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Effect of Dietary Plant Lipids on Conjugated Linoleic Acid (CLA) Concentrations in Beef and Lamb Meats

Pilar Teresa Garcia and Jorge J. Casal

Additional information is available at the end of the chapter

1. Introduction

Beef and lamb, are a food category with positive and negative nutritional attributes. Ruminant meats are major sources for many bioactive compounds including iron, zinc and B vitamins. However they are associated with nutrients and nutritional profiles that are considered negative including high levels of saturated fatty acids (SFA) and cholesterol. It is well know that the low PUFA/SFA and high n-6/n-3 ratio of meats contribute to the imbalance in the in the fatty acid intake of today consumers [1]. Consumers are becoming more aware of the relationships between diet and health and this has increased consumer interest in the nutritional value of foods. Nutritionist advisers recommended a higher intake of polyunsaturated fatty acids (PUFA), especially n-3 PUFA at the expense of n-6 PUFA.

The nutritional beef and lamb profile could be further improved by addition of potentially health promoting nutrients. There are many references of improved fatty acid composition in grass fed beef. Besides the beneficial effects of n-3 fatty acids on human health one fatty acid that has drawn significant attention for its potential health benefits in the last two decades is conjugated linoleic acid (CLA). Conjugated linoleic acids (CLA) are implicated as anti-carcinogenic, anti-atherosclerosis, and anti-inflammatory agents in a variety of experimental model systems. It has been shown that in ruminants grazing have potential beneficial effects on PUFA/SFA and n-6/n-3 ratios, increasing the PUFA and CLA content and decreasing the SFA concentration of beef [2].

The total CLA content of beef varies from 0.17 to 1.35% of fat [3]. This wide range is related to the type feed, breed differences, and management strategies used to raise cattle [3, 4]. Grazing beef steers on pasture or increasing the amounts of forage (grass or legumes hay) in the diet has been shown to increase the CLA content in the fat of cattle. Also, supplementing

high-grain diets of beef cattle with oils (e.g., soybean oil, linseed oil, sunflower oil) may in-crease the CLA content of beef [3, 5].

There has been an increased interest in the substitution of animal fat sources with vegetable oils in animal nutrition. Vegetable oils have been attributed with reducing the level of satu-ration in monogastric animal tissues due to their unsaturated fatty acid concentration when compared with animal fat. In ruminants, dietary lipids were undergo two important trans-formations in the rumen. The initial transformation is the hydrolysis of the ester bond by microbial lipases. This initial step is a pre requisite for the second transformation, the biohy-drogenation of unsaturated fatty acids [6, 7].

Several factors influence the CLA content of beef as breed, sex, seasonal variation, type of muscle, production practices but diet plays the most important role. Dietary CLA from beef can be increased by manipulation of animal diets. CLA concentration in beef can be influ-enced by dietary containing oils or oilseeds high in PUFA, usually linoleic or linolenic fatty acids.These dietary practices can increase CLA concentrations up to 3 fold [5, 8]. Moreover, trans-11 18:1 (vaccenic acid,VA) is the precursor of cis-9,trans-11 18:2 (rumenic acid, RA) is the major CLA isomer in animal and humans and, therefore, it might be considered as a fat-ty acid with beneficial properties.

Soybean oil is one of the few plant sources providing ample amounts of both essential fatty acids 18:2 n-6 and 18:3 n-3. The fatty acid content of soy foods is often unrecognized by health professionals, perhaps because there is so much focus on soy proteins. Soybeans are used in cattle, poultry and pigs diets and could be a more important source of 18:3 n-3 for animal nutrition and also increase 18:3 n-3 and its fatty acids metabolites in meats. Genom-ics, specifically marker assisted plant breeding combined with recombinant DNA technolo-gy, provided powerful means for modifying the composition of oilseeds to improve their nutritional value and provide the functional properties required for various food oils [9].

Thus, the manipulation of the fatty acid composition in ruminant meat to reduce SFA con-tent and the n-6/n-3 ratio whilst, simultaneously increasing the PUFA and CLA contents, is the major importance in meat research. The supplementation of ruminant diets with PUFA rich lipids is the most effective approach to decrease saturated FA and promote the enrich-ment of CLA and n-3 PUFA.

2. CLA structure, biosynthesis and potential beneficial effects on human health

The CLA acronym refers to a group of positional and geometric isomers of linoleic acid, in which the double bands are conjugated. At least twenty four different CLA isomers have been reported as occurring naturally in food, especially from ruminant origin [10]. Isomeri-sation and incomplete hydrogenation of PUFA in the rumen produce several of octadeceno-ic, octadecadienoic and octadecatrienoic isomeric fatty acids [11] and, at least some of them, have powerful biological properties. The formation of conjugated dienes in the rumen dur-

ing biohydrogenation of lipids in feed was observed previously, however, the anticarcinogenic effect of beef extracts was first observed and later identified [12, 13, 14].

The dominant CLA in ruminant meats is the cis-9, trans-11 isomer (RA) which has being identified as possessing a range of health promoting biological properties including antitumoral and anticarcinogenic activities [15]. The rumenic acid is mostly produced in tissues by delta 9 desaturation of trans-11 18:1, (VA) and by ruminal biohydrogenation of dietary PUFA. The higher deposition of CLA in the neutral lipid fraction, 88% of total CLA relatively to phospholipid fraction, has been reported [16].The majority of the main natural isomer cis-9,trans-11 CLA does not originate directly from the rumen. Instead, only small amounts of CLA escape the rumen and trans-18:1 isomers are the main biohydrogenation intermediates available. El absorbed trans-11 18:1 is desaturated in the tissues by Δ9-desaturase to form RA [17]. Stearoyl-CoA (SCD) is a rate-limiting enzyme responsible for the conversion of SFA into monounsaturated fatty acids (MUFA). This enzyme, located in the endoplasmic reticulum, inserts a double band between carbons 9 and 10 into SFA and affects the fatty acid composition of membrane phospholipids, triglycerides and cholesterol esters [18]. SCD is also a key enzyme in the endogenous production of the cis-9,trans-11 isomer of conjugated linoleic acid (CLA). Trans octadecenoates (trans 18:1) are the major intermediates formed during rumen biohydrogenation of C18 PUFA. High trans-10 18:1 have been observed in tissues of concentrated-fed ruminants, whereas vaccenic acid is consistently associated with forage feeding [11, 19]. Evidence is accumulating that different trans 18:1 isomers have differential effects on plasma LDL cholesterol. Trans-9 and trans-10 18:1 are more powerful in increasing plasma LDL cholesterol than trans-11 18:1 [20]. Comparison of antiproliferative activities of different CLA isomers present in beef on a set of human tumour cells demonstrates that all CLA isomers possess antiproliferative properties. It appears that important to determine the variations of the distribution of CLA isomers in beef since these proportions could influence the biological properties of bioformed CLA [21].

3. Factors influencing CLA concentrations on beef lipids

Amounts of CLA in beef vary mainly with feeding conditions, nature and quality of forages, proportions between forage and concentrate, oil-seed supplementations, but also with intrinsic factors such as breed and sex and age of animals [22].

3.1. Breed, sex and age (Table 1)

Breed or genotype and production system are determinant factors of the fatty acid composition of the ruminant meats. Breed affects the fat content of meat and fat content itself is a factor determining fatty acid composition. Genetic variability relates to differences between breeds or lines, variation due to the crossing of breeds and variation between animals within breeds reported that it can be difficult to assess the real contribution of genetics to variation in the CLA content.

	CLA	Reference
Breed		
LD Limousin	2.24 g/100g	[29]
LD Angus	1.96 g/100g	[29]
LD Angus	0.51 b% FAME	[25]
LD Charolais x AA	0.57 a% FAME	[25]
LD Holando x AA	0.58a% FAME	[25]
LD Nguni grass	0.34% FA	[28]
LD Bonsmara grass	0.31% FA	[28]
LD Angus grass	0.33% FA	[28]
LD Holstein grass	0.84 % FA	[24]
LD Simmental grass	0.87% FA	[24]
LD Holstein concentrate	0.75% FA	[24]
LD Simmental concentrate	0.72% FA	[24]
SM Pasture and Silage Steers Longhorn	6.75a mg/100g	[23]
SM Pasture and Silage Steers Charolais	3.29b mg/100g	[23]
SM Pasture and Silage Steers Hereford	2.93b mg/100g	[23]
SM Pasture and Silage Steers B. Gallowey	5.09a mg/100g	[23]
SM Pasture and Silage Steers Beef Shorton	4.01ab mg/100g	[23]
Sub Pasture and Silage Steers Longhorn	1210a mg/100g	[23]
Sub Pasture and Silage Steers Charolais	651b mg/100g	[23]
Sub Pasture and Silage Steers Hereford	584b mg/100g	[23]
Sub Pasture and Silage Steers B. Gallowey	796 b mg/100g	[23]
Sub Pasture and Silage Steers Beef Shorton	808b mg/100g	[23]
Mertolenga PDO beef	0.39ab g/100g FA	[27]
Mertolenga PDO veal	0.46a g/100g FA	[27]
Vitela Tradicional do Montado PGI veal	0.35b g/100g FA	[27]
LT & LL Veal Limousin	1.09% FAME	[26]
LT & LL Veal Tudanka x Charolais	1.00% FAME	[26]
Sex and age		
LL bulls 14 month	0.37 % FA	[31]
LL bulls 18 month	0.39% FA	[31]
LL heifers 14 month	0.44% FA	[31]
LL heifers 18 month	0.41 % FA	[31]
L lumborum steers	0.20 % FA	[32]
L.lumborum bulls	0.21 % FA	[32]

Table 1. Conjugated linoleic acid (CLA) concentrations on beef according to breed, sex and age .a b Indicates a significant differences (at least $p < 0.05$) between breed, sex or age reported within each respective study. Abbreviations LD: Longissimus dorsi ; SM: Semimembranosus ; LL Longissimus lumborum; LT Longissimus thoracis; Sub: Subcutaneous fat.

Significant between-breed differences in CLA content were observed in both muscle and subcutaneous adipose tissue of five breeds of cattle with the highest values in Longhorn and with the lowest in Hereford [23]. German Holstein bulls accumulated a higher amount of CLA compared with German Simmental bulls [24]. CLA percentages were affected by breed with the low values for Angus beef compared with Charolais x Angus and Holstein Argentine steers [25]. The content of trans -10 C18:1 isomer tended to be higher in Limousin compared to Tudanca meat when expressed as mg/100g of meat, and the difference was only significant when expressed in terms of relative percent. The higher level of trans-10 C18:1 was consistent with the greater consumption of concentrate by Limousin calves [26]. Within a similar production system the age/weight, gender and crossbreeding practices have minor effects on muscle FA composition but Mertolenga-PDO veal has higher total CLA contents that PDO beef and PGI veal [27]. On the contrary the cis-9, trans-11 CLA levels among steers of Nguni, Bonsmara and Angus breeds raised on natural pasture were similar [28]. Similar results were found comparing the CLA content of Limousin and Aberdeen Angus beef [29].

Sex and age differences in muscle FA contents are often be explained by the degree of fatness and associated changes in the triacylglycerol/phospholipid ratio [30]. Sex-dependent differences in the FA composition of muscle an adipose tissue from cattle slaughtered at different ages were demonstrated [31]. Concentration CLA in meat beef not affected by castration [32].

3.2. Type muscle and anatomical location (Table 2)

Little work has been conducted to assess the effects of slaughter season and muscle type on meat CLA profile. The type of muscles strongly influenced proportions of total CLA and of all CLA isomers classes in intramuscular fatty acids (Table 2). CLA is mainly associated to the triacylglycerol fraction which is linked to the fat content of tissues [21]. VA and CLA percentages were lower in lean muscle than subcutaneous fat or marbling [33]. The CLA content of steaks differs depending on the location of the fat, CLA level was almost doubled in outer subcutaneous fat compared to lean muscle [34]. There was significant differences in the concentration of CLA among depot sites through-out a bovine carcass. The brisket contained a higher concentration of cis-9, trans-11 CLA but no significant differences in the concentrations of trans-10, cis-12 CLA among the locations [35].

3.3. Season and pasture type (Table 2)

No differences between dietary grass silage and red clover silage were detected on CLA content of LD muscle of dairy cull cows [36]. Total CLA content was lower (p< 0.05) in intensively produced beef than in Carnalentejana-PDO meat, which did not show significant differences (p<0.05) when the slaughter season was compared. Furthermore *Longissimus thoracis* (LT) muscle had a higher (p<0.001) total CLA content relative to *Longissimus dorsi* (LD) muscle. In addition no significant differences (p<0.05) regarding specific CLA content were observed when slaughter season, production system and muscle type were analyzed [37]. Significant interactions between the slaughter season and muscle type were obtained for several fatty acid and CLA isomers and for total lipid and CLA. Mirandesa –PDO veal showed seasonal differences in the levels of CLA isomers but the CLA content was affected by much more influence by the muscle type [38]. The variation of CLA milk fat content during pasture season

might be related to the alfa-linolenic/linoleic acid ratio in the pasture. The ratio in the average pasture sample decreased from 4.36 in May to 1.97 in August, and subsequently it increased to 3.14 in September, thus close to that at the beginning of pasture season. Thus the seasonal variation of the ratio in pasture were directly proportional to the corresponding content of CLA in ewe milk fat [39].

	CLA	Reference
Muscle and adipose tissue location		
Steak muscle	0.30b % FA	[33]
Steak marbling	0.50a % FA	[33]
Outer subcutaneous fat	0.50a % FA	[33]
Inner subcutaneous fat	0.50a% FA	[33]
Seam	0.40ab % FA	[33]
Adipose tissue brisket	0.70a g/100g FA	[35]
Adipose tissue chuck	0.62ab g/100g FA	[35]
Adipose tissue flank	0.56b g/100g FA	[35]
Adipose tissue loin	0.53b g/100g FA	[35]
Adipose tissue plate	0.57b g/100g FA	[35]
Rib	0.52b g//100g FA	[35]
Round	0.63ab g/100g FA	[35]
Sirloin	0.57b g/100g FA	[35]
LT concentrate	4.45 mg/g fat	[37]
ST concentrate	3.88 mg/g fat	[37]
Season		
LT Autumn	5.07 mg/g fat	[37]
LT Spring	4.92 mg/g fat	[37]]
ST Autumn	3.82 mg/g fat	[37]
ST Spring	5.06 mg/g fat	[37]
L L Spring	0.30 a g/100g FA	[34]
L L Autumn	0.31a g/100g FA	[34]
ST Spring	0.23 b g/100g FA	[34]
ST Autumn	0.19b g/100g FA	[34]]
Pasture type		
LD Tall fescue	0.28%	[91]
LD Alfalfa	0.37%	[91]
LD Red clover	0.30%	[91]
LD cull cows grass silage	0.22 % TFA	[36]
LD cull cows red clover silage	0.17 % TFA	[36]

Table 2. Conjugated linoleic acid (CLA) concentrations on beef according to muscle and adipose tissue location, season and grass composition a b Indicates a significant differences (at least p<0.05) between anatomical location, season or pasture type reported within each respective study. Abbreviations LD: Longissimus dorsi ; SM: Semimembranosus ; LL Longissimus lumborum; LT Longissimus thoracis

3.4. Grass vs. concentrate (Tables 3 & 5)

A direct linear relation between grass percentage in cattle diet and meat CLA content has been described by [2] although the mechanism remains controversial. They suggested that grass in the diet enhances the growth of ruminal bacterium *Butyrivibrio fibrisolvens* which convert 18:2 n-6 into cis-9, trans-11 CLA isomer through the action of a linoleic acid isomerase. Others [40] proposed that the increased content of CLA in animals fed forage- based diets is associated with an increase in trans-11 18:1, which is the substrate of stearoyl-CoA desaturase in tissues. It is generally accepted that the concentrations of CLA can be increased in beef by increasing the forage to concentrate ratio, and by feeding fresh grass instead of grass silage [4, 22] (Table 3). Beef contains both of the bioactive CLA isomers, namely, cis-9, trans-11 and trans-10, cis-12. Many reports demonstrated that cis-9, trans 11 CLA is a major fatty acid in tissue and little or no trans-10, cis 12 CLA was detected [5,41]. High trans-10 18:1 have been observed in tissues of concentrated-fed ruminants, whereas vaccenic acid is consistently associated with forage feeding [11, 19]. Significantly higher contents of trans-18:1 were found in animals fed on concentrate diets relative to the pasture diet. This is mainly due to the trans-6, trans-8, trans-9 and trans-10 isomers, since the trans-11 and trans-12 18:1 remains unaffected by the dietary treatments. The feeding systems, pasture only, pasture feeding followed by 2 or 4 months of finishing on concentrate, and concentrate only, had a major impact on the concentration of CLA isomers from bull LD muscles. Beef fat from pasture-fed animals had a higher nutritional quality relative to that from concentrate-fed bulls and the feeding regimen had a major impact on the CLA isomeric distribution of beef affecting 10 of 14 CLA isomers. The CLA isomeric profile showed a clear predominance of the cis-9, trans-11 isomer for all diets [42]. The grass silage diets increased the proportions of trans-11 18:1 and cis-9, trans-11 18:2. Feeding a high forage diet may therefore have increased the rate of appearance of trans-11 18:1 in the rumen, providing more substrate for the endogenous production and deposition of CLA in bovine tissues [43]. This hypothesis is consistent with an increase of trans-11 18:1 concentration with no effect on cis-9, trans-11 in duodenal content of Hereford steers fed increasing levels of grass hay [44]. The relative flow of PUFA through the major biohydrogenation pathways, trans-10 or trans-11, 18:1, can be judged by the 11t-/10t- 18:1 ratio with a higher ratio denoting an improvement in its healthfulness to its human consumers [45]. Backfat composition was compared in steers fed either a control (barley grain based) diet or diets containing increasing levels of corn or wheat derived dried distillers'grains with solubles (DDGS). Back fat from control and wheat derived DDGS fed steers had lower levels of trans-18:1 and a higher 11trans/10 trans 18:1 ratio compared to back fat from corn derived DDGS fed steers [45]. The explanation might be found in ruminal biohydrogenation pathway of LA and ALA. Most of the cis-9, trans-11 CLA isomer present in tissues derive from endogenous desaturation of trans-11, 18:1, which originates during biohydrogenation of 18:2 n-6 and 18:3 n-3. The CLA concentrations in three different muscles of pasture- or feedlot-finished cattle were greater from pasture-finished than from cattle feedlot-finished [46]. The absolute cis-9, trans-11 CLA was about twice as high in Asturiana de la montaña (AV) and Asturiana del Valle (AV) animals than in other AV genotypes, probably due to the much higher fat content of the AM and AV animals [47]. This effect was also found in other studied were cis-9, trans-11 content variation was influenced by the total lipid content, and hence with variation in the neutral lipid fraction [48]. A linear correlation between VA and cis-9, trans-11 CLA was observed in several studies [8] and in other studied no significant correlation was found [49]. Breed or genotype effects could act by enhancing or

inhibiting the Δ9-desaturase activity. The major isomers in beef fed a high barley diet is trans-10, 18:1 rather than trans-11, 18:1. In feedlot finished beef fed a diet containing 73% barley was found 2.13 % of trans-10 18:1 and only 0.77% of trans-11 18:1 in subcutaneous fat [19, 50]. Feeding ruminants diets with high levels of barley (low fiber, high starch) reduces rumen pH, alters the bacterial flora and causes a shift in the biohydrogenation pathway towards producing trans-10 18:1 instead of trans-11 18:1 [51]. Subcutaneous fat is quite sensitive to changes in diet and rumen function. This is due to adipose tissue having a high proportion of neutral lipids which accumulate greater levels of PUFA biohydrogenation products relative to polar lipids [24]. In addition, subcutaneous fat is easily accessible, inexpensive and levels of trans-18:1 have been reported to be linearly related to those found in muscle [52]. Vaccenic acid made up the greatest concentration of total trans fats in grass-fed beef, whereas CLA accounted for approximately 15% of the total trans fats [53].

	CLA	Reference
LD Grazing	10.8a mg/g fat	[2]
LD Concentrate-fed	3.7b mg/g fat	[2]
LD Grazing	5.3a mg/g fat	[90]
LD Concentrate-fed	2.5 b mg/g fat	[90]
LD Pasture	0.72 % FAME	[25]
LD Pasture +0.7% corn	0.61 % FAME	[25]
LD Pasture+1.0 %corn	0.58% FAME	[25]
LD Feedlot	0.31 % FAME	[25]
LD Grass silage (GS)	3.62% FA	[43]
LD GS +Low concentrate	2.50% FA	[43]
LD GS+ High concentrate	2.72% FA	[43]
LT Semi-intensive 12 month	0.49a %	[92]
LT Semi-intensive 14 month	0.49a %	[92]
LT Intensive 12 month	0.25b %	[92]
LT Intensive 14 month	0.29b %	[92]
Ground control	0.50b g/100g	[53]
Ground grass	0.94a g/100g	[53]
Steaks control	0.38b g/100g	[53]
Steaks grass	0.66a g/100g/	[53]
Control	0.82 % FA	[45]
Back fat 20% DDGS corn	0.88 % FA	[45]
Back fat 20% DDGS wheat	0.88 % FA	[45]
Back fat 40%DDGS corn	0.97 % FA	[45]
Back fat 40% DDGS wheat	0.81 % FA	[45]
LT concentrate	4.45 mg/g fat	[37]
ST concentrate	3.88 mg/g fat	[37]

Table 3. Conjugated linoleic acid (CLA) concentrations on beef under dietary grass or concentrate a b Indicates a significant differences (at least p<0.05) between dietary grass or concentrate reported within each respective study. Abbreviations LD: Longissimus dorsi ; Longissimus thoracis ; ST: Semitendinosus

3.5. Oil supplementation (Tables 4 & 5)

The most common method of enhancing the CLA and VA content of ruminant meat and dairy products is to provide the animal with additional dietary unsaturated fatty acids, usually from plants oils such as soybean oil (SBO), for use as substrates for ruminal biohydrogenation [4]. Steers fed a corn-based diet supplemented with SBO may enhance TVA without impacting CLA, while reducing the MUFA content of lean beef [54]. Both oilseed and free oils affect CLA content in a similar manner. Free plant oils with high PUFA concentrations are normally not included in ruminant diets as high levels of dietary fat disturb the rumen environment and inhibit microbial activity. The main sources of supplementary fatty acids in ruminant rations are plant oils and oilseeds, fish oils, marine algae and fat supplements. Since dietary inclusion of fatty acids must be restricted to avoid impairment of rumen function, the capacity to manipulate the fatty acid composition by use of ruminally available fatty acids is limited [55]. Many researchers have found higher CLA content in muscle lipids by supplementing with different oils. However, some studies reported no significant differences in CLA content due to oil supplementations. The differences in responses to plant oils were probably due to variations in stage of growth of cattle, levels of oil supplementation, levels of oil in total ration and amount of linoleic acid in oils. Researchers have successfully increased CLA content by supplementation of different oils [4,48,56]. Others [3] supplementing with 4% SBO to diets did not affect the CLA. Similar to [41] who reported that feeding 5% SBO no affected CLA but increased trans10-cis-12 CLA. The addition of different vegetable oils to the bulls diet (soybean or linseed, either protected or not protected from rumen digestion) increased the CLA content, with an average CLA value of 0.72 %. The increase of CLA was also due to the addition of oils presenting large quantities of its precursor LA in diets with unprotected soybean and linseed oils [57]. Diets containing silage and concentrate or sugarcane and sunflower seeds fed Canchim- breed animals, produce an improvement in CLA levels (0.73g/100g vs. 0.34g/100) [58]. Rapeseed oil and whole rapeseed do not seem to have positive effects. Of the three studied none showed increased CLA concentrations in the LDi after supplementation with 6% rapeseed oil [41]. Soybean oil (SBO) has been used as a source de LA throughout the finishing period to promote greater CLA accretion in lean tissues with equivocal results [56, 41]. and where CLA accretion was increased with SBO addition, growth performance was reduced [56]. Fed steers with 5% of soybean oil in a finishing experiment for 102 days had no effects in meat cis-9, trans, 11 CLA [41]. In a study with steers, supplementation of 4% soybean oil to a finishing diet based on concentrate and forage (80:20) resulted in a depression of the CLA deposition in muscle tissues (2.5 vs. 3.1 mg/g FAME) compared to the same diet without soybean oil On the other hand, comparing 4% with 8% added soybean oil in a 60:40 concentrate : forage diet showed a numerical increase of the CLA content with the higher soybean supplementation (2.8/3.1 mg/g FAME) [59]. The inclusion of sunflower oil in the diets (80% barley, 20% barley silage) of finishing cattle at 0%, 3%, or 6% increased the CLA content of the beef by 75% when cattle were fed 6% sunflower oil [4]. Although supplementation with oil or oil seeds increased CLA content in muscle, the inclusion of linoleic acid –rich oil or oilseeds such as safflower or sunflower, in the diet of ruminants appears to be the most effective [60]. Supplementation of cattle with a blend of oils rich in n-3 PUFA and linoleic acid results in a synergistic accumulation of rumi-

nal and tissue concentrations of TVA [61]. VA is the substrate for Δ9 –desaturase- catalyzed de novo tissue tissue synthesis of cis-9 trans-11 isomer of CLA. However, despite increases in its substrate, muscle tissue concentrations of cis-9, trans-11 CLA have not increased by using this strategy [62]. Inclusion of extruded linseed in the diet of Limousin and Charolais cattle, increase CLA [63]. The importance of the contribution of TVA to total CLA intake is further reinforced by a French study [64] in which a huge 233% increase of VA was shown, along with 117% increase of RA, which was caused by adding extruded linseeds into the animal fodder. Several authors reported that diets containing proportionally high levels of linolenic acid, such as fresh grass, grass silage, and concentrates containing linseed, resulted in increased deposition of the cis-9, trans-11 CLA isomer in muscle [65]. The biohydrogenation by rumen microorganism does not include the cis-9, trans-11 CLA isomer as an intermediate. The trans-11 18:1 is the common intermediate during the biohydrogenation of dietary linoleic acid and linolenic acid to stearic acid [6]. Since only a relatively small percentage the cis-9, trans-11 CLA isomer, formed in the rumen, is available for deposition on the muscles, the major source of this isomer in muscle results from the endogenous synthesis involving Δ 9 desaturase and vaccenic acid [17].. Hereford steers cannulated in the proximal duodenum were used to evaluated the effects of forage and sunflower oil level on ruminal biohydrogenation and conjugated linoleic acid. Flow of trans-10 18:1 decreased linearly as dietary forage level increased whereas trans -11 18:1 flow to the duodenum increased linearly with increased dietary forage. Dietary forage or sunflower oil levels did not alter the outflow of cis-9, trans-11 CLA [44]. Linseed supplementation was an efficient way to increase CLA proportion in beef (+22% to 36%) but was highly modulated by the nature of the basal diet, and by intrinsic factors as breed, age/sex, type of muscle, since these ones could modulate CLA proportions in beef from 24% to 47% [21]. Soybean oil, which is rich in linoleic acid, has been found in several studied [66,67,] to be more efficient than linseed oil, which is rich in linolenic acid, in increasing the CLA content of milk. In beef cattle the addition of 3% and 6% sunflower oil to a barley based finishing diet results in increased CLA content in LD muscle: 2.0 vs 2.6 vs. 3.5 mg/g lipid for control, 3%,, and 6% sunflower oil, respectively. A more substantial increase in the CLA concentration was found when sunflower oil was added to both the growing and finishing diet of beef cattle.[68,69]. 4.3, 6,3 and 9.1 mg CLA / g FAME in LD muscle lipids of heifers, were found, after supplementing the feed with 0, 55, and 110 g sunflower oil per kg of the diet for 142 days before slaughter [48]. Supplementation of a high forage fattening diet with either soybean oil or extruded full fat soybeans at a level of 33g added oil per kg of diet DM resulted in a 280-410 % increase in the concentration of CLA in the intramuscular and subcutaneous lipid depots of fattening Friesian bull calves The content of VA in both lipid depots were also increased about three-fold by this oil supplementation [70].

	CLA	Reference
Concentrate IMF fat	3.4 b mg/g fat	[70]
Soybean oil IMF fat	13.0 a mg/g fat	[70]
Extruded soybean IMF fat	15.4 a mg/g fat	[70]

	CLA	Reference
Concentrate Sub fat	5.2 c mg/g fat	[70]
Soybean oil Sub fat	20.3 b mg/g fat	[70]
Extruded soybean Sub fat	26.6 a mg/g fat	[70]
LD concentrate / silage	0.41d % FA	[93]
LD Grass	0.70c % FA	[93]
LD grass +sunflower oil	1.34a % FA	[93]
LD grass +linseed oil	0.93b% FA	[93]
LD Wagyu Control	0.27 b % FA	[68]
LD Wagyu 6% sunflower oil	1.29a % FA	[68]
LD Limousin Control	0.28b % FA	[68]
LD Limousin 6% sunflower oil	1.19a % FA	[68]
LM grass	0.73c % FA	[48]
LM grass+ sunflower oil	1.78a % FA	[48]
LM grass+linseed oil	1.26b % FA	[48]
LM Corn oil 0%	0.68b % FA	[94]
LM Corn oil 0.75%	0.85a % FA	[94]
LM Corn oil 1.5%	0.81ab % FA	[94]
LT Control	0.33% FA	[95]
LT Control + Vit E	0.36 % FA	[95]
LT Control	0.34 % FA	[95]
LT Control+ flaxseed	0.34 % FA	[95]
LD Control	0.35 c mg/100g FA	[57]
LD Soybean oil	0.94a mg/100g FA	[57]
LD Linseed oil	0.80a mg/100g FA	[57]
LD Protected linseed oil	0.55b mg/100g FA	[57]
LM grass NL	0.78c g/100g FA	[48]
LM grass+sunflower oil NL	1.90a g/100g FA	[48]
LM grass+linseed oil NL	1,35b g/100g FA	[48]
LM grass PL	0.32c g/100g FA	[48]
LM grass+sunflower oil PL	0.71a g/100g FA	[48]
LM grass+linseed oil PL	0.51b g/100g FA	[48]

Table 4. Conjugated linoleic acid (CLA) concentrations on beef under dietary oils supplementation. a b Indicates a significant differences (at least p<0.05) between dietary oil supplementations reported within each respective study. Abbreviations LD: Longissimus dorsi ; SM: Semimembranosus ; LT Longissimus thoracis; Sub: Subcutaneous fat; NL: neutral lipids; PL: Phospholipids.

Diet P vs. C	Trans- 11 18:1	Trans- 10 18:1	Reference
C	0.92	1.21b	[42]
P+4month C	1.10	0.81b	[42]
P+2month C	1.15	0.98b	[42]
P	1.35	0.20a	[42]
Ground control	1.14	2.69	[53]
Ground grass	4.14	0.75	[53]
Steaks control	0.51	3.60	[53]
Steaks grass	2.95	0.60	[53]
Grass silage (GS)	2.03a	Na	[43]
GS +Low C	1.37b	Na	[43]
GS+ High C	1.15b	Na	[43]
Control	0.65	2.02	[45]
20% DDGS corn	0.78	2.37	[45]
20%DDGS wheat	0.74	1.60	[45]
40% DDGS corn	0.92	3.16	[45]
40%DDGS wheat	0.69	1.33	[45]
LT et LL Tudanca x Charolais	2.68	0.36b	[26]
LT et LL Limousin	2.24	1.01 a	[26]

Table 5. Trans-11 and trans 10 C18:1 isomer proportions on beef under different conditions. a b Indicates a significant differences (at least p<0.05) between trans-10 C18:1 and trans-11 C18:1 reported within each respective study. "na" indicates that the value was not reported in the original study. Abbreviations LD: Longissimus dorsi ; LL Longissimus lumborum LT: Longissimus thoracis.

4. Factors influencing CLA concentrations on lamb lipids

In lamb production, more than other species, each country or region has its own specific weight/age and type of carcass criteria, depending on the culture and the customs of the people. Many factors including breed, gender, age/body weight, fatness, depot site, environmental condition, diet and rearing management influence lamb fat deposition and composition. Further studied are needed to understand how animal circadian rhythms, diurnal rumination patterns and daily changes in herbage chemical composition could affect lamb fatty composition [71].

4.1. Production system (Tables 6 & 8)

No differences were detected in the muscle CLA/ trans-11 18:1 index of herbage or concentrate –fed lambs but the supplementation of tanino produced strong effects on the accumulation of fatty acids which are involved in the biohydrogenation pathway [72]. During two years (Y1 and Y2) lambs were under four diets. Only silage both pre and post weaning (SS), only silage until weaning, silage plus concentrate thereafter (SC), silage plus concentrate both pre and post weaning (CC) and silage plus concentrate before weaning, only silage after (CS). Treatment differences for trans-11 18:1 were presented only in Y1, with muscle from the lamb fed silage before weaning having the highest levels. The same groups has the highest levels of cis-9, trans 11 CLA in Y1. Similar in Y2 the group SS has the highest CLA level, while the CC group has the lowest [73]. The feeding strategy around parturition influence the CLA and VA content of lamb meat. Pre-partum grazing, regardless of post-partum feeding, can improve the fatty acid composition, increasing the CLA content in lamb meat [74]. The meat of lambs slaughtered at Christmas has a higher CLA content than those reared in winter (slaughtered at Easter) as a result of the traditional feeding system which provided that lambs born and reared in autumn receive milk from ewes permanently pastured while those reared in winter are suckled by ewes permanently stall-fed [75]. The grazing on *T.subterraneum* as monoculture, associated with *L. multiflorum* in the proportion T/L=66/33 incremented cis-9,trans-,11 CLA of *L. dorsi* muscle of lambs [76]. The meat fatty acid profile was affected by the grazing management: compared to a morning-grazing or to a whole day-grazing management. Allowing lambs to gaze in the afternoon resulted in a meat fatty acid profile richer in CLA. In particular, in the 4hPM meat there is a greater proportion of those fatty acids arising from ruminal biohydrogenation, among them the CLA [71].

	CLA mg/g fat	Reference
LD Grass pellets	1.29a % FA	[40]
LD Concentrate diet	1.02a % FA	[40]
LD Concentrate diet *Ad libitum*	0.74b % FA	[40]
Muscle Concentrate+concentrate CC	0.46b g/100g lipids	[73]
Muscle Silage+concentrate SC	0.61a g/100g lipids	[73]
Muscle Concentrate+silage CS	0.45b g/100g lipids	[73]
Muscle Silage+silage SS	0.65a g/100g lipids	[73]
LT Pre-partum hay	1.42b % FA	[74]
LT Pre-partum grazing	1.66a % FA	[74]
LT Post-partum hay	1.35b % FA	[74]
LT Post –partum grazing	1.73a % FA	[74]
LD Grassed 9 am to 5 pm	1.85b g/100g FAME	[71]
LD Grassed 9 am to 1 pm	1.45b g/100g FAME	[71]
LD Grassed 1 pm to 5 pm	2.39a g/100g FAME	[71]
LL Sucking lamb Autumn	1.10% IM Fat	[75]
LL Sucking lamb Winter	0.56 % IM Fat	[75]

	CLA mg/g fat	Reference
LD Grazing subterraneous clover	0.46a % FA	[76]
LD Grazing Italian rye grass	0.26 b % FA	[76]
Pasture LD	0.90b % FAME	[96]
Pasture Leg muscles	1.27a % FAME	[96]
Pasture LD total lipids	0.90 % total FAME	[96]
Pasture LD Triacylglycerols	0.62 %total FAME	[96]
Pasture LD Phospholipids	0.11 % total FAME	[96]

Table 6. Conjugated linoleic acid (CLA) concentrations on lamb meat according to concentrate, pasture, muscle type and season. a b Indicates a significant differences (at least p<0.05) between values reported within each respective study. Abbreviations LD: Longissimus dorsi ; ST: Semitendinosus ; LL Longissimus lumborum; ; LT: Longissimus toracsis; SM: Semimembranosus.

4.2. Oil supplementation (Tables 7& 8).

Several strategies have been tested in recent years to improve CLA isomers in meat of inten-sively–reared lambs, keep indoors and fed high-concentrate diets rich linoleic acid and poor in linolenic. Incorporating linseed rich, in linolenic acid, the proportion of trans-11, 18:1 and cis-9, trans-11 18:2 were higher in the muscle and in the adipose tissues of linseed –fed lambs than in control lambs [77]. This increased is in contrast to results of [78] but in agree-ment with [79]. Discrepancias between these studies may due to differences in the level of intake the linoleic and linolenic acids or the different level of Δ9- desaturase inhibition as it has been shown that Δ9 desaturase is inhibited by PUFA with increasing inhibition as the degree of fatty acid unsaturation increases. Fed lambs from weaning to slaughter with diets that contained 5% supplemental from high oleic acid safflower or normal safflower in-creased the meat cis-9,trans,11 CLA compared with the control group [80] In lambs inclu-sion of 8% of soybean oil to a lucerne hay-based diet resulted in an intramuscular (M. L*ongissimus thoracis*) CLA content of 23.7 compared with 5.5 mg/g FAME in the control group [81]. Feeding soybean and linseed oils to lambs pre and post weaning did not increase CLA content of muscle, whereas post weaning oil supplementation minimally increased CLA concentration in subcutaneous fat [82]. Conflicting results have been reported on alter-ing FA content of meat supplementing ruminant diets with lipid sources high in linoleic and linolenic acids. Some research suggests supplementing CLA, linoleic or linolenic acids in high concentrate fed to lambs can increase CLA content in muscle [83], whereas supplemen-tation of linoleic in finishing diets fed to cattle had no effects on CLA in adipose or muscle tissue [3,41]. Feeding lipid sources rich in linoleic and linolenic increases the cis-9,trans-11 18:2 content of ruminant meats [21,81, 83,84]. However, feeding linseed oil, rich in linolenic acid, seems to be less effective in the increases of cis-9, trans-11 18:2 in muscle than sunflow-er oil, rich in linoleic acid [45,48]. Seems to be that a blend of sunflower and linseed oils may be a good approach to obtain an enrichment in CLA in lamb meat. Maximun CLA concen-trations (42.9 mg/100 g fresh lamb tissue) was observed with 100% of sunflower, decreasing linearly at 78% by sunflower oil with linseed oil replacement [86]. A consistent significant increase in CLA content in lamb tissues was observed with dietary supplementation with

6% of safflower oil. The CLA concentration in several lamb tissues was increased by more than 200% [39]. These results indicated that supplementation of lamb feedlot diets with a source of LA was a successful method of increasing CLA content of tissues. Merino Branco ram lambs initially fed with concentrate showed a lower proportions of cis-9,trans-11 18:2 CLA (0.98% vs. 1.38% of total fatty acids) than lambs initially fed with Lucerne. Initial diet did not compromise the response to the CLA promoting diet (dehydrated lucerne plus 10% soybean oil) and the proportion of cis-9,trans-11 C18:2 CLA in intramuscular fat increased with the duration of time on the CLA-promoting diet (1.02% vs. 1.34% of total fatty acids) [87]. Supplementation of oilseed with different levels of oleic (rapeseed), linoleic (sunflower and safflower seeds), and linolenic acid (linseed) on trans-11 18:1 and CLA isomers on ewe different tissues showed that the percentage of trans-11 C18:1 averaged around 4.56 % of total fatty acids for all supplements and tissues [88]. Increasing dietary forage and soybean oil did not change the sheep mixed ruminal microbes concentration of vaccenic acid but increased rumenic acid [89].

	CLA mg/g fat	Reference
LD Sunflower oil	2.13a mg/100 g muscle	[86]
LD Sunflower oil+ 33% linseed oil	2.06 a mg/100 g muscle	[86]
LD Sunflower oil + 66% linseed oil	1.84b mg/100g muscle	[86]
LD Linseed oil	1.56 c mg/100g muscle	[86]
Leg control	1.78a mg/g fat	[97]
Leg CLA	1,50a mg/g fat	[97]
Leg Safflower oil	4.41b mg/g fat	[97]
Adipose tissue Control	2.77 b mg/g fat	[97]
Adipose tissue CLA	2.60 b mg/g fat	[97]
Adipose tissue Safflower oil	7.33 a mg/g fat	[97]
LD control	3955 b ppm in muscle	[98]
LD Control + 5% sunflower oil	8491a ppm in muscle	[98]
Fat control	4947b ppm in fat	[98]
Fat control +5% sunflower oil	11313a ppm in fat	[98]
LT Control	0.75c % of FA	[87]
LT Control+Lucerne+10% soybean oil	1.21b % of FA	[87]
LT Lucerne	1.28 b% of FA	[87]
LT Lucerne+Lucerne+10% soyben oil	1.47 a% of FA	[87]
Muscle Control no fat	0.05 b mg/g muscle	[77]
Muscle control+wheat+linseed	0.11 a mg/g muscle	[77]
Muscle control+corn+linseed	0.12 a mg/g muscle	[77]
LL Control	0.60b g/100g FAME	[88]
LL Linseed	0.72b g/100g FAME	[88]
LL Rapeseed	0.70b g/100g FAME	[88]
LL Safflower seed	0.96a g7100g FAME	[88]
LL Sunflower seed	0.98a g/100g FAME	[88]

Table 7. Conjugated linoleic acid (CLA) concentrations on lamb meat according to dietary oil. a b Indicates a significant differences (at least p<0.05) between valus reported within each respective study. Abbreviations LD: Longissimus dorsi ; LL Longissimus lumborum; LT Longissimus thoracis

	Trans- 11 18:1	Trans- 10 18:1	Reference
LD Grazing subterraneous clover	4.22	Na	[76]
LD Grazing Italian rye grass	3.65	Na	[76]
LD Grass 9 am to 5 pm	1.55a	Na	[71]
LD Grass 9 am to 1 pm	1.06b	Na	[71]
LD Grass 1 pm to 5 pm	1.60a	Na	[71]
LD grass pellets	2.25a	0.38b	[40]
LD concentrate	1.39b	1.54a	[40]
LD concentrate ad lib	0.85b	1.73a	[40]
LD concentrate	79.4	Na	[72]
LD Herbage	31.4	Na	[72]
Silage-silage	1.54a	Na	[73]
Silage-concentrate	1.45a	Na	[73]
Concentrate-concentrate	1.08b	Na	[73]
Concentrate-silage	1.14b	Na	[73]
LM Pre Control	0.25	5.13b	[82]
LM Pre Control +oil	0.29	6.02a	[82]
LM Post Control	0.25	4.30b	[82]
LM Post Control+ oil	0.29	6.81a	[82]
Sub Pre Control	0.29	9.85b	[82]
Sub Pre Control +oil	0.31	8.25b	[82]
Sub Post Control	0.28	7.09b	[82]
Sub Post Control+ oil	0.33	11.01a	[82]

Table 8. Conjugated linoleic acid (CLA) isomer proportionson lamb under different condicions. a b Indicates a significant differences (at least $p<0.05$) between values reported within each respective study. "na" indicates that the value was not reported in the original study. Abbreviations LD: Longissimus dorsi ; LM: Semimembranosus; Sub: Subcutaneous

5. Conclusions

Several factors influence the CLA content of ruminant meats as breed, sex, seasonal variation, type of muscle, production practices but diet plays the most important role. CLA concentration in beef and lamb can be influenced by dietary containing oils or oilseeds high in PUFA, usually linoleic or linolenic fatty acids. The supplementation of ruminant diets with PUFA rich lipids is the most effective approach to decrease saturated FA and promote the enrichment of CLA and n-3 PUFA. The differences in responses to plant oils were probably due to variations in stage of growth of animals, levels of oil supplementation, levels of oil in total ration and amount of linoleic acid in oils. Thus, the manipulation of the fatty acid composition in ruminant meat to reduce SFA content and the n-6/n-3 ratio whilst, simultaneously increasing the PUFA and CLA contents, is the major importance in meat research.

Author details

Pilar Teresa Garcia[1*] and Jorge J. Casal[2]

*Address all correspondence to: pgarcia@cnia.inta.gov.ar

1 Area Bioquimica y Nutricion. Instituto Tecnologia de Alimentos. Centro de Investigacion en Agroindustria. Instituto Nacional Tecnologia Agropecuaria. INTA Castelar, CC77(B1708WAB) Moron, Pcia Buenos Aires, Argentina

2 Universidad de Moron. Facultad de Ciencias Agroalimentarias. Cabido 137. Moron , Pcia Buenos Aires, Argentina

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