

Diet and Genotype Effects on n-3 Polyunsaturated Fatty Acids of Beef Lipids

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Abstract

The aim of this study was to study the effects of four feeding systems on n-3 Polyunsaturated Fatty Acids (PUFAs) in Longissimus dorsi muscle lipids from three different steer genotypes and discuss the current finding concerning the role of these factors on alfa-linolenic acid conversion into n-3 long chain PUFAs. The feeding systems described here, represent productive models widespread in Argentina Pampeana region, ranging from pasture exclusive diet, to the feedlot system. C18:2 n-6 (LA) increased whereas C18:3 n-3 (ALA) decreased according to the increase in grain in the diet. The LA/ALA and n-6/n-3 PUFAs ratios increased as the grain in the diet increased. We concluded that by feeding animals correctly, we address the problem of consuming a high n-6/n-3 PUFAs ratio without virtually changing normal diets. Although these changes appear modest, for non-sea fish eaters, animal products such as beef are the only sources of long-chain n-3 PUFAs, and any improvement in the fatty acid composition of beef will result in an increase in the consumption of n-3 fatty acids.

Keywords

healthy beef, linoleic acid, linolenic acid, LA/ALA ratio, n-6/n-3 PUFAs

1. Introduction

Studies in meat science are advancing the development of foods with characteristics that benefit human health. Both Linoleic Acid (LA) and Alpha-Linolenic Acids (ALA) belong to the n-6 (omega 6) and n-3 (omega 3) series of Polyunsaturated Fatty Acids (PUFAs) respectively. LA and ALA are defined as essential fatty acids because they are not synthesized in the human body and thus have to be obtained from the diet. Hence, it is essential to provide these two acids in the diet, because they are precursors for Long Chain Polyunsaturated Fatty Acids (LC-PUFAs) (Williams, 2000). LA, which is practically found in every food, is the most prominent PUFA in the Western diet. As LA is the metabolic precursor of Arachidonic Acid (ARA) and derived bioactive eicosanoids, there is concern that dietary LA could increase tissue ARA, eicosanoid formation and subsequently enhance the risk of and/or exacerbate

conditions associated with acute and chronic diseases (Whelan, 2008).

According to recent studies the conversion of ALA into n-3 LC-PUFAs, especially 22:6 n-3 (DHA) in humans is scarce and is affected by the ratio LA/ALA, the total PUFAs and the amounts of ALA in the diet (Barcelo-Coblijn & Murphy, 2009). The conversion of ALA into LC-PUFAs is decreased by high dietary ratios of LA/ALA. Moreover, n-6 fatty acids intake influences tissue concentrations of n-3 LC-PUFAs (Brenna et al. 2009). Competitive interactions among n-6 and n-3 PUFAs at the level of formation and action of eicosanoids, appear to relate directly to potential benefits attributed to an eligible dietary balance of n-3 and n-6 PUFAs. With higher dietary ratios of n-6/n-3 PUFAs, the pro-inflammatory/aggregatory eicosanoids from LA are favoured over those from ALA, showing anti-inflammatory/aggregatory effects. Controlling the synthesis of n-3 LC-PUFA, such as EPA (20:5 n-3) and DHA, from ALA, as well as controlling the respective eicosanoids by consumption of the optimized ratio of n-6 to n-3 is, therefore, the particular nutritional focus of interest. Since members of the n-6 and n-3 PUFAs fatty acid families compete for the corresponding enzyme systems the conversion of LA or ALA into their longer chain homologues is significantly influenced by the composition of dietary fats. Most of the fat intake in humans comes from animal products (meat, milk, eggs, etc.). This has changed because animals used to eat mainly forages (high ALA and low LA) but now they typically eat less forage and consume more cereals and soy bean (high LA and low ALA). As a result, the animal products are now much higher in n-6 PUFAs and lower in n-3 PUFAs. Smink et al. (2013) suppressed the effect of ALA intake on n-3 LC-PUFAs by LA intake and suppressed the concentration of n-6 LC-PUFAs in blood plasma by dietary ALA. They found that the inhibiting effect of ALA in blood ARA, at equal increasing intakes, was stronger than the stimulating effect of LA as a precursor. Analysis of n-3 PUFA intake, intake recommendations, and health claims are often limited to ALA, EPA and DHA and omit Docosapentaenoic Acid (DPA). DPA is an intermediate in the production of DHA from EPA and its contribution in beef lipids is higher than that of EPA and DHA. The functional and nutritional attributes of DPA are largely unknown but some studies have shown that DPA appears to be a primary end point for the synthesis of n-3 PUFAs from ALA (Howe et al., 2006).

Data from a large number of studies regarding LA and ALA proportions in beef lipids indicate that the fatty acid composition vary largely across production systems (Garcia & Casal, 2012). The fatty acid composition of adipose tissue and muscle in cattle is affected by several factors as diet, total fat content, breed, genotype, age and gender. Genetic variation in the fatty acid composition of adipose tissue triacylglycerols, with breed differences were observed in several studies. However, in the phospholipid fraction, the total amount of PUFAs does not differ among crossbreed groups, because certain amounts of unsaturated fatty acids are required to maintain the physical properties of the cell at the body temperature of mammals (Malau-Aduli et al., 1998). Sexton et al. (2012) showed that there is a sire breed x time of weaning interaction for percent total PUFA, with the Charolaise steers having a greater PUFAs percentages than other treatment groups. Humada et al. (2012) studied the effects of two production systems (semi-intensive vs. intensive), and two slaughter ages (12 vs.14 months) on LA and

ALA proportions on beef fatty acids composition. The slaughter age affected LA or ALA proportions. LA and ALA are strongly positively correlated to intramuscular fat of *Longissimus dorsi* muscle at the quantitative level (mg/100g fresh tissue), but there are negative correlation coefficients for the relative proportion of these fatty acids to intramuscular fat. The relationships between intramuscular fat and the fatty acid profile of triglycerides and phospholipids fractions are weaker compared to total muscle fat. The aim of this work was to study the effects of four feeding systems on LA, ALA, n-6 and n-3 PUFAs and in LA/ALA and n-6/n-3 PUFAs ratios in *Longissimus dorsi* muscle lipids from three different genotypes of steers and discuss the current finding concerning the role of these factors on ALA conversion into n-3 LC-PUFAs in humans. The feeding systems described here, represent productive models widespread in Argentina Pampeana region, ranging from pasture exclusive diet, to the feedlot system. The British purebred or their crossbreds, which are the main components of the meat consumed domestically, were represented by Abedeen Angus (AA) steers. The crossbred Charolais x Aberdeen Angus (CHAxAA) steers represents the most advisable crossbred for production of heavy steers for export markets, and Argentine Holstein (HA) steers are potentially adequate for commercial purpose.

2. Materials and Methods

2.1 Animal Management of the Animals and Their Diets

A total of 144 Aberdeen Angus (AA), Charolais x Aberdeen Angus (CHAxAA) and Argentine Holstein (HA) steers were allotted from 5-7 months old to slaughter weight to the four following treatment:

Pasture: steers grazed on pasture exclusively (mainly alfalfa and festuca).

Cn0.7%LW: steers fed with a similar pasture but supplemented daily with cracked corn grain (0.7% of live weight).

Cn1%LW: steers fed with a similar pasture but supplemented daily with cracked corn grain (1% of live-weight).

Cn85%: steers fed with a diet based in corn grain (85%), alfalfa hay (8.6%), soybean meal (8.4%) and, minerals and vitamins (0.5%).

The forage in Pasture, Cn0.7% and Cn1% treatments were provided by a mixed pasture of alfalfa (*Medicago sativa*) and tall fescue (*Festuca arundinacea Schreb*) grazed under a rotational system of six paddocks with seven days of permanency in each one.

Twelve steers for treatment were assigned and they were conventionally slaughtered in a conventional abattoir at a similar degree of finishing estimated by visual evaluation. Data from the animal performance within each breed and treatment group are presented in Tables 1 and 2.

Table 1. Animal Data: Initial and Final Live Weights and Experimental Period (EP)

Genotype	Initial live weight (kg)			Final live weight (kg)			EP (days)		
	AA	CHAxAA	HA	AA	CHAxAA	HO	AA	CHAxAA	HA
N	12	12	12	12	12	12	12	12	12
Pasture	190a	181a	195a	388b	449a	505b	365	382	391
Cn0.7%L	188a	171a	194a	408a	467a	512ab	333	378	367
W									
Cn1%LW	188a	174a	194a	420a	483a	541a	289	356	354
Cn85%	189a	168a	193a	350a	399b	374c	152	192	170

Note. abc means within a column with different letters significantly differ ($p < 0.05$).

Table 2. Experimental Animals: Growth Rate (g/d) and EAC (Estimated Average Contribution) of Grain to Total Energy Intake

Genotype	Growth rate (g/d)			
	AA	CHAxAA	HA	EAC
Pasture	546c	707c	800d	0%
Cn0.7%LWt	675bc	787c	875cd	33.6%
Cn1%LW	808b	871bc	983bc	44.9%
Cn85%	1055a	1223a	1093ab	85.8%

Note. abcd means within a column with different letters significantly differ ($p < 0.05$).

2.2 Methods of Analysis

After 24 h at 4°C, 12 steaks, 2-25 cm of thickness, from *Longissimus Dorsi* muscles at the 11th rib were taken from each group, carefully dissected and used for chemical analysis. All samples were frozen and stored at -20°C until analysis were performed. Aliquot samples of 10g each, trimmed of external fat, minced carefully, dried and extracted in a Tecator apparatus using hexane as the extraction solvent according to official methods (AOAC, 1992), were used to determine total intramuscular fat (IMF). Aliquot samples of 5g each were extracted using the Folch et al. method (Folch et al., 1957). Fatty Acid Methyl Esters (FAME) were prepared according to the method of Pariza et al. (2001) and measured using a Chrompack CP 900 equipment (Chrompack Inc., Middleburg, The Netherlands) fitted with a flame ionization detector. Fatty acid methyl esters were separated with a fused silica capillary column CP-Sil 88 (100m x 0.25mm i.d.) from Chrompack Inc., Middleburg, The Netherlands, with N₂ as the carrier gas. The oven temperature was programmed at 70° C for 4 min, increased from 70 to 170°C at a rate of 13°C/min and then increases from to 170° to 200°C at 1°C/min. Individual fatty acids were identified by comparing relative retention times with individual fatty acids standard (PUFA-2 Animal Source. Supelco). Analytic results were expressed as percentages of total fatty acids

and mg of fatty acid contribution in 100g of fresh *Longissimus Dorsi* muscle.

2.3 Statistical Analyses

Data were subjected to analysis of variance using the GLM procedure of SAS v.9.1 statistical software package. The two factorial design were considered: breed with three levels (AA, CHAxAA and AH) and diet with four levels (Pasture, pasture + 0.7% grain, pasture + 1% grain and grain) and their interactions. Tukey's test was used for means comparison.

3. Results

The effects of the diet on the LA and ALA proportions, n-3 and n-6 PUFAs, IMF%, LA/ALA and ratios n-6/n-3 PUFA are shown in Tables 3 and 4.

Table 3. Effects of Genotype and Diet, on LA, ALA, n-6 and n-3%, and LA/ALA and n-6/n-3 Ratios in Steer *Longissimus Dorsi* Lipids

	LA%	ALA%	n-3%	n-6%	n-6/n-3	LA/ALA	IMF%	PUFA%
AA	4.10	0.91a	2.07a	5.97	3.84b	6.36a	3.57	8.04
CHAxAA	4.30	0.93a	2.03ab	6.24	3.78b	6.72a	3.80	8.27
HA	4.29	0.81b	1.82b	6.41	4.71a	8.05b	3.59	8.22
Pasture	3.55b	1.38a	3.09a	5.48b	1.08	2.59c	2.89b	8.57ab
Cn0.7%	3.76b	0.99b	2.09b	5.61b	2.69	3.84bc	3.58ab	7.70b
Cn1.0%	3.94b	0.77c	1.69c	5.91b	3.61	5.31b	4.25a	7.61b
Cn85%	5.65a	0.39d	1.01d	7.82a	8.32	16.43a	3.90a	8.83a
RMSE	0.97	0.20	0.49	1.40	1.34	3.59	1.32	1.76
Genotype	0.53	0.008	0.028	0.307	0.001	0.057	0.043	0.797
Diet	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005
GxD	0.611	0.267	0.348	0.537	0.004	0.381	0.135	0.609

Note. *Significance of main effects (breed, diet) and their interaction (G x D)

abcd means within a column with different letters significantly differ.

RMSE: Root mean square error n-3 (18:3+20:5+22:5+22:6); n-6 (18:2+18:3+20:3+20:4+22:4);

PUFA%=n-6+n-3%.

IMF: Intramuscular fat

Table 4. Effects of Genotype and Diet on n-6 and n-3 PUFAs (Percent of Total Fatty Acids) on Steer *Longissimus Dorsi* Muscle Lipids

	18:2 n-6	20:3 n-6	20:4 n-6	18:3 n-3	20:5 n-3	22:5 n-3	22:6 n-3
AA	4.10	0.44	1.10b	0.91a	0.31	0.52	0.33
CHAxAA	4.30	0.42	1.16b	0.93a	0.31	0.50	0.29
HA	4.29	0.44	1.41a	0.81b	0.35	0.52	0.13
Pasture	3.55b	0.44	1.12	1.38a	0.57a	0.76a	0.38
Cn0.7%	3.76b	0.41	1.18	0.99b	0.34b	0.53b	0.23
Cn1.0%	3.94b	0.42	1.23	0.77c	0.35b	0.47b	0.20
Cn85%	5.65a	0.46	1.35	0.39d	0.14b	0.30c	0.17
RMSE	0.97	0.16	0.48	0.20	0.13	0.20	0.13
Genotype	0.53	0.851	0.005	0.008	0.241	0.725	0.000
Diet	0.000	0.588	0.236	0.000	0.000	0.000	0.000
GxD	0.611	0.071	0.708	0.267	0.076	0.237	0.000

Note.*Significance of main effects (breed, diet) and their interaction (G x D).

abcd means within a column with different letters significantly differ.

RMSE: Root mean square error

The diet significantly affected all these variables. The proportions of LA increased ($p < 0.001$) according with the increase in grain in the diet, whereas the ALA proportions decreased ($p < 0.001$). Total n-3 LC-PUFAs decreased significantly whereas total n-6 LC-PUFAs increased as the grain in the diet increased. Total LC-PUFAs were higher in Pasture and Cn85% than in Cn07% and Cn1%. The LA/ALA and n-6/n-3 PUFA ratios were ($p < 0.001$) lower in pasture and supplement-fed beef than in the grain-fed beef. The LA/ALA ratio increased linearly as the grain in the diet increased. The n-6/n-3 PUFAs ratio followed a similar pattern. IMF% was significantly affected by the diet, being lower in pasture-fed beef than in the others.

The effects of the genotype on LA, ALA, n-3 and n-6 PUFAs, IMF percentages and LA/ALA and n-6/n-3 PUFA ratios are shown in Tables 3 and 4. The genotype affected ALA, n-3 LC-PUFAs, and n-6/n-3 PUFA and LA/ALA ratios. The beef lipids from HA showed ($p < 0.001$) lower ALA proportions than those from AA and CHAxAA, but no significant differences in the LA proportions. Total n-6 LC-PUFAs were similar in all genotypes whereas total n-3 LC-PUFAs were lower in HA than in AA and CHAxAA, which were similar. The LA/ALA and n-6/n-3 ratios were higher in HA than in the other two genotypes. The effects of genotype and diet on the estimated contributions (mg/100g fresh *Longissimus dorsi* muscle) of LA, ALA and n-3 and n-6 PUFAs are presented in Table 5. The effects of the diet on the contributions of LA, ALA, n-3 and n-6 and total PUFAs were more important than the genotype effects.

Table 5. Estimated Contribution of *Longissimus Dorsi* Fatty Acids (mg/100g Fresh Tissue) According to Genotype and Diet

	LA	ALA	ARA	EPA	DPA	DHA
AA	144	31a	37c	10	17	10A
CHAxAA	159	33a	41b	10	17	10A
HA	148	27b	47a	11	17	5B
Pasture	101c	40a	31c	16a	21a	11A
Cn0.7%	126c	34b	37b	11a	18b	8AB
Cn1.0%	158b	32b	48a	9c	18b	8AB
Cn85%	216a	15c	49a	6c	11c	6B
RMSE	45,9	11.3	15.1	4.1	6.3	5.4
Genotype	0.263	0.026	0.005	0.334	0.995	0.000
Diet	0.000	0.000	0.000	0.000	0.000	0.008
GxD	0.847	0.945	0.640	0.478	0.473	0.000

Note. The estimated values were calculated according with the levels of intramuscular fat

*Significance of main effects (breed, diet) and their interaction (B x D)

abc means within a column with different letters significantly differ.

RMSE: Root mean square error

4. Discussion

It is well know that fatty acid composition of beef, and hence its nutritional value, can be manipulated by genetic and nutritional approaches although it is acknowledge that genetic factors provide smaller differences than dietary factors. In this study, as in a previous one, we found some evidence of genetic variation in LA and ALA proportions in beef lipids (Garcia et al. 2008). Nuernberg et al. (2005) found that the percentages of ALA in German Simmental and German Holstein bulls fed with grass or concentrate increased whereas the percentage of LA were only affected by breed being higher in German Simmental intramuscular fat. In contrast, Muchenje et al. (2009) found no differences due to the breed in the proportions of LA and ALA among Nguni, Bonsmara and Angus steers. Corazzin et al. (2012), found that Italian Simmental and Holstein bulls presented dietary differences in *Longissimus thoracis* muscle for LA in phospholipids and neutral lipids fractions.

We found significant increase in most of the individual muscle n-3 PUFAs and decrease in n-6 PUFAs in the case of the diet supplemented with grain when compared with pasture group. In our study beef PUFAs content was higher than the values reported by others (Scollan et al., 2006) LA is the primary PUFAs in both grass/forage-fed and grain-finished beef providing 60-85% of total PUFAs (Duckett et al., 2013). Usually the percentage of PUFAs in beef is increased by as much as 25% in response to grass-feeding due to the lower intramuscular total fat of most grass-fed beef. However, the total

estimated amount of PUFAs in steaks from U.S. grass/forage-fed cattle may be up to 75mg lower per 100g of beef, primarily as LA (Daley et al., 2010). Only 16 to 26 mg ALA have been reported in various lean cuts for grass/forage cattle versus 4-13 mg ALA from U.S. grain-finished beef, whereas but only traces of n-3 LC-PUFAs are noted in meat from U.S. grass/forage-fed cattle (Duckett et al., 2013).

The n-6/n-3 FA ratio in grass-fed beef is beneficially low, typically less than 3 (Howe et al., 2006). The decreased n-3/n-6 ratio in grazing beef is a consequence of increased ALA proportion and a relative constant proportion of LA in adipose tissues from steers on pasture compared with those finished with feedlot diets. Steers finished on a grain diet show a 2.5 times higher n-6/n-3 PUFAs ratio than those finished on pasture (Basarab et al., 2007; Duckett et al., 2009). However, the fold of increase in the n-6/n-3 FA ratio can be higher when examined in beef muscles (Garcia et al., 2008). Similar results have been found for others with crossbreed heifers offered a control diet of grass silage and concentrate (Sarries et al., 2009). Alfaia et al. (2009) used Alentejano bulls to research the effect of four feeding systems (pasture feeding followed by 2 or 4 months of finishing on concentrate and concentrate only) on the fatty acid composition of muscle of *Longissimus dorsi* muscle and found no significant differences in the proportions of LA among all treatments. Conversely, they found that the meat from the bulls fed the pasture diet had the highest percentages of ALA. Varela et al. (2004) found that both a pasture finishing system and an indoor finishing system (maize, silage and concentrate) affected the intramuscular fatty acid profile of Rubia Gallega steers. They also found that intramuscular fat fatty acids of finished steers contained higher concentrations of LA but no differences in those of ALA. In addition, Alfaia et al. (2006) found differences in LA between meat from bullocks reared in a semi-intensive system and that from bullocks produced in a conventional intensive concentrate-based system. On the other hand, Faucitano et al. (2008) found that cattle fed high concentrate diets showed a significantly decreased the proportion of ALA in *Longissimus dorsi* muscle lipids. Lee et al. (2009) studied the effects of diets with grass or red clover silage on the proportions of LA and ALA in *Longissimus dorsi* muscle in dairy cull cows and found that red clover silage increased the percentages of LA and ALA. Herdman et al. (2010) studied the effects of two diets, one rich in LA and the other rich in ALA and found that both affected the fatty acid composition of the lipid classes in the IMF of German Simmental bulls. The bulls fed the ALA rich-diet had higher concentrations of ALA in phospholipids and triglycerides. Leheska et al. (2008) in an USA study for inclusion in the USDA National Database for Standard Reference compared the fatty acid composition of grass-fed beef and conventionally fed (control) beef. They concluded that the two production systems, semi-intensive vs. intensive, affected the LA and ALA concentrations.

While the contribution of ALA to cardiovascular diseases is debatable, evidence regarding the role of n-3 LC-PUFAs in the prevention of heart diseases is convincing (FAO, 2010). Due to the importance of n-3 LC-PUFAs in cardiovascular health, intake recommendations suggest a minimum of 250 mg EPA+DHA/day (EFSA, 2010). It has been shown that both grass/forage and grain-finished-fed beef can

contribute n-3 LC-PUFAs to the U.S. diet, averaging between 5.33 and 2-19 mg/100 respectively, primarily as EPA+DPA (Van Elswyk & McNeillet, 2014).

5. Conclusions

The genotype and animal diet, are the main factors considered in the current systems of beef production, affected the nutritional value of beef lipids. The LA and ALA proportions, and therefore the LA/ALA ratio of beef lipids were generally more healthy in grass-fed than grain-fed beef. Considering the importance of LA and ALA competition in the synthesis of LC n-3 PUFAs, and therefore in the concentrations of EPA, DPA and DHA in beef lipids, it is possible to optimize genotypes and animal diets. The dietary design will be of great importance to improve the nutritional value of beef. By feeding animals correctly, we address the problem of consuming too much n-6 fatty acids at the expense of too little n-3 fatty acids, without virtually changing of diet. In conclusion, it is possible to manipulate the fatty acid composition of beef toward a more healthy profile. Although these changes appear modest, for non-fish eaters, animal products such as beef are the only sources of long-chain n-3 PUFAs, and any improvement in the fatty acid composition of beef will result in an increase in the consumption of n-3 fatty acids.

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