

# Influence of dietary supplementation with conjugated linoleic acid, rosemary and oregano extracts on fatty acid profile of fresh pork meat

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**Summary.** The impact of feeding containing conjugated linoleic acid (CLA), in combination with oregano (*Origanum vulgare* L.) and/or rosemary (*Rosmarinus officinalis* L.) water extracts, on fatty acid profile of fresh pork meat was evaluated. The qualitative fatty acid composition of loin (*Longissimus lumbrorum muscle*) meat samples was determined with capillary gas chromatography coupled with mass spectrometry (GC/MS) whereas the quantitation of fatty acids (weight % of total fatty acids) was carried out using GC/Flame Ionization Detector. The results indicated that the qualitative fatty acid composition of meat was not affected by the dietary administration of oregano and/or rosemary water extracts. Twenty-one fatty acids were identified and the amount of fatty acid classes increased in the order polyunsaturated (PUFA) < monounsaturated (MUFA) < saturated (SFA), in all meat samples. The administration of diet containing cis-9, trans-11 and trans-10, cis-12 CLA isomers (1 g CLA/Kg fed diet) provided a pork meat having CLA levels accounting for about 1% of total fatty acids. The addition of oregano and/or rosemary water extract into CLA-enriched diet had no effects on CLA deposition in *Longissimus lumbrorum* tissues. Statistically significant differences were not stated between the tested groups. Thus, the feeding containing CLA, in combination with oregano and/or rosemary water extracts, applied in the present study, resulted able to enhance the CLA content in pork meat, providing a potentially healthier meat product. Anyway, it not proved efficient in bringing the PUFA/SFA ratio of meat closer to the recommended value, such as >0.7. The PUFA/SFA ratio revealed in all samples was 0.2.

**Keywords:** *Origanum vulgare* L., *Rosmarinus officinalis* L., water extract, fatty acid composition, pork meat, *Longissimus lumbrorum muscle*, CLA

«INFLUENZA DELLA SUPPLEMENTAZIONE DIETETICA CON ACIDO LINOLEICO CONIUGATO, ESTRATTI DI ORIGANO E ROSMARINO SUL PROFILO IN ACIDI GRASSI DELLE CARNI FRESCHE DI MAIALE »

**Riassunto.** L'effetto della somministrazione dietetica di isomeri dell'acido linoleico coniugato (CLA) in combinazione con estratti acquosi di origano (*Origanum vulgare* L.) e/o rosmarino (*Rosmarinus officinalis* L.), è stato valutato sul profilo in acidi grassi di carne suina fresca (*Longissimus lumbrorum*). La composizione qualitativa della frazione acidica carne della carne è stata determinata mediante gas cromatografia capillare accoppiata alla spettrometria di massa (GC/MS), mentre l'analisi quantitativa è stata realizzata mediante un sistema gas cromatografico dotato di detector a ionizzazione di fiamma (FID). I risultati mostrano come il profilo acidico delle carni non è influenzato dalla somministrazione di estratti di origano e rosmarino. In tutti i campioni sono stati individuati ventuno acidi grassi e le quantità delle classi degli acidi grassi variano nel seguente ordine: acidi grassi polinsaturi (AGP) < acidi grassi monoinsaturi (AGM) < acidi grassi saturi (AGS). La somministrazione della dieta contenente cis-9, trans-11 e trans-10, cis-12 isomeri del CLA (1 g CLA/Kg di mangime) ha consentito di ottenere carni suine con livelli di CLA che si sono attestati attorno al

1% degli acidi grassi totali. L'aggiunta di estratti di origano e/o rosmarino alla dieta già arricchita di CLA non ha prodotto alcun tipo di effetto sull'accumulo di CLA nei tessuti muscolari del *Longissimus lumborum*. Tra i gruppi sperimentali infatti non sono emerse differenze statisticamente significative. Pertanto, l'alimentazione di maiali con diete contenenti CLA, in combinazione con estratti di rosmarino e/o origano, usata nel presente studio, è risultata efficiente nell'arricchire le carni con CLA, quindi nel fornire carni potenzialmente più salutari. Comunque essa non è risultata efficiente nel portare il rapporto AGP/AGS nelle carni vicino al valore raccomandato, ovvero >0.7. Infatti in tutti i campioni di carne il rapporto AGP/AGS era 0.2.

**Parole chiave:** *Origanum vulgare* L., *Rosmarinus officinalis* L., estratti acquosi, acidi grassi, carne suina, *Longissimus lumborum*, CLA

## Introduzione

Red meat is considered a highly nutritious and valued food because is an important dietary source of bioactive compounds, such as high biological value proteins, vitamins and minerals of high degree of bio-availability (1). At the same time, red meat represents a major source of fat in the diet, especially of saturated fatty acids (SFA) and cholesterol which have been implicated in cancer and coronary heart diseases. Thus, the perception of the role of red meat, in the global diet is dichotomous. While recommendations to limit red meat are often rooted in the belief that red meat, as a source of SFA, contributes significantly to cardiovascular disease, evidence suggests otherwise. In fact, red meat's contribution of many key nutrients relative to the energy it provides supports the role of lean red meat as an important nutrient-rich food in the fight against many chronic diet-related non-communicable diseases (NCD) (2). However, despite the beneficial role of red meat in improving the nutritional status of developing nations is continuously supported by experimental evidences, the association between red meat and the incidence of diseases overshadow the positive attributes of the red meat.

Taking into account this trend and inspired by the health conscious consumer, newest strategies in meat producing domesticated animals have been studied. They have aimed at changing the fatty acid composition by bringing the polyunsaturated fatty acid PUFA/SFA ratio of meat closer to the recommended value (>0.7), as well as for the PUFA  $\omega 6/\omega 3$  ratio (<5). For this purpose, the innovative farming techniques are focusing towards the use of feed enriched with natural sources of PUFA, especially of  $\omega 3$  PUFA and conju-

gated linoleic acids (CLA), in view of their potential benefits for consumers (3-6). As far as pork meat is concerned, due to the monogastric nature of pigs, the feed exerts a relevant effect on the meat composition, not only in the amount of fat but also on its composition in fatty acids. In fact, dietary fatty acids are incorporated practically unchanged into the adipose tissue of monogastrics whereas dietary fatty acids are hydrogenated in the rumen of ruminants.

Different types of cereals as well as dietary oils and their effects on the proportions in fatty acid composition of pork meat have been studied (7-11). The addition of fish oil seem to be the effective way to increase the content in eicosapentaenoic (EPA, C22:5  $\omega 3$ ) and docosahexaenoic (DHA, C22:6  $\omega 3$ ) and sensibly reduces the PUFA  $\omega 6/\omega 3$  ratio to near 2 in pork meat. Differently, the use of canola or linseed oils produce a substantial increase in the content of linolenic acid (Ln, C18:3 $\omega 3$ ) and slightly increase of EPA and DPA. This evidence has been ascribed to the limited conversion of Ln to its longer chain metabolites EPA and DPA which occurred in pigs. Hoz et al. 2003 (12) demonstrated that higher PUFA/SFA (around 0.6-0.7) and PUFA  $\omega 6/\omega 3$  (near 2.0) ratios can be obtained in pork tender loin by using linseed oil alone or a mixture of linseed and olive oils, as supplement in pig diet.

Regarding the CLA, extensive studies testified that dietary CLA increased the content of SFA, decreased the level of MUFA and led to a CLA enrichment of pork loin meat (13, 14). Four weeks of dietary supplement of 1%, 2.5% and 5% of synthetic CLA increased the CLA concentration from 0.1 mg/g fatty acids in control to 3.7, 10.1 and 11.6 mg/g fatty acids respectively in pig *Longissimus dorsi* muscle (15). Eggert et al. 2001 (16) reported that the somministration of diet

containing 1% CLA resulted in 5.5 mg CLA/100 g fatty acids present in the pig muscle.

Unfortunately, the PUFA enrichment of meat is restricted by the resulting decrease in lipid oxidative stability (17, 18). Lipid oxidation is one the major causes of quality deterioration of meat, with adverse effect on flavour, colour, texture and nutritional value. For example, the increase of the linoleic acid content in the meat led to the lowering the melting point of the fat and reduced the oxidative stability, adversely affecting the technological properties (19) and sensory (20).

In view of this fact, the dietary supplementation with a proper amount of antioxidants could provide a good alternative to enhance the oxidative stability of meat with a high content of PUFA (21, 22). It is well established that dietary supplementation of pigs with vitamin E ( $\alpha$ -tocopheryl acetate) enhanced muscle  $\alpha$ -tocopherol concentration and stabilizes PUFA and cholesterol in muscle against oxidative deterioration (23). Depending on the concentration (typically around 100–200 mg/kg feed) and time of supplementation the content of such vitamin may be proportionally increased in the muscles (24). Typical values near 13 mg/kg dry muscle or 0.038 mg/g lipids from pork loin (*Longissimus lumborum*), may be reached (8, 25).

Alternatively to the employment of vitamin E, recent researches have suggested that supplementation with phenolic compounds may have beneficial effects on the antioxidant defence system of the animal and protects cell biological membranes against lipid oxidation also preserving the welfare and food quality. Additionally, some plants with high content in phenolic compounds have demonstrated antimicrobial activity and delayed lipid oxidation of meat when they are included in the diet of the animal (26,27). With this regard, special attention has been paid to rosemary (*Rosmarinus officinalis* L.) and oregano (*Origanum vulgare* L.), herbs commonly used as a flavouring agent. Rosemary resulted rich in phenolic diterpenes such as carnosol, carnosic acid, rosmanol, epirsomanol isorosmanol, methyl carnosate and other phenolic acids, such as rosmarinic acid (28–31) whereas oregano mainly contains carvacrol, thymol, and their precursors, c-terpinene and p-cymene (32).

Liotta et al. 2007 (33) evaluated the effect of dietary supplementation with rosemary extract on the

oxidative stability and fatty acid composition of *Longissimus dorsi* muscle from Nero Siciliano pig. They found a PUFA/SFA ratio significantly higher in the muscle ( $P=0.004$ ) of the rosemary supplemented group with respect the control group. Moreover, the oxidative stability of the meat was unaffected by the dietary treatment. The results testified a possible anti-oxidative activity of the rosemary extract. Differently, Simitzis et al. (34) reported that the dietary administration of different levels of oregano essential oil did not exert any effect on pig meat quality parameters (pH, colour, intramuscular fat, cooking loss and shear force value), probably indicating that components of oregano essential oil were not introduced into the cell phospholipids membranes in pig muscles.

In view of these evidences, the present work was aimed to acquire a deeper knowledge about of impact of feeding containing CLA, in combination with oregano and rosemary water extracts, on fatty acid profile of fresh pork meat (*Longissimus lumborum* muscle). Especially, the impact of different feeding will be evaluated in order to establish if the addition of plant extract into the CLA enriched diet promote the CLA enrichment of meat. The effects of individual extracts were tested, as well as combinations of extracts to infer possible additive or synergistic effects between these antioxidants.

The experimental work involved one trial test. The test provided a four diets, 1) CLA enriched diet (Ctrl), 2) CLA enriched diet + rosemary (R), 3) CLA enriched diet + oregano (O) and 4) CLA enriched diet + oregano + rosemary (OR). The qualitative fatty acid composition of loin (*Longissimus lumborum*) meat samples was determined with capillary gas chromatography coupled with mass spectrometry (GC/MS) whereas the quantitation of each fatty acids was carried out using GC/Flame Ionization Detector.

## Material and methods

### *Sampling and experimental Diets*

In the trial test 64 Italian swine (*Duroc x Large*, 45 kg initial weight) were divided in four groups, each composed by 8 males and 8 females. The rearing consisted in two stages of growing feed, the first until 90 kg and

the second until 120 kg and after that a finishing allowed to reach 160-170 kg.

The control group (Ctrl) was feed a diet consisting of degermed corn (55%), barley (20%), soy (15%), finely sieved bran (7.25%), calcium carbonate (1.25%), dicalcium phosphate (0.75%), premix pig-g (0.3%), sodium chloride (0.25%), lysine (0.2%), methionine (0.05%), LodeStar™CLA (1%). The O supplemented group received a control diet plus oregano water extract Phenbiox® (2 g/Kg fed diet) whereas the R group received a control diet plus rosemary water extract Phenbiox® (2 g/Kg fed diet). Finally, OR group receiving a control diet plus rosemary water extract Phenbiox® (1 g/Kg fed diet) and oregano water extract Phenbiox® (1 g/Kg feed). The source of conjugated linoleic acids (LodeStar™CLA, Berg+Schmidt Functional lipids) contained min. 50% of CLA, including c9 t11 (min. 24%) and t10 c12 (min. 24%) isomers. The oregano and rosemary water extracts were provided by Phenbiox S.r.l. (Bologna, Italia).

All diets containing equal amount of CLA and all supplemented diets contained the same amounts of the plant water extract. Anyway, the plant water extract was added both to the growing and the finishing feed whereas LodeStar™CLA was exclusively added to the feed during finishing stage. Feed was administered in liquid form, the water/feed ratio was 3:1 at a starting dose of 1.9 kg/pig/day, with an increase of 0.1/kg/pig/day for 11 weeks, and 0.05 kg/pig/day for a further 4 weeks to reach a dose of 2.97 kg/pig/day which was maintained until the end of the test (184 days). The pigs were reared until they reach of slaughter weight typical of heavy pigs (160-170 kg). Afterwards, the pork loins were sliced to obtain two chops of about 200 g of weight, which were packed and stored at -18°C for few days until fat extraction.

#### *Extraction of total lipids*

Four fresh pork loins (*Longissimus lumborum*), as concerns each feed groups, were randomly collected (two from male and two from female pigs) and utilized for lipid extraction. An aliquot (20 g) of each muscle tissue was homogenized in chloroform - methanol (1:2, v/v) and the lipids were extracted according to Bligh and Dyer (35).

#### *Analysis of fatty acid profile*

Fatty acid methyl esters (FAMES) were obtained from total lipids through alkaline transmethylation (36). The qualitative analysis of FAMES was carried out using a Focus gas chromatograph (Thermo Electron Corporation, West Palm Beach, FL, USA) equipped with a CP-Sil88 fused silica capillary column (100 m × 0.25 mm i.d., film thickness 0.2 µm, Chrompack, Middelburg, The Netherlands) and a quadrupole mass detector (FocusDSQ). The carrier gas was helium at a flow rate of 1.6 mL min<sup>-1</sup>; the oven temperature program started from 160°C, raised to 240°C at a rate of 4°C min<sup>-1</sup> and remained at 240°C for 10 minutes. The injector temperature was 260°C. The sample was injected into a split/splitless system. The ion source temperature of the mass detector was set at 260°C. The mass spectrum was acquired using Xcalibur Data System ver. 1.4. Peaks were identified by comparison with known standards and using the NIST mass spectral database. The quantitative analysis of FAMES was performed by means of gas chromatography using a CP-9002 apparatus (Chrompack, Middelburg, The Netherlands) equipped with a flame ionization detector (FID) and the same column and operative conditions reported above. The temperature of the detector was set at 260°C. A Supelco (Bellefonte, PA, USA) standard solution containing a mixture of 37 FAMES was used for identification of peaks and for the calculation of correction factor of the individual fatty acid peak areas. Fatty acid compositions (wt %) were calculated by the corrected peak area normalization method.

#### *Statistical analysis*

The data collected were grouped into feed samples, in order to point out the effect of different feeds on fatty acids profile of pork loins. All data were presented as group mean values ± standard deviation (SD). The statistical analysis of data was performed by ANOVA carried out with the GraphPad InStat ver. 3.0 system (GraphPad Software, San Diego, CA, USA). Tukey - Kramer's test was used for comparison of the means among the groups of diet, differences were detected in order to get an overview of the differences in the fatty acid composition among the fresh meat. Significance

was accepted at a probability of 0.05 ( $P < 0.05$ ), according to the MSD (minimum significant differences) test.

## Results and discussion

The lipid average expressed on a wet weight basis and the fatty acid composition (weight % of total fatty acids) of pork loins (*Longissimus lumborum* muscle) were reported in Table 1.

The lipid content ranged from 11.2 to 15.9 g/100g of the wet tissue. Although no statistically significant differences ( $P > 0.05$ ) were detected among the samples

obtained from different trial diets, the highest lipid content was revealed in meat samples from OR group. Analogously to the lipid content, the quali-quantitative fatty acid composition of *Longissimus lumborum* muscle was not affected by the dietary administration of oregano and/or rosemary water extracts. Twenty-one fatty acids were identified and the amount of fatty acid classes increased in the order PUFA < MUFA < SFA in all meat samples.

The SFA portion presented the half of total fatty acid composition in all samples, whereas the MUFA fraction constituted nearly forty percent of the total fatty acids. The palmitic acid (C16:0) resulted the most rep-

**Table 1.** Lipid content (g/100 wet tissue weight) and fatty acid composition (weight % of total fatty acids) of pork loins (*Longissimus lumborum* muscle) obtained from animal belonging to different feeding groups.

	Diet			
	Ctrl	O	R	OR
Lipid content	13.9±3.7	11.2±2.6	13.2±0.3	15.9±0.5
Fatty acid (%)				
C12:0	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0
C14:0	1.9±0.2	1.9±0.2	2.2±0.2	2.1±0.3
C16:0	28.4±0.6	27.5±2.2	30.2±1.5	28.3±1.9
C17:0	0.2±0.0	0.2±0.1	0.2±0.1	0.2±0.0
C18:0	18.5±1.7	19.5±1.4	18.8±0.5	17.3±0.6
C20:0	0.2±0.0	0.3±0.0	0.2±0.0	0.2±0.0
∑ SFA	49.3±1.9	49.7±2.8	51.6±2.1	48.1±2.6
C16:1 Δ <sup>9c</sup>	2.6±0.5	2.5±0.4	2.9±0.2	3.0±0.3
C16:1 iso	0.3±0.1	0.3±0.1	0.3±0.1	0.3±0.0
C18:1 Δ <sup>9t</sup>	0.3±0.0	0.4±0.1	0.4±0.0	0.4±0.0
C18:1 Δ <sup>9c</sup> + C18:1 Δ <sup>11c</sup>	36.2±1.8	35.3±3.4	33.4±1.6	37.3±3.4
C20:1 Δ <sup>11c</sup>	0.9±0.1	0.9±0.1	0.8±0.1	0.8±0.1
∑ MUFA	40.5±2.2	39.4±3.5	37.9±1.5	42.0±3.3
C18:2 Δ <sup>9t,12t</sup>	0.1±0.0	0.1±0.1	0.1±0.1	0.1±0.1
C18:2 Δ <sup>9c,12c</sup> [ω6]	7.8±0.5	8.2±0.7	7.9±1.4	7.4±0.7
C18:3 Δ <sup>9c,12c,15c</sup> [ω3]	0.4±0.1	0.5±0.1	0.4±0.1	0.4±0.1
C18:2 Δ <sup>9c,11t</sup> [CLA]	0.7±0.0	0.7±0.1	0.7±0.1	0.8±0.1
C18:2 Δ <sup>10t,12c</sup> [CLA]	0.3±0.0	0.3±0.1	0.3±0.1	0.3±0.1
C20:2 Δ <sup>11,14</sup> [ω6]	0.4±0.1	0.4±0.0	0.3±0.0	0.3±0.0
C20:3 Δ <sup>8c,11c,14c</sup> [ω6]	tr	0.0±0.1	0.1±0.1	tr
C20:4 Δ <sup>5c,8c,11c,14c</sup> [ω6]	0.4±0.0	0.6±0.1	0.7±0.5	0.4±0.0
C22:4 Δ <sup>7c,10c,13c,16c</sup> [ω6]	tr	0.1±0.1	tr	tr
∑ PUFA	10.2±0.5	10.9±1.1	10.5±2.0	9.9±0.7
PUFA/SFA	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0
PUFA ω3/PUFA ω6	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0

The lipid content was determined on a fresh weight basis. Value are given as means ± S.D. (n = 3); tr, lower than 0.1%.

Legend for fatty acids: C12:0, lauric; C14:0, myristic; C16:0, palmitic; C16:1, palmitoleic; C16:1 iso, isomer of hexadecenoic; C18:0, stearic; C18:1 Δ<sup>9c</sup>, oleic; C18:1 Δ<sup>11c</sup>, vaccenic; C18:2 Δ<sup>9t,12t</sup>, linoleic; C18:2 Δ<sup>9c,12c</sup>, α-linolenic; C18:2 Δ<sup>9c,11t</sup> + C18:2 Δ<sup>10t,12c</sup>, isomer of conjugated linoleic; C18:1 Δ<sup>9t</sup>, vaccenic; C18:1 Δ<sup>9c</sup>, oleic; C18:1 Δ<sup>11t</sup>, cis-vaccenic; C20:0, eicosanoic; C20:1 Δ<sup>11c</sup>, gondoic; C18:3 Δ<sup>9c,12c,15c</sup>, α-linolenic; C20:2 Δ<sup>11,14</sup>, eicosadienoic; C20:3 Δ<sup>8c,11c,14c</sup>, dibomo-γ-linolenic; C20:4 Δ<sup>5c,8c,11c,14c</sup>, arachidonic; C22:4 Δ<sup>7c,10c,13c,16c</sup>, adrenic. SFA, saturated fatty acids; PUFA, polyunsaturated fatty acid.

representative SFA whereas the C18:1, as a sum of oleic and vaccenic acids (C18:1 $\Delta^{9c}$  + C18:1 $\Delta^{11c}$ ), resulted the most abundant fatty acid in all samples. Although higher level of MUFA occurred in meat from OR dietary trial with respect all the other samples, no statistically significant differences between tested groups of animals were observed on MUFA content.

As regard to the PUFA fraction, all meat samples presented the  $\alpha$ -linoleic acid (C18:2 $\Delta^{9c,12c}$ ) as the most abundant PUFA. No significant differences related to the dietary treatment were observed for PUFA content of meat.

Differently, a previous study (33) pointed to that the addition of rosemary extract (1 g/Kg fed diet) to the diet of Nero Siciliano pigs led to significant increase of PUFA content in *Longissimus dorsi* muscle. Specifically, the PUFA increase was a primary consequence of the linoleic, arachidonic and docohaxaenoic (C22:6  $\omega$ 3) acids increase. Haak et al (37) revealed a converse trend of PUFA variation. They displayed that the rosemary supplementation of pig diet led to a significant decrease of C22:6  $\omega$ 3 level in *Longissimus toracis* muscle.

Taking into account all data set, it is possible to emphasize that the influence of dietary supplementation with plant extracts, in particular rosemary extract, on pork meat fatty acids profile is different according to the anatomical part (muscle) from which the meat is originated.

Unfortunately, previous studies evaluating the effect of oregano extract on pork meat fatty acid composition were not found in the literature, thus the comparison of the results is not allowed.

From a nutritional perspective, a special attention was paid to CLA portion. The dietary CLA supplementation with 1g CLA/Kg fed diet provided a pork meat with CLA levels accounting for 1% of total fatty acids. The addition of oregano and/or rosemary in the CLA enriched diet had no effects on CLA deposition in *Longissimus dorsi* tissues. The CLA isomeric profile showed a clear predominance of bioactive *cis*-9, *trans*-11 CLA isomer in all samples, followed by the *trans*-10, *cis*-12 CLA. The ratio of the two CLA isomers in all samples resulted 0.2. It strongly differed from that in the diet, which was 1. This evidence supports the work by Bee (38) suspecting that in pig a

higher incorporation is observed for *cis*-9, *trans*-11 CLA than for *trans*-10, *cis*-12 CLA.

Anyway, although the CLA dietary supplementation performed in our experimental study resulted able to enhance the CLA content in meat, providing a potentially healthier meat product, it not proved efficient in bringing the PUFA/SFA ratio of meat closer to the recommended value, such as >0.7. The PUFA/SFA ratio revealed in all samples was 0.2

## Conclusions

Despite several studies reported the influence of feeding with essential oil and extracts from rosemary and oregano on quality parameters (i.e. pH, colour, Warner-Bratzler shear force values, sensory attributes) of a variety of meats, such as turkey (39), pork (40), chicken (41), and lamb meat (42), little information is available concerning supplementation of pig diets with aromatic plants and their subsequent effects on meat fatty acid composition.

Our set of results shown that the addition of rosemary and /or oregano extracts (2 g/Kg fed diet) into a CLA enriched diet did not affect the fatty acid composition of pork meat from *Longissimus lumborum* muscle, as well as, the CLA accumulation. Statistically significant differences were not stated between the tested groups. Despite the well-know and testified antioxidant activity of rosemary and oregano, in the present experimental condition, the dietary supplementation with rosemary and oregano water extracts were not able to provide a greater stability of PUFA and consequently a selective increase of PUFA, especially of CLA, was not revealed in meat samples.

Further studies should be performed in order to evaluate if the lack of plant extract effect, may be attributed to the level of inclusion in the diets (2 g plant extract/Kg fed diet) used in the present study.

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