Conjugated linoleic acid content in certified Argentine Angus beef

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Abstract – The impact of the presence of polyunsaturated fatty acids in forage with respect to the cereals and the time the animals are subjected to the pastoral diet modifies the concentration of Conjugated Linoleic Acid (CLA). The aim of the joint investigation between the Asociación Argentina de Angus and the Meat Center of INTI was to carry forward the analytical determination and quantification of the presence of CLA and of CLA 9-cis, 11-trans on commercial samples of certified angus beef destined for consumption which comes from animals that had been fed different diets during the primary production phase. The data of the different samples were subjected to a ANOVA which resulted in determining that there existed a significant difference between the concentration and qualitative patterns of CLA found among the samples that came from animals that fed on pastures and rangeland with or without grain supplements and the samples of animals that came from feedlots, significant differences.

Key Words – CLA, Forage, pastures

I. INTRODUCTION

Argentina beef is characterized by the fact that it comes from cows and heifers fed on pastures and grassland. It's possible to get differentiations of meat products in international commerce in order to increase the offers of specialty products. Differentiating the beef by the level of conjugated linoleic acid (CLA) due to the feeding method is an advantage over other countries with different feeding methods.

CLA refers to isomers form conjugated linoleic acid, which constitute the family of polyunsaturated fatty acids. Studies have identified 28 CLA, although only two CLA cis – 9, trans – 11 and trans – 10, cis – 12 were studied due to their greater presence in beef and its health benefits. Griinari & Bauman held that CLA found in beef and milk of ruminants originated from the incomplete bihydrogenation of linoleic acid inside the rumen.

CLA is produced naturally inside the rumen of beef cattle by the bacterial fermentation action that isomerizes the linoleic acid transforming it into CLA. Beef cattle are able to synthesize CLA from 18:1 trans – 11 by the action of the enzyme delta 9 desaturase that is estimated to produce the greatest source of cis – 9, trans – 11 in fat tissue.

Research has found anticancer properties (carcinogenesis inhibitor induced chemically) and anti-inflammatory from CLA C18:2 cis - 9, trans - 11 (also called Vacenic acid) in the range of 72% to 94% of the total of CLA in cattle. Also CLA C18:2 trans - 10, cis – 12 have anti-obesity properties and activate anti-arteriosclerosis although the level is comparatively less to the overall percentage of CLA. Both compounds promoted the emergence of dietary supplements by the pharmaceutics industry for human consumption due to the proven results. Pharmaceutical products have both CLA at a ratio of 1:1. The endogenous synthesis of Vacenic acid is estimated to be the resource of C18:2, the most important CLA in animal tissue.

The isomers are found naturally in high concentrations in food derivatives from ruminants due to the fact they are formed from intermediate compounds from lipolysis and bihydrogenation from linoleic acid. Some research noted that the CLA level is higher in ruminants than monogastric animals. This phenomenon is explained by the microorganism activity in the rumen.

Breda argues that among the ruminal microorganisms the most relevant CLA producer is Butyrivibrio fibrisolvens even though the regulating factors of CLA synthesis inside the rumen haven't been well defined. He maintained that CLA isomers are absorbed in the small intestine with other fatty acids and then esterified and finally circulates throughout the animal's body. Although in 1999 Griinari and Bauman suggested that the largest portion of CLA originates from the endogenous synthesis.

Various investigations show divers average daily intake levels depending on what country is studied. A study in Germany carried out by Jahreis maintains that an approximant daily intake of 1/3 of CLA (0.36 - 0.44 g/day) required for the metabolism is contributed by beef products. Meanwhile Ritzenthaler et al. estimated that intake levels of CLA in the USA were on average 0.2g/day while the average intake of Canada was 0.1g/day.

The number differences depend on the level of daily intake of beef and are directly affected by the food supply offered from cattle. According to data publicized by the FAO the average consumption of beef in Germany was 12.80 Kg per capita/year in 2009. The intake in the USA and Canada was 39.80 and 30.90 Kg per capita/year respectively during the same year. While Schmid et al. shows that the beef and beef products contribute 25% – 30% of the total CLA consumed in western countries. This level would depend on the per capita consumption, because in Argentina the increased level of consumption results in the incorporation of more CLA than other countries.

Eynard estimated that the increased quantity of CLA consumed in Argentina could reach more than 1 g/day per person. The minimum requirement to get a beneficial effect is estimated to be 0.62 g/day. This level of consumption is explicated by the per capita annual consumption of 54.10 Kg in 2009 and 59.1 Kg in 2012. The natural consumption of CLA by nutritional means can enable an effect of chemopreventive against illness without additional cost of oral supplements or to change diet habits which indirectly reduces the cost of government health services.

There is scientific evidence that the addition of enriched CLA in diets or its precursor (Vacenic acid) induce the increase of the concentration of CLA in tissues of monogastric animals. [Schmid, Collomb, Sieber, & Bee, 2006]. Polygastric animals exposed to pastures diets increases the concentration of CLA in the beef in comparisons to animals from feedlots with grains and commercially balanced feed. The introduction of oilseed in the diet can be an efficient method of increasing the concentration of CLA in the tissue. Animal diets supplemented with grains did not present a significant difference with animals from pastoral systems. Both production systems, Pastures/grassland and Pastures/grassland with grain supplementation, are the most important production system in Argentina.

The productive system and the nutritional strategy applied to cattle modifies the content of fatty acids of the beef. The consequence of polyunsaturated fatty acids present in the forage with respect to the cereals and the time exposed to the pastoral regiment modifies the deposits of intramuscular fat and especially the concentration of CLA. Rumen is able to saturate most of the fatty acids, although not being complete, which allows the absorption of acids that escape to the hydrogenation ruminal assuring a high concentration of CLA or its precursor susceptible to the enzymatic action.

The monogastric meats have a CLA value less than 1 mg/g of lipids. Concentrations vary in beef meat due to the sex, age, breed and diet of the animal. The range of variations is substantial, depending on which country, between 1.2 y 10.0 mg/g of lipids with differences in the CLA concentration up to 70% (3.6mg/g of fat – 6.2 mg/g of fat). For example, Argentina beef will have higher levels of CLA than USA beef due to different production systems, but especially due to diets. [Schmid, Collomb, Sieber, & Bee, 2006] [Eynard & Lopéz, 2003]

According to Schmid et al. many factors affect the CLA level, the season, the breed, sex of the animal and management practices. The highest incident factor is the diet because the substrate to format the CLA originates with the forage.

The high concentration of CLA in the ruminants muscles is associated with high concentrations of intramuscular fat which influence the CLA content in the fat of the fatty acids. [Schmid, Collomb, Sieber, & Bee, 2006]

According to Schmid et al. heifers fed on pasture and grassland during termination had more than 5 times the CLA levels in tissues than heifers that were fed with grains in feedlots. The CLA level found in *Longissimus dorsi* muscle were 5.3 mg/g of lipids from pasture fed animals compared to 2.5mg/g lipids from feedlot fed animals.

Shantha et al. have demonstrated that cooking methods that do not elevate the internal temperature above 80°C, freezing and oxidation do not significantly modify the quantity of CLA as expressed by mg/g of lipids. This leads to the conclusion that consumers could ingest the necessary CLA from their daily diet by eating beef products. [Shantha, Crum, & Decker, 1994]

According to Hur et al. CLA is stable and does not participate in the oxidation process, does not suffer structural changes from being stored, while the content of other polyunsaturated fatty acids decrees during oxidation.

F.D.A. (USA) recognizes the health benefits of CLA but excludes these fatty acids from the trans fats in the nutrition labeling required on foods. Meanwhile Eynard & Lopez pose that CLA is the only natural source of the fatty acids with anticancer properties that are recognized by the National Academy of Sciences of the USA (NAS) when the level of CLA intake is from 0.25% to 1% of total lipids. This natural source is found in beef or beef products or in polygastric animals in interstitial tissue (not visible), fat distributed in muscle fiber and subcutaneous deposits. Jointly the American Dietetic Association recognizes beef as a functional food because of its high concentration of CLA. The level of CLA correlates to the feed strategy offer to cattle.

The objective of the investigation was to determine analytically the total CLA and CLA 9 – cis, 11 – trans in commercial certified Angus beef, destined for commercial use. The research is the product of an alliance between Argentina Angus Association and the Meat Center of the National Institute of Industrial Technology (INTI).

II. MATERIALS AND METHODS

Samples

Beef samples were taken from 475.378 metric tons (t) of certified Angus beef. The certification is regulated by Resolution 280 published by the Servicio Nacional de Sanidad y Calidad Agroalimentaria Argentino (SENASA) and is compatible with regulation 1760 of the European Union that regulates optional labeling. The samples were taken at random during the months of June, July and August in 2013 from the Huges slaughterhouse located in Santa Fe Provence, Argentina and January, February, March and April in 2014 from Mattievich slaughterhouse located in Santa Fe Provence, Argentina. The production distribution during the six months was June 48.5 metric tons t, July 46.700 t, August 33.200 t, January 85.704 t, February 52.362 t, March 132.430 t and April 76.482 t.

The analysis were done on 81 samples taken from animals no older than 2 years (4 teeth) selected at random and stored frozen no longer than 14 days. The samples were of the *Longissimus dorsi* muscle between 12th – 13th rib, where the marbling "slight" was measured. The samples came from farms located in Buenos Aires, Santa Fe, Córdoba and Santiago del Estero and La Pampa.

Sample preparation

The samples were defrosted and fat was extracted applying the Soxhlet method. Molten fat extracted weighed from 0.04 g a 0.08 g to which methanol saturated in potassium hydroxide was added. The solution was placed in a bath at 35° +2°C for 40 minutes then left at ambient temperature. 10 ml of Sulfuric acid 3.5 M was added and agitated followed by placement in a bath to 35°+2°C for 20 minutes. They were removed from the bath and again left at ambient temperature. 5 ml of hexane was added and the samples were spun for 25 minutes. 2.5 ml were extracted from the upper hexane and placed in a glass tube to evaporate with circulating nitrogen at 35°C. Finally hexane was added and the samples were agitated for 1 minute then transfer to vials which were submitted to a Gas Chromatography with a flame ionization detector (GC – FID).

Sample Analysis

The analysis was performed with a GC – FID Shimadzu 2010 Plus with a Capillary Column Supelco SP - 2560, 100 m by 0.25 mm ID and 0.2 microns. The calibrations curves were made with Supelco 37 Component FAME mix 10 mg / ml in dichloromethane, Fluka Conjugated 9Z, 11E (Linoleic acid) and Fluka Conjugated 10E, 12Z (Linoleic acid) that was stored in a freezer at -20°C.

The quantification was performed with standard injections, checking the samples for the presence of peaks with identical retention time in order to calculate the area percentage by the ratio of the individual to the total. The individual areas have been correct for the FID response factor and conversion factor (FTG). The values calculated from concentrations are expressed as the percentage of fat content.

Statistical analysis

The statistic design used to analyze the information was Unifactorial with 3 treatments. The data obtained was submitted to analysis of variance (ANOVA) utilizing the software Minitab version 15.

III. RESULTS AND DISCUSSION

CLA

By the first analysis of the data collected it can be seen that the average of the samples of the group fed a diet from pasture or grassland, referred to in Argentina as “farm finish” – was 4.3789 mg of CLA/g of lipids with a standard deviation of 1.2921 mg of CLA/g.

The next group was fed a diet from pasture and grain or grassland and grain, referred to as “rationed” or “supplementation” had an average of 3.0335 mg of CLA/g of lipids with a standard deviation was 0.8937 mg de CLA/g. The diet with or without grain demonstrated a significant difference between the three groups.

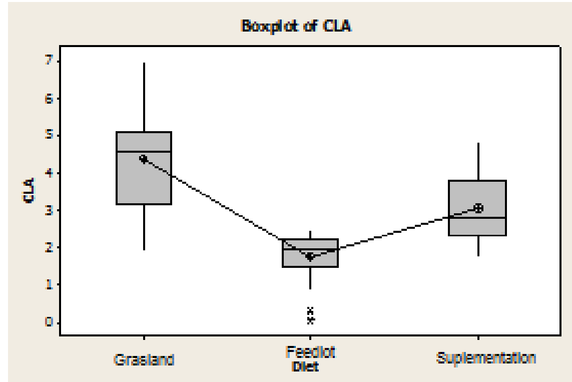
Table 1 CLA Samples with different feed were compared

Average from Samples from Feedlot Animals	Average from Samples from Pasture/grassland with grain Supplementation	Average Samples from Pasture/grassland
1.7437 CLA mg /g of lipid	3.0335 CLA mg /g of lipid	4.3789 CLA mg /g of lipid
Significant difference	Significant difference	Significant difference

The third group included beef cattle fed in feedlot, the average was 1.7437 mg de CLA/g of lipids with a standard deviation of 0.8937 mg of CLA/g.

The next graph shows the boxplots of the CLA concentration versus the three diets which demonstrates the averages, the medians, the boxes that represent the middle 50% of the results, and the max and the minimum values. The graph shows how samples from pasture and grassland present the largest dispersion respect to the average and median. Samples from diets of pastures or grassland supplemented with grain present a smaller dispersion and those from feedlots show the lowest dispersion concentration and values close to the average.

Figure 1. Boxplot from variance of concentration of CLA



The next table shows the variance analysis (ANOVA table), where the CLA concentration verses diet offer to animals shows significant differences in CLA form the three diets with a significance level of the 0%(p-value= 0%).

Table 2 Variance Analysis (ANOVA)

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One-way ANOVA: CLA versus Diet

Source  DF      SS      MS      F      P
Diet     2  93,755  46,878  49,36  0,000
Error   78  74,070   0,950
Total   80 167,825
S = 0,9745   R-Sq = 55,86%   R-Sq(adj) = 54,73%

                              Individual 95% CIs For Mean Based on
                              Pooled StDev
Level          N   Mean   StDev   ----+-----+-----+-----+
Grassland     27  4,3789  1,2921                   (--*-)
Feedlot       27  1,7437  0,6169 (-*--)
Supplementation 27 3,0335 0,8937          (-*--)
                                  ----+-----+-----+-----+
                                     2,0   3,0   4,0   5,0
Pooled StDev = 0,9745
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The Kruskal – Wallis test, simultaneous confidence intervals, shows individual levels of confidence of 97.45% and simultaneous 95% for pairwise comparisons between diets. With these we are able to establish the result that the concentration of CLA from “farm finish”, “rationed” and feedlot animals differs statistically.

Table 3 Font sizes and styles

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Kruskal-Wallis Test: CLA versus Diet

Diet             N   Median   Ave Rank     Z
Grassland       27    4,561       62,4   5,80
Feedlot         27    1,940       17,8  -6,28
Supplementation 27    2,806       42,8   0,48
Overall         81                41,0

H = 48,89  DF = 2  P = 0,000
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Summarizing the data, different studies from diverse countries explain that the CLA in beef samples fed with pasture and with pasture supplemented with grains have not presented significant differences between them. P.T. García et al. has published papers about Argentina beef cattle arriving at the same response to differential diets. Even though the perspective or vision of analysis from international bibliography is different the results are the same as with this paper, finding no significant differences between pastures and grassland diets with or without supplementing with grains. We and the others found statistically significant differences between beef cattle from feedlot and pastures or grasslands with or without grain supplied. Grouping the samples from pastures or grassland with or without grains, to us is not possible.

C 18:2 9 – Cis, 11 trans

By the first analysis of the data collected it can be seen that the average of the samples of the group fed a diet from pasture or grassland, referred to in Argentina as "farm finish" – was 3.9166 mg of CLA 9 - cis, 11 - trans/g of lipids with a standard deviation of 1.4158 mg of CLA 9 - cis, 11 - trans/g of lipid.

Table 4 CLA 9 – cis, 11 – trans Samples with different feed were compared

Average from Samples from Feedlot Animals	Average from Samples from Pasture/grassland with grain Supplementation	Average Samples from Pasture/grassland
1.6758 mg CLA 9 – cis, 11 - trans /g lipids	3.0031 mg CLA 9 – cis, 11 - trans /g lipids	4.3572 mg CLA 9 – cis, 11 - trans /g lipids
Average Percentage of CLA 9 – cis, 11 – trans divided total of CLA: 96.11%	Average Percentage of CLA 9 – cis, 11 – trans divided total of CLA: 99.00%	Average Percentage of CLA 9 – cis, 11 – trans divided total of CLA: 99.50%
Significant difference.	Significant difference.	Significant difference.

The next group was fed a diet from pasture and grain or grassland and grain, referred to as "supplementation," had an average of 3.0031 mg of CLA 9 - cis, 11 - trans /g of lipids with a standard deviation was 0.9102 mg of CLA 9 - cis, 11 - trans /g. The diet with or without grain demonstrated a significant difference between both.

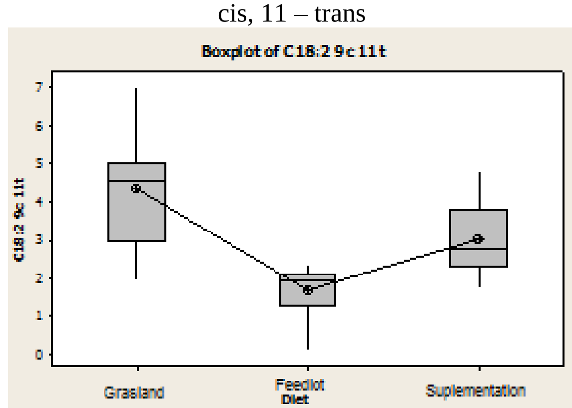
The third group included beef cattle fed in feedlot, the average was 1.6758 mg of CLA/g of lipids with a standard deviation of 0.6025 mg of CLA/g.

Data on the presents of CLA 9 – cis, 11 trans in certified Angus beef certificated that samples from feedlots were estimated at 96.11% of total CLA. International bibliography holds that the incident of CLA 9 – cis, 11 – trans was between 72%– 94% which was less than found in this work.

Samples from animals that fed on pastures and grasslands with or without grain were demonstrated to exceed values found by different authors, demonstrating that in the samples obtained almost the totality of CLA was CLA 9 – cis, 11 trans.

The next graph shows the boxplots of the CLA concentration versus the three diets which demonstrates the averages, the medians, the boxes that represent the middle 50% of the results, and the max and the minimum values. The graph shows how samples from pasture and grassland present the largest dispersion in respect to the average and median. Samples from diets of pastures or grassland supplemented with grain present a smaller dispersion and those from feedlots show the lowest dispersion concentration and values close to the average.

Figure 2. Boxplot variance concentration of CLA 9 – cis, 11 – trans



The next table shows the variance analysis (ANOVA table), where the CLA 9 – cis, 11 - trans concentration verses diet offer to animals shows significant differences in CLA 9 – cis, 11 - trans form the three diets with a significance level of the 0%(p-value= 0%).

Table 5 Variance Analysis (ANOVA)

```
One-way ANOVA: C18:2 9c 11t versus Diet

Source   DF       SS       MS      F       P
Diet      2   97,066   48,533  50,25   0,000
Error    78   75,338    0,966
Total    80  172,404

S = 0,9828   R-Sq = 56,30%   R-Sq(adj) = 55,18%

                            Individual 95% CIs For Mean Based
                            Pooled StDev
Level           N   Mean   StDev ---+----+----+-----+
Grassland       27  4,3572  1,3062              (-*-)
Feedlot         27  1,6758  0,6025(-*-)
Supplementation 27  3,0031  0,9102        (-*-)
                                ----+-----+----+-----+-
                                   2,0    3,0   4,0   5,0
Pooled StDev = 0,9828
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The Tukey test, simultaneous confidence intervals, shows individual levels of confidence simultaneous 95% for pairwise comparisons between diets. With these we are able to establish the result that the concentration of CLA 9 – cis, 11 - trans from “farm finish”, “rationed” animals and feedlot animals shows statistical differences.

Table 6 Test de Tukey with 95% confidence intervals on the diets comparison

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Tukey 95% Simultaneous Confidence Intervals
Grouping Information Using Tukey Method

Diet             N   Mean  Grouping
Campo            27  4,3572  A
Supplementation  27  3,0031    B
Feedlot          27  1,6758      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Diet

Individual confidence level = 98,07%

Diet = Grasland subtracted from:
Diet              Lower   Center   Upper
Feedlot          -3,3207  -2,6814  -2,0421
Supplementation  -1,9934  -1,3541  -0,7148

Diet = Feedlot subtracted from:
Diet              Lower  Center  Upper
Supplementation  0,6880  1,3273  1,9665
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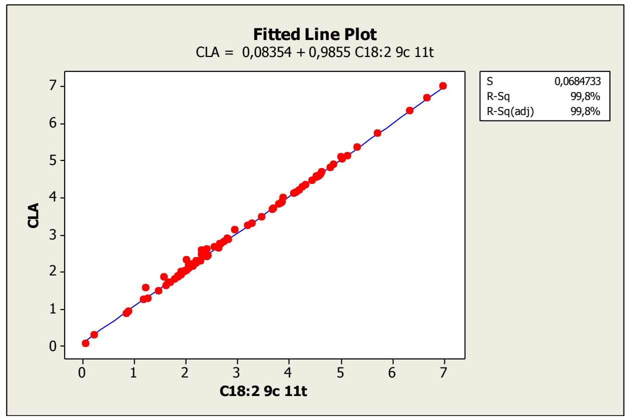
Lineal Regression between the CLA and CLA 9 – cis, 11 - trans concentration

The CLA variable depend on various types of CLA, however the concentration of CLA 9 – cis, 11 - trans being present at 96.11% in samples from feedlots animals, 99.50% in samples fed with pasture or grassland animals with grain supplementation and 99.00% in samples from animals fed with pasture and grassland allows for linear regression between CLA as a dependent variable and CLA 9 – cis, 11 – trans as an independent variable with adjustment of R2 of 99.8% and the next equation:

CLA = 0,08354 + 0,9855 C18:2 9c 11t.

Figure 3. Lineal Regression between the CLA and the CLA 9 – cis, 11 - trans



To resume up; CLA 9 – cis, 10 – trans concentration would be a good predictor to the concentration of CLA in samples. According to analyses it can be said that the dependent variable has lineal correlations with the variable dependent on adequate R2.

Table 7 Information and data about lineal regression between the CLA and the CLA 9 – cis, 11 - trans

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Regression Analysis: CLA versus C18:2 9c 11t

The regression equation is
CLA = 0,08354 + 0,9855 C18:2 9c 11t

S = 0,0684733   R-Sq = 99,8%   R-Sq(adj) = 99,8%

Analysis of Variance
Source       DF       SS        MS          F      P
Regression    1  167,455   167,455   35715,31  0,000
Error        79    0,370     0,005
Total        80  167,825
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IV. CONCLUSION

The analysis carried out on samples of animals from different treatments concludes that samples taken from commercial consignments of certified Angus beef fed on pastures or grasslands with and without grain supplementation and feedlots animals exhibited significant statistical differences. Taking into account the incidence level of CLA cis – 9, trans – 11 is above 96% of the total CLA which justifies mentioning that the compound is the most important substance found almost entirely naturally.

The higher concentrations of CLA could be taken as an intrinsic attributes in beef and can be promoted by marketing and differentiate the products with the idea that the consumer can acquire functional products in the national and international marketplace.

Data demonstrates the differences between groups of animals fed on pasture-based grazing and those fed in feedlots would permit commercial information showing the significant differences based on diets. However if it is decided to elevate the CLA value of feedlot animals then research could be carry out on adding to the diet industrial products such as sunflowers, soybean and linen in order to increase the natural level of CLA in beef.

REFERENCES

1. 1. Alvarez, María I. y Moreira dos Santos, Wagner L. (2005). Evaluación de la terneza del bife angosto de novillos tipo Nelore de diferentes edades. Revista Veterinaria , 16 (2), 57.
2. 2. Christie, W. W., Sébédio, J. L., & Pierre, J. (2001). A practical guide to the analysis of conjugated linoleic acid (CLA). Inform (12), 147-152.
3. 3. Dunshea, F. R., D´Souza, D. N., Pethick, D. W., Harper, G. S., & Warner, R. D. (2005). Effects of dietary factors and other metabolic modifiers on queality and nutitional Value of Meat. Meat Science (71), 8-38.
4. 4. Eynard, A. R., & Lopéz, C. B. (29 de August de 2003). Conjugated linoleic acid (CLA) versus saturated fats/cholesterol: their proportion in fatty and lean meats may effect the risk of developing colon cancer. Biomedic Central , 1-5.
5. 5. Ferguson, L. R. (2010). Meat and Cancer. (M. Science, Ed.) 84 (84), 308-313.
6. 6. García, P. T., Pensel, N. A., Sancho, A. M., Latimori, N. J., Kloster, A. M., Amigone, M. A., y otros. (2008). Beef lipids in relation to animal breed and nutrition in Argentina. (M. Science, Ed.) Meat Science (79), 500-508.
7. 7. Grigioni, G. (2011). Manual de procedimientos: determinación de los parámetros de calidad física y sensorial de carne bovina. CABA, Argentina: INTA.
8. 8. Griinari, M. J., & Bauman, D. E. Capítulo 13 Biosynthesis of Conjugated Linoleic Acid and Its Incorporation into Meat and Milk in Ruminants. Estados Unidos.
9. 9. Higgs, J. D. (2000). The Changing nature of red meat: 20 years of Improving Nutritional Quality. Trends in Food Science & Technology (11), 85 - 95.
10. 10. Hur, S. J., Ye, B. W., Ha, Y. L., Park, G. B., & Joo, S. T. (2004). Effects of conjugated linoleic acid on color and lipid oxidation of beef patties during cold storage. Meat Science (66), 771-775.
11. 11. Jahreis, G., Fritsche, J., Möckel, P., Schöne, F., Möller, & Steinhart, H. (1999). The potential Anticarcinogenic Conjugated Linoleic Acid, Cis - 9,trans - 11 C18:2, in milk of different Species: Cow, Goat, Ewe, Sow, Mare, Woman. Nutrition Research , 19 (10), 1541 - 1549.
12. 12. Ma, D. W., Wierzbicki, A. A., Field, C. J., & Clandinin, M. T. (1999). Conjugated linoleic acid in Canadian dairy and beef Products. (A. C. Society, Ed.) Journal Agric. Food Chem. (47), 1956-1960.
13. 13. Morales, R., Folch, C., Delgado, N., & Flores, P. Atributos de la carne bovina generada en sistemas pastoriles de la zona sur de Chile. INIA Remehue.
14. 14. Mulvihill, B. (2001). Ruminant meat as a source of conjugated linoleic acid (CLA). British Nutrition Foundation , 295-299.
15. 15. Mulvihill, B. (2001). Ruminant meat as a source of conjugated linoleic acid (CLA). British Nutrition Foundation (26), 295 - 299.
16. 16. National Cattlemen´s Beef Association. (2008). Conjugated Linoleic Acid and Dietary Beef. Research RKM & Knoledge Management .
17. 17. Poulson, C. S., Dhiman, T. R., Ure, A. L., Cornforth, D., & Olson, K. C. (2004). Conjugated linoleic acid content of beef from cattle fed diets containing high grain, CLA, or raised on forages. Livestock Production Science (91), 117-128.
18. 18. Raes, K., De Smet, S., & Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat: a review. Animal Feed Science and Technology (113), 199 - 221.
19. 19. Realini, C. E., Duckett, S. K., Brito, G. W., Rizza, M. D., & De Mattos, D. (2004). Effect of pasture Vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. Meat science , 567-577.

20. 20. Schmid, A., Collomb, M., Sieber, R., & Bee, G. (2006). Conjugated linoleic acid in meat and meat products: A review. Meat Science (73), 29-41.
21. 21. Scollan, N., Hocquette, J. F., Nuernberg, K., Dannenberger, D., Richardson, I., & Moloney, A. (2006). Innovations in beef production suytems that enhance the nutritional and heath value of beef lipids and their relationship with meat quality. Meat Science (74), 17-33.
22. 22. Shackelford, S.D.; Wheeler, T.L. y Koohmaraie, M. (2004). Technical Note: Use of belt grill cookery and slice shear force for assessment of pork longissimus tenderness. Journal of Animal Science , 1 (82), 238 - 241.
23. 23. Shantha, N. C., Crum, A. D., & Decker, E. A. (1994). Evaluation of Conjugated Linoleic Acid Concentrations in Cooked Beef. (A. C. Society, Ed.) Journal Agric. Food Chem. (42), 1757 - 1760.
24. 24. Shantha, N. C., Crum, A. D., & Decker, E. A. (1994). Evaluation of Conjugated Linoleic Acid Concentrations in Cooked Beef. J. Agric. Food Chem. (42), 1757 - 1760.
25. 25. Silvestre, M. P., Lopes, D. C., Silva, M. R., & Silva, V. D. (2013). Availability of food sources of conjugated Linoleic Acid in Brasil. (A. N. Information, Ed.) Pakistan Journal of Nutrition , 08 -11.
26. 26. Teira, G. A. (2004). Actualidad y perspectivas de un componente principal de la calidad de las carnes bovinas. 15 (28), 215 - 244.
27. 27. Vásquez, R. E., Abadía, B., Arreaza, L. C., Ballesteros, H. H., & Muñoz, C. A. (2007). Factores asociados con la calidad de la carne. II parte: perfil de ácidos grasos de la carne bovina en 40 empresas ganaderas de la región Caribe y el Magdalena Medio. Revista Copoica - Ciencia y Tecnología Agropecuario , 8 (2), 66 - 73.