles EA, Evans C, Stock TE, Yaqoob P and Calder PC 2001. Dietary fatty acids influence the production of Th1- but Not Th2-type cytokines. Journal of Leukocyte Biology 69, 449–457.

Wojdasiewicz P, Poniatowski ŁA and Szukiewicz D 2014. The Role of Inflammatory and Anti-Inflammatory Cytokines in the Pathogenesis of Osteoarthritis. Mediators of Inflammation 2014, 1–19.

Zhao G, Etherton TD, Martin KR, Vanden Heuvel JP, Gillies PJ, West SG and Kris-Etherton PM. 2005. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochemical and Biophysical Research Communications 336, 909–917.