Agricultural Journal 9 (2): 103-107, 2014
ISSN: 1816-9155

Conjugated Linoleic Acid (CLA) in Samples of Beef and Factors That Affect

[1]Jose Luis de la Cruz Gamez, [1]Jose A. Toca Ramirez, [1]Esperanza Herrera Torres,
[1]Manuel Murillo Ortiz, [2]Juan Ramon Esparza Rivera and [1]Francisco O. Carrete Carreon
[1]Facultad de Medicina Veterinaria Y Zootecnia,
[2]Facultad de Ciencias Quimicas, UJED, 58130 Morelia, Michoacan, Mexico

Abstract: The positive sensory and nutritious attributes of ruminant meat have been overshadowed in recent years by the perception involving a high contribution of saturated fat to the diet with negative health effects. However along with dairy products, beef has been the largest source of Conjugated Linoleic Acid (CLA) which could have several benefits for human health. This study evaluated in laboratory data from 50 beef samples taken at random from a beef slaughtering plant of Federal Inspection type (TIF) for CLA determination, using the Gas Chromatography method (GC), the impact of factors related to animals (feeding system, feed type, sex, age, breed and origin) on the CLA proportion in the longissimusdorsi muscle. Among these factors, only significant differences ($p<0.05$) were found for the origin of the animals in the proportion of CLA content in different places of origin. However, intrinsic factors (breed, age, sex and type of muscle) could modulate the CLA proportion in beef from 24-47%. The specific biological properties of these isomers should determine the understanding and consequences in the variations of intramuscular CLA isomers for health of consumers.

Key words: Beef, conjugated linoleic acid isomers, breed, gender, age, diet

INTRODUCTION

Linoleic acid is an unsaturated 18-carbon fatty acid with two double bonds in positions 9 and 12 and both are in the cis configuration (Ip *et al*., 1994). Conjugated Linoleic Acid (CLA), contains cis and trans isomers in carbons 8-12 (Ip *et al*., 1994) and is naturally produced during the naturalbiohydrogenation of linoleic acid (18:2) by bacteria in the rumen (Lock *et al*., 2005; Parodi, 2004).

Cis-9 and 12, trans-10 and 11 are considered as potential anti-oxidants, anti-carcinogen, anti-obesity and immune modulator agents (Park *et al*., 1999). The cis-9, trans-11 $C_{18:2}$ isomer, present in milk or meat of ruminants can be absorbed as such into the gastrointestinal tract or endogenously synthesized from vaccenic acid (trans-11 $C_{18:1}$ (Bauman *et al*., 2000a), although the latter pathway is that of greater relative importance. In both cases the precursors of these isomers (dietary polyunsaturated fatty acids, linoleic and linolenic acids), once ingested undergo a process of incomplete hydrogenation in the rumen. As a consequence of this unique process of ruminants vaccenicacid is accumulated due to the fact that its hydrogenation and conversion to stearic acid (C18:0) is slower and constitutes a limiting step in the rumen (Grummer and Rabelo, 1999).

Ritzenthaler reported that meat and dairy products are the predominant source of cis-9, trans-11 of CLA isomersin human diet and meat contributes over 25% of the total diet within the cis-9, trans-11 of CLA. CLA levels could have effects onhuman health. Conjugated linoleic acid and trans-octadecenoic acids are produced in the rumen as intermediates in the biohydrogenation of thediet of linoleic acid for stearic acid (Bauman *et al*., 2000b).

Fat of cattle products (meat and milk) in many cases is considered unhealthy because of its high content in saturated fat, in recent years it has been found that a component of this fat may have beneficial effects for human health (McGuire and McGuire, 2000). Pariza *et al*. (1979, 1983) detected that the supply of ethereal extract (fat) of friedor raw meat inhibited carcinogenesis in mice. Subsequently (Ha *et al*., 1987) established that this effect was due to the presence of fatty acid derivatives with double conjugated bonds (conjugated linoleic acid), in this case in cis-9 position, trans-11.

CLA may come from various natural or synthetic sources, the single isomer that has been proven to actually have anticancer effects, even at very low concentrations is the cis-9, trans-11 isomer found in products from ruminants (McGuire and McGuire, 2000). Another effect of CLA, specifically of the trans-10, cis-12

Corresponding Author: Jose A. Toca Ramirez, Unidad Academica de Medicina Veterinaria Y Zootecnia, Universidad Juarez del Estado de Durango,11.5 km C.P. 34700 Carretera Durango-Mezq., Mexico

C_{18-2} isomer is to modify the partition of energy by reducing the fat deposition (Pariza *et al*., 1979, 1983) due to this effects against obesityare given to CLA. Although, not known exactly which of the isomers is responsible, CLA would also have positive effects on the immune system, atherosclerosis, on the processes of ossification and diabetes (Bauman *et al*., 2001). The hypothesis of this research was to determine that CLA concentration in fresh beef is within an optimal profile.

The objective of this study was to determine the CLA concentration in the area of the longissimusdorsi muscle and back fat in beef.

MATERIALS AND METHODS

Experimental samples: Commercial samples of fresh beef were obtained at the slaughter plant TIF No. 65 of the Regional Livestock Union of Durango and various samplings for the acquisition of 50 samples were conducted with approximately 200 g each taken from the area of the longissimusdorsi muscle and back fat during the months of October, 2011 through April, 2012 in addition, the field information for each of the animals that were sampled was obtained sex: Age, feeding type, feeding system and breed.

ALC quantification by gas chromatography: Quantification of fatty acids by gas chromatography was performed by a modification of the method cited by Meza based on the method published by Roach *et al*. (2002).

Lipid extraction: Lipid extraction was performed according to modifications of the methods of Bligh and Dyer (1959) and Folch *et al*. (1957). Each gram of sample was mixed with chloroform, methanol and distilled water in the following ratio: 8:4:3 (mL). The sample was allowed to stand for 30 min, mixing every 10 min for 30 sec. Then, the mixture was homogenized in a vortex for 1 min. Then, it was centrifuged at 3000 rpm for 10 min. The upper layer was withdrawn and water was added again in the same initial ratio, vortexed for 1 min and centrifuged, repeating this last step 2 more times. Finally, the bottom layer obtained was filtered through what man No. 1 filter paper into a 30 mL test tube for total removal of the solvent by heating in a water bath at a temperature of 70-75°C. The fat sample obtained was stored at freezing temperature until preparation of Fatty Acid Methyl Esters (FAME).

Methylation of fatty acid esters: For the methylation of esters the AOAC (1969) procedure was used. For each sample gram, 3 mL of 0.25 N NaOH were mixed in methanol (HPLC grade), vortexed for 1 min and placed in a boiling water bath for 1 h at 70°C. Then, the mixture was cooled at room temperature. The 2 mL of Boron trifluoride (BF3) (14% in methanol) of Sigma-Aldrich® was added, vortexed for 1 min. It was allowed to stand for 30 min. The mixture was transferred to 15 mL corning tubes, 3 mL of hexane (HPLC grade) were added, vortexed for 1 min and centrifuged at 3000 rpm for 15 min. Then, the top layer (hexane-FAME) was removed with a pasteur pipette, 3 mL of hay were added again, vortexed and centrifuged at the same conditions for 2 more times and then diluted with hexane at 10 mL. FAME samples were finally stored at 5°C until their analysis in the gas chromatograph.

Quantification of fatty acids by gas chromatography: The 1 μL of the solution of fatty acid methyl esters was injected in hexanein a gas chromatograph Hewlett Packard 6820 equipped with a flame ionization detector. FAMEs were separated using a supelco SP 2560 capillary column (length: 125 m Igauge: 0.25 mm Iparticle size: 0.2 μm) using helium as carrier gas at a pressure of 40 psi. With an initial column temperature of 120°C which was maintained for 5 min increased to 210°C (3°C min^{-1}) and then increased to 225°C (1°C min^{-1}) remaining so for 15 min. The injector temperature was of 260°C with a detector temperature of 260°C. Fatty acid peaks were identified by their retention times with reference to pure methyl ester standards (SUPELCO, USA) and the fatty acid contents assessed by a calibration curvewere quantified.

Fatty acids were quantified by gas chromatography according to a standard of methyl ester with 5 fatty acids (palmitic, stearic, oleic, linoleic and linolenic acid) and methyl ester standard of CLA.

Fat extraction: The fat extraction was performed according to modifications of the methods of Bligh and Dyer (1959) and Folch *et al*. (1957). The fresh sample is mixed with methanol, distilled water and chloroform (chromatographic grade reagents) in the following ratio: 1 (g): 0.9:1:2:0.6 (mL) and the mixture is homogenized for 2 min at 2000 rpm adding then the double of volume of initial chloroform, stirring for 2 more min at 2000 rpm. It is then centrifuged for 5 min at 3000 rpm extracted the formed lower layer to be filtered. The grease sample was obtained in test tubes for the complete removal of the solvent by heating in a boiling water bath at 70°C. Fat sample obtained was stored at 20°C until the preparation of fatty acid methyl esters.

Methylation of fatty acid esters: Methylation of esters was done using the AOAC (1969) method. The 1 mL of sample and 2 mL of 1N NaOH were mixed in methanol, it

was vortexed for 30-45 sec and then heated for 15 min in a boiling water bath. Once the sample was cooled 1 mL of boron trifluoride (14%) was added, vortexed for 1 min and allowed to stand for 30 min. Subsequently, 4 mL of hexane (HPLC grade) and 1 mL of distilled water were added and the sample is centrifuged at 3000 rpm for 15 min. The top layer was then removed with a pasteur pipette and diluted with 10 mL hexane. The sample of fatty acid methyl esters wasfinally transferred to a flask and stored at -20°C until analysis by gas chromatography.

Quantification in gas chromatograph: The 1 microliter of the solution of fatty acid methyl esters in hexane was injected into a gas chromatograph Hewlett Packard 6820 equipped with a flame ionization detector. Fames are separated into a capillary column Supelco SP2560 (125×0.25×0.2 mm) using helium as carrier gas at a pressure of 40 psi. The initial column temperature was 180°C and was increased to 215°C (1°C min^{-1}) maintained for 25 min and then increased to 230°C (5°C min^{-1}) and remaining so for 10 min. The initial temperature of the injector was 220°C with a detector temperature of 260°C. The peaks of each fatty acid are identified by their retention times with reference standards of pure methyl esters (SUPELCO, USA) and CLA content evaluated using a calibration curve was quantified.

Statistical analysis: Samples were analyzed using a completely randomized design and Tuckey test, using the statistical package SAS version 9.1.

RESULTS AND DISCUSSION

Factors influencing the ALC content in beef: Management strategies in feed have been shown to have strong effects on the composition of fatty acids in animal tissues (Demeyer and Doreau, 1999; Mir *et al*., 2004). To determine the impact of diet on intramuscular deposition of CLA, diet effects were investigated in 50 experimental samples of the feed type fed cattle prior to slaughter and in Table 1 can be seen that the feeding systems did not present significant differences ($p<0.05$) for the findings of CLA content in the longissimusdorsi inter muscular area. This research was conducted during October, 2011 to April, 2012 and given that in 2011 the state of Durango presented a severe drought during the rainy season, causing a shortage in the production of fodder for the extensive system in Durango, being this production system the most important for meat production. However, the lipid content of the plant raw materials used in feeding of ruminants is highly variable depending on their origin. The ethereal extract of green fodder ranges from 4-12% of dry matter, however in dry forage and corn silage only varies between 1.5 and 5% (Morand-Fehr and Tran, 2001).

Table 1: Effect of the feed system on the content of CLA in beef

N	Feed system	Mean
17	Intensive	4.007[a]
9	Semi-intensive	5.197[a]
6	Extensive	1.948[a]

Table 2: Effect of type of feeding on the proportion of CLA content in beef

N	Type of feeding	Mean
27	Forage-concentrate	4.255[a]
5	Forage	2.338[a]

Table 3: Effect of the influence of sex on CLA content in beef

N	Sex	Mean
5	H	5.403[a]
27	M	3.688[a]

Table 4: Effect of the influence of age on CLA content in beef

N	Age	Mean
2	5.0	6.089[a]
1	7.0	5.693[a]
8	3.0	5.626[a]
6	1.6	4.355[a]
4	1.5	4.121[a]
1	3.5	2.354[a]
4	1.8	2.171[a]
3	2.0	2.145[a]
3	4.0	1.203[a]

[a]Equal letters do not present significant differences

The impact of the effect of diet on the content in the CLA composition in beef samples is shown in Table 2 and was investigated in the longissimusdorsi muscle to compare the content concentration of CLA in the diet of cattle, consisting of aforage based diet and a forage-concentrate diet.

In dairy cows grazing fresh forage (Kay *et al*., 2004) showed that most of the cis-9, trans-11 CLA isomer derived from endogenous synthesis, the precursor being the acid C18: 1, trans-11 (vaccenic acid, AV) and this process includes the D9-desaturase enzyme (Griinari *et al*., 2000).

In the green fodders the linolenic acid (C 18:3 n-3) predominates which is >50% of total fatty acids and the linoleic acid that ranges from 10-20%, however in the same conserved fodders the amount of linoleic acid (C 18:2 n-6 and oleic acid (C 181N-9) increase their proportion (+5 and +2 pointsof mean, respectively) whereas the acid decreases linolenic a mean of 20% points (Morand-Fehr and Tran, 2001). In the non-forage raw materials, the ethereal extract is composed mainly of triglycerides (Bondi, 1989).

Influence of factors linked to animals: Other factors that may modify the CLA content in beef are intrinsic factors for animals, age, sex, breed and type of muscles. (De la Torre *et al*., 2006). Difference in tissues of fattening cattle is influenced by genotype, sex and age of animals (De Smet *et al*., 2004; Nurnberg *et al*., 1998). In Table 3 and 4, it can be seen that sex and breed are not significantly different ($p<0.05$) for the analysis of their individual effect. In other studies, De la Torre *et al*. (2006)

Table 5: Effect of the influence of breed on CLA content in beef

N	Breed	Mean
1	Holstein	5.693[a]
3	Brangus	5.296[a]
26	Cruza-crossbred	4.038[a]
1	Brahaman	0.000[a]
1	Angus	0.000[a]

*Equal letters do not present significant differences

Table 6: Effect of the influence of origin on CLA content in beef

N	Origen	Mean
7	Nuevo-ideal	5.451[a]
23	Durango	3.844[ab]
2	Canatlan	0.000[b]

*Different letters represent significant differences

found that when age and sex were taken together the total proportion of CLA in intramuscular fat ($p<0.004$) was significantly influenced and cis, cis-and trans, trans-of the proportions of isomers of CLA ($p<0.02$ and 0.001, respectively) finding that the intramuscular fat of young Charolais bulls contained high proportions of CLA (+41% on average) than mature Charolais cows. Sex has a long effect on the fatness of animals, bulls are more agile than steers and they themselves can be more agile than females (Enser, 1991; Nurnberg *et al*., 1998).

Moreover, the effect of breed was tested to compare the proportions of CLA and lipids present in different sampled breeds. Table 5 shows that results are not significantly different ($p<0.05$) for the effect of breed on the CLA content in beef. In other studies with cows of Charolais and Holstein breeds, it was found that intramuscular fat of Charolais cows contained more total CLA than those of Holstein cows (on average +47%, $p<0.001$) and particularly morecis, transisomers (on average +55%, $p<0.001$). These effects could be explained by differences in the muscle for fat deposition (De la Torre *et al*., 2006). However, corrections should be made previously to eliminate the effect of fatness to find significant differences that occur between breeds for concentrations of Fatty Acids (FA) in intramuscular fat (Angus vs. Simmental, Wagyu vs. Wagyux, Limousin vs. Limousine) (Laborde *et al*., 2001; Mir *et al*., 2002).

On the other hand, significant differences ($p<0.05$) were found for the origin of the animals in the proportion of CLA content in different places of origin and no other research information for the variable origin of the animals was found and we can explain that the Nuevo Ideal Municipality has the highest proportion of CLA and corresponds to a dairy area where Holstein breed dominates and where the feeding system is semi-intensive (forage-concentrate) throughout the year and it is different from Canatlan and Durango where beef production depends heavily on the extensive system and dependent on the rainy season that occur annually in the months from June to September (Table 6).

CONCLUSION

The proportion of CLA in meat from ruminants depends on the ruminal formation of CLA and the vaccenic acid on the ability of the tissue to desaturate vaccenic acid in CLA. Extrinsic factors (lipid supplementation and nature of the basal diet and/or intrinsic factors (breed, age and sex of animals and type of muscle) are factors that influence the amount but also the composition of CLA isomers in fat of beef cattle. In other studies, these factors modified also the proportion of cis, trans-CLA related to cis, cis and trans, trans isomers (De La Torre *et al*., 2006).

The results show that the combination of these factors should be taken into account within the framework of development strategies aimed at raising cattle.

REFERENCES

AOAC, 1969. AOAC official method 969.33: Fatty acids in oils and fats: Preparation of methyl esters boron trifluoride method, first action 1969. AOAC International, Gaithersburg, MD., USA. http://tera.chem.ut.ee/~ivo/Chrom/GC/AOAC_Me_BF3.pdf.

Bauman, D.E., L.H. Baumgard, B.A. Corl and J.M. Griinari, 2000a. Biosynthesis of conjugated linoleic acid in ruminants. J. Anim. Sci., 77: 1-15.

Bauman, D.E., D.M. Barbano, D.A. Dwyer and J.M. Grinari, 2000b. Production of butter with enhanced conjugated linoleic acid for use in biomedical studies with animal models. J. Dairy Sci., 83: 2422-2425.

Bauman, D.E. and J.M. Griinari, 2001. Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome. Livestock Prod. Sci., 70: 15-29.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Bondi, A.A., 1989. Nutricion Animal [Animal Nutrition]. Editorial Acribia, Zaragoza, Spain, ISBN-13: 9788420 006628, Pages: 546 (In Spanish).

De Smet, S., K. Raes and D. Demeyer, 2004. Meat fatty acid composition as affected by fatness and genetic factors: A review. Anim. Res., 53: 81-98.

De la Torre, A., D. Gruffat, D. Durand, D. Micol, A. Peyron, V. Scislowski and D. Bauchart, 2006. Factors influencing proportion and composition of CLA in beef. Meat Sci., 73: 258-268.

Demeyer, D. and M. Doreau, 1999. Targets and procedures for altering ruminant meat and milk lipids. Proc. Nutr. Soc., 58: 593-607.

Enser, M., 1991. Animal Carcass Fats and Fish Oils. In: Analysis of Oilseeds, Fats and Fatty Foods, Rossel, J.B. and J.L.R. Pritchard (Eds.). Elsevier Application Science, USA., ISBN-13: 9781851666140, pp: 329-395.

Folch, J., M. Lees and G.H.S. Stanley, 1957. A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem., 226: 497-509.

Griinari, J.M., B.A. Corl, S.H. Lacy, P.Y. Chouinard, K.V.V. Nurmela and D.E. Bauman, 2000. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Δ^9-desaturase. J. Nutr., 130: 2285-2291.

Grummer, R.R. and E. Rabelo, 1999. Factors affecting digestibility of fat supplements. Proceedings of the 34th Annual Pacific Northwest Animal Nutrition Conference, October 12-14, 1999, Portland, OR., USA., pp: 159-173.

Ha, Y.L., N.K. Grimm and M.W. Pariza, 1987. Anticarcinogens from fried ground beef: Heat-altered derivatives of linoleic acid. Carcinogenesis, 8: 1881-1887.

Ip, C., D.J. Lisk and J.A. Scimeca, 1994. Potential of food modification in cancer prevention. Cancer Res., 54: 1957S-1959S.

Kay, J.K., T.R. Mackle, M.J. Auldist, N.A. Thomson and D.E. Bauman, 2004. Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci., 87: 369-378.

Laborde, F.L., I.B. Mandell, J.J. Tosh, J.W. Wilton and J.G. Buchanan-Smith, 2001. Breed effects on growth performance, carcass characteristics, fatty acid composition and palatability attributes in finishing steers. J. Anim. Sci., 79: 355-365.

Lock, A.L., P.W. Parodi and D.E. Bauman, 2005. The biology of trans fatty acids: Implications for human health and the dairy industry. Aust. J. Dairy Technol., 60: 134-142.

McGuire, M.A. and M.K. McGuire, 2000. Conjugated Linoleic Acid (CLA): A ruminant fatty acid with beneficial effects on human health. J. Anim. Sci., 77: 1-8.

Mir, P.S., Z. Mir, P.S. Kuber, C.T. Gaskins and E.L. Martin *et al*., 2002. Growth, carcass characteristics, muscle Conjugated Linoleic Acid (CLA) content and response to intravenous glucose challenge in high percentage Wagyu, Wagyu×Limousin and Limousin steers fed sunflower oil-containing diets. J. Anim. Sci., 80: 2996-3004.

Mir, P.S., T.A. McAllister, S. Scott, J. Aalhus and V. Baron *et al*., 2004. Conjugated linoleic acid-enriched beef production. Am. J. Clin. Nutr., 79: 1207S-1211S.

Morand-Fehr, P. and G. Tran, 2001. La fraction lipidique des aliments et les corps gras utilises en alimentation animale [The lipid fraction of foods and fats used in animal feed]. INRA Prod. Anim., 14: 285-302.

Nurnberg, K., J. Wegner and K. Ender, 1998. Factors influencing fat composition in muscle and adipose tissue of farm animals. Livestock Prod. Sci., 56: 145-156.

Pariza, M.W., S.H. Ashoor, F.S. Chu and D.B. Lund, 1979. Effects of temperature and time on mutagen formation in pan-fried hamburger. Cancer Lett., 7: 63-69.

Pariza, M.W., L.J. Loretz, J.M. Storkson and N.C. Holland, 1983. Mutagens and modulator of mutagenesis in fried ground beef. Cancer Res., 43: 2444s-2446s.

Park, Y., J.M. Storkson, K.J. Albright, W. Liu and M.W. Pariza, 1999. Evidence that the trans-10, cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids, 34: 235-241.

Parodi, P.W., 2004. Milk fat in human nutrition. Aust. J. Dairy Technol., 59: 3-59.

Roach, J.A.G., M.M. Mossoba, M.P. Yurawecz and J.K.G. Kramer, 2002. Chromatographic separation and identification of conjugated linoleic acid isomers. Analitica Chimica Acta, 465: 207-226.