Journal MVZ Cordoba
2023; may-august. 28(2):e3090.
https://doi.org/10.21897/rmvz.3090

Original

Candidate gene polymorphism effects on the fatty acid composition of Wagyu-Cross beef

Luis Enrique Sánchez-Ramos[1]; Ana Maria Sifuentes-Rincón[1]; Juan Gabriel Magaña-Monforte[2]; Víctor Ricardo Moreno-Medina[1]; Gaspar Manuel Parra-Bracamonte[1]*.

[1]Instituto Politécnico Nacional. Centro de Biotecnología Genómica. Laboratorio de Biotecnología Animal. Tamaulipas, México.
[2]Universidad Autónoma de Yucatán. Facultad de Medicina Veterinaria y Zootecnia. Yucatán, México.
*Correspondencia: gparra@ipn.mx

Received: January 2023; Accepted: April 2023; Published: May 2023.

ABSTRACT

Objective. To assess the fatty acids composition (FA) in Wagyu beef and its crosses with Angus, Beefmaster, Brangus and Hereford, and to analyze its relationship with genetic markers related to lipid metabolism. **Materials and methods**. 111 *Longissimus dorsi* samples were collected and grouped by genetic group. FA were extracted and quantified in a gas-liquid chromatography and DNA markers theoretically associated to FA were typed. Hardy-Weinberg equilibrium and linkage disequilibrium were examined, the effect of crosses and the effect of genotypes were estimated. **Results.** The crosses did not show substantial differences in FA composition. Nine SNPs showed association with FA composition, and a significant effect was found in the SLC2A4 marker ss62538460 which influenced SFA, MUFA and MUFA/SFA; PLTP ss77832104 and IGF2R ss77831885 markers, influenced C16:0, MYOZ1 ss77832104 on C17:1 and PPARGC1A c.1892+19 on C18:2. In addition, previously described effects of MEF2C ss38329156 and SCD c.878 were supported. **Conclusions.** These results are first evidence on FA deposition in Wagyu cattle and their crosses, and proposes some *loci* in candidate genes with the possibility of implementation in assisted selection strategies.

Keywords: Candidate gene; crossbreeding; fatty acid; marbling; marker-assited selection; meat quality; red meat; single nucleotide polymorphism (Sources: MeSH, FAO).

RESUMEN

Objetivo. Comparar la composición de ácidos grasos (FA) en la carne Wagyu y sus cruzas con Angus, Beefmaster, Brangus y Hereford, y analizar su relación con marcadores genéticos del metabolismo lipídico. **Materiales y metodos.** Se colectaron 111 muestras de *Longissimus dorsi*, las cuales se agruparon por grupo genético, se cuantificaron los FA (cromatografía de gases líquidos) y se tipificaron marcadores de ADN asociados teóricamente a FA. Se examinó el equilibrio de Hardy-Weinberg y

How to cite (Vancouver).
Sánchez-Ramos LE, Sifuentes-Rincón AM, Magaña-Monforte JG, Moreno-Medina VR, Parra-Bracamonte GM. Candidate gene polymorphism effects on the fatty acid composition of Wagyu-Cross beef. Rev MVZ Cordoba. 2023; 28(2):e3090. https://doi.org/10.21897/rmvz.3090

ISSNL: 0122-0268

desequilibrio de ligamiento de los marcadores y se estimó el efecto de las cruzas y el efecto de los genotipos. **Resultados.** Las cruzas no mostraron diferencias substanciales en la composición de FA con respecto a Wagyu. Nueve SNPs mostraron asociación con la composición de FA, y se encontró un efecto importante en el marcador SLC2A4 ss62538460, el cual influyó sobre SFA, MUFA y MUFA/SFA. Los marcadores, PLTP ss77832104 y IGF2R ss77831885 influyeron sobre C16:0, MYOZ1 ss77832104 sobre C17:1 y PPARGC1A c.1892+19 sobre C18:2. Además, se comprobaron efectos previamente descritos de MEF2C ss38329156 y SCD c.878. **Conclusiones.** Los presentes resultados representan una de las primeras evidencias sobre la deposición de FA en ganado Wagyu y sus cruzas y propone algunos *loci* en genes candidatos con posibilidad de implementación en estrategias de mejoramiento asistido.

Palabras clave: Ácidos grasos; calidad de la carne; carne; genes candidatos; polimorfismo de un solo nucleótido; selección asistida por marcadores; veteado (*Fuentes: MeSH, FAO*).

INTRODUCTION

The term "Wagyu" means Japanese cattle, so this term includes the four breeds of cattle of Japanese origin, (Japanese Black [BJ], Japanese Coffee, Japanese Polled and Japanese Shorthorn) (1). Wagyu beef is recognized for its high content of intramuscular fat (IMF); however, of the four breeds, only BJ can produce meat at the marbling level at which this meat is recognized. Because of this, in Japan, BJ occupies more than 90% of the total livestock population and is the only breed that is raised on a large scale in countries such as Australia, Spain and the United States (2). The term Wagyu outside Japan is usually used to refer to the meat of BJ cattle.

The level of IMF or marbling is closely related to consumer sensory perception, so it is used as a visual indicator of sensory quality (3), and an increase in IMF in meat has been associated with greater palatability (4,5). Similarly, the fatty acid (FA) composition in the IMF influences the sensory and nutritional characteristics. In specific breeds, marbled beef not only has excellent sensory quality but also contains a large amount of health-promoting FAs (6).

Generally, saturated fatty acids (SFAs) have been associated with negative health effects for consumers and satisfactory meat taste (7,8); conversely, monounsaturated fatty acids (MUFAs) have been associated with positive health effects (6) and sensory characteristics, and polyunsaturated fatty acids (PUFAs) have been associated with various positive health effects. It has been suggested that consumers prefer and demand meat with higher amounts of MUFAs and lower amounts of SFAs (8). Due to the above preferences, the meat industry has sought to improve the MUFA/SFA ratio. Some studies have indicated that Wagyu beef contains a higher concentration of MUFAs and a lower concentration of SFAs than Angus, Charolais, and Holstein beef (9,10).

In Mexico, the Wagyu breed was introduced through embryos imported from the United States and is currently produced using crossbreeding with meat breeds with the intention of improving carcass traits and adaptability to production conditions. Currently, its meat is marketed under the name of Wagyu-Cross, which uses the crossbreeding strategy with other beef cattle breeds to combine conformation and carcass size traits with the capability of marbling and fat deposition (11); however, the relative advantages of this strategy have not been evaluated. Therefore, the objective of this study was to characterize and compare the composition of FAs in Wagyu beef and its crosses and to study the effect of a panel of genetic markers in candidate genes of lipid metabolism on the composition of FAs.

MATERIALS AND METHODS

Sample origin. We collected 111 beef samples of the section between the 11th and 12th rib from the *longissimus dorsi* muscle of animals from the "Exhacienda Cañas" ranch, located in the municipality of Canatlán Durango, Mexico, 24.806 N, -104.815 W, with a semiarid climate that is warm with rains in summer.

The samples were grouped according to the genetic group in Wagyu (WAG, n=39) and its crosses with Angus (WAN, n=18), Beefmaster (WBM, n=12), Brangus (WBR, n=29) and Hereford (WHF, n=13). The sampled animals were fed during the fattening stage with a diet with a special formulation based on corn, hay, bran, soybean paste, dry grains, silage, alfalfa and calcium. The animals were on average 33.6±8.9 months old and were fattened for an

average of 428 ± 149.6 days and slaughtered in a Federal Inspection Type (TIF) slaughterhouse.

Quantification of fatty acids. Meat samples were processed with the Lipid Extraction Kit (Catalog No. MAK174, Sigma–Aldrich®, St. Louis, MO) using the manufacturer's suggested protocol and boron-methanol trifluoride solution for the transesterification process. The quantification of FAs was performed in a liquid–gas chromatograph/mass spectrometer (GC/MS) (GC7890A, Agilent Technologies, Santa Clara, CA), with a DB-23 column (123-2332, Agilent Technologies, Santa Clara, CA) (30 m x 0.32 mm, 0.25 µm), following previously reported parameters (11), where the injection temperature was 50°C and was increased at 10 °C/min until reaching 180°C, where it was maintained for 5 min, followed by increases of 5°C/min until reaching 220°C, where it was maintained for 20 min. The reading time was 48 min at a maximum temperature of 240°C. The carrier gas used was helium with a total flux of 29.174 mL/min. The chromatograms were read with the Agilent G1701EA ChemStation GC/MSD program (Agilent Technologies, Santa Clara, CA) based on the retention times of the FAs in the Supelco 37 Component FAME mix standard (Supelco®, Burlington, MA). The concentrations of FAs in the samples (g/100 g of FA) were obtained from the percentages of the quantified peak areas (11).

Genotyping. A panel of 20 SNPs located in 17 candidate genes was selected to determine their association with FA deposition based on the design by Vela (11). The SNPs were selected considering their contribution in some metabolic pathways associated with FAs and their significantly reported effect on the FA composition in meat or milk (Table 1). DNA was extracted from the samples with the commercial Genelute Mammalian Genomic DNA kit (Cat. No. G1N350, Sigma–Aldrich®, St. Louis, MO). Genotyping was carried out by the company NEOGEN Corp. with Sequenom MassARRAY® system technology (Agena Bioscience, San Diego, CA).

Statistical analysis. The Hardy-Weinberg equilibrium (HW) of each locus studied by genetic group was examined, in addition to the linkage disequilibrium using GENEPOP software (17).

Table 1. Panel of 22 selected SNPs for the fatty acid association assessment.

Gene	Chromosome	Locus	Alleles	Ref
ACACA	19	ss64381883	G/A	12
DGAT1	14	ss77831745	G/A	13
HNF4A	13	ss61961144	T/C	12
IGF2R	9	ss77831885	G/A	12
INSIG2	2	rs134478878	C/G	14
INSIG2	2	rs133396141	C/T	14
LPL	8	ss65478732	T/C	12
MEF2C	7	ss38329156	G/T	12
MYOZ1	28	ss77831945	T/C	12
PLTP	13	ss77832104	G/A	12
PPARG	22	ss62850198	A/G	12
PPARGC1A	6	c.1847	C/T	15
PPARGC1A	6	c.1892+19	T/C	15
PRKAR2A	22	ss62837580	T/C	12
PRKAR2A	22	ss62837667	T/C	12
SCD	26	c.702	A/G	16
SCD	26	c.762	T/C	16
SCD	26	c.878	C/T	16
SCD5	6	rs109937152	C/T	14
SLC2A4	19	ss62538460	G/A	12
SREBF1	19	ss62543518	T/C	12
SRPR	29	rs110036978	C/G	14

To estimate the effect of Wagyu crosses and adjust the FA concentration for the subsequent association with genotypes at the *loci* studied, a mixed model was fitted using the MIXED procedure, as follows:

$$Y_{ijklmn} = +S_i+GG_j+E_k+S_l+A_m+D_m+_{ijklmn}$$

where Y= random variable of FA concentration, = overall mean, S_i= random effect of the sire, GG_j= fixed effect of the j-th genetic group, E_k= fixed effect of the k-th FA extraction, S_l= fixed effect of the l-th sex of the animal, A_m= fixed effect of the m-th year, D_n= fixed effect of the linear covariate of the n-th fattening day, and $_{ijklmn}$= residual random error, subsequently considered as the random variable for adjusting the FA concentration in meat (aY_i). The least squares means of each genetic group were estimated, and a comparison of means was made with a t test of minimal significant difference and considering an alpha = 0.05.

Subsequently, a polygenic linear model was adjusted to estimate the effect of genotypes on the studied loci as follows:

$$aY_i = +G1... i+_i$$

where aY= adjusted random variable of FA concentration, = overall mean, G= fixed polygenic effect of n= 1... i loci, and = residual random error. The least squares means of the genotypes at each locus were estimated, and a comparison of means was performed with a t test of minimal significant difference with Bonferroni adjustment for multiple comparisons, considering an alpha = 0.05.

All statistical procedures were performed using SAS v.9.4 software (SAS Institute Inc., Cary, CN).

RESULTS

Composition of fatty acids. The composition of the following FAs was determined: lauric (C12:0), myristic (C14:0), myristoleic (C14:1), pentadecanoic (C15:0), palmitic (C16:0), palmitoleic (C16:1), heptadecanoic (C17:0), heptadecenoic (C17:1), stearic (C18:0), oleic (18:1), linoleic (C18:2), α-linolenic (C18:3), eicosanoic (C20:0), eicosenoic (C20:1), eicosadienoic (C20:2) and eicosatrienoic (C20:3), in addition to the MUFA/SFA and PUFA/SFA proportions of Wagyu beef and Wagyu crosses. The means of FAs of the population studied are presented in table 2.

The least squares means of the effect of crosses on the FA deposition in the longissimus dorsi of the WAN, WBM, WBR, WHF and WAG groups were analyzed (Table 3). WBM had the highest concentration of C16:0 and C18:0 and ΣSFA, where a difference was observed (5.3%, 6.2% and 13.0%, respectively) with respect to WBR, which was the group with the lowest concentrations; however, no significant differences ($p \geq 0.05$) were obtained between the groups. Additionally, C15:0 and C17:0 were found in higher concentrations in WBM, but only C17:0 in WBM had a significant difference with respect to the other studied genetic groups ($p<0.01$). On the other hand, WBM had the lowest concentrations of C18:1, ΣMUFA and C18:2, and a difference was observed (10.213%, 11.039%, and 2.754%, respectively) with respect to WBR; however, there was no statistical significance ($p \geq 0.05$).

Table 2. Fatty acid profile (g/100 g) in Wagyu beef and its crosses. n=83.

Fatty acid	Symbol	Mean±SD
Lauric Acid	C12:0	0.120±0.075
Myristic Acid	C14:0	3.759±1.076
Myristoleic Acid	C14:1	0.746±0.408
Pentadecanoic Acid	C15:0	0.466±0.210
Palmitic Acid	C16:0	27.509±5.713
Palmitoleic Acid	C16:1*c*	3.710±1.505
Heptadecanoic Acid	C17:0	0.837±0.377
Heptadecenoic Acid	C17:1	0.467±0.253
Estearic Acid	C18:0	16.731±6.122
Oleic Acid	C18:1	40.877±6.433
Linoleic Acid	C18:2	4.348±1.608
α-Linolenic Acid	C18:3	0.218±0.100
Araquidic Acid	C20:0	0.160±0.140
Eicosenoic Acid	C20:1	0.289±0.131
Eicosadienoic Acid	C20:2	0.102±0.138
Dihomo-γ-linolenic Acid	C20:3	0.183±0.171
Saturated fatty acids	ΣSFA	49.421±5.905
Monounsaturated fatty acids	ΣMUFA	45.801±6.393
Polyunsaturated fatty acids	ΣPUFA	4.776±1.922
MUFA/SFA		0.952±0.224
PUFA/SFA		0.097±0.040

Effect of candidate gene markers on the concentration of beef cattle fatty acids. From the SNPs analyzed, it was found that HNF4A ss61961144 and SCD c.762 deviated from HW equilibrium ($p<0.001$ and $p<0.05$, respectively). On the other hand, it was found that SCD c.702 was in linkage disequilibrium with SCD c.762, and SCD c.702 and SCD c.762 showed linkage to SCD c.878 ($p<0.001$); in the same way, the SNPs ss62837667 and ss62837580 located in the PRKAR2A gene showed linkage disequilibrium ($p<0.001$).

Nine markers were found to have an effect on the composition of FAs in Wagyu and Wagyu-Cross beef (Table 4). Among the genes that interact in the catabolic pathway of AMP-activated protein kinase (AMPK), the marker MEF2C ss38329156 was found to be associated with C14:0 ($p<0.05$), PPARGC1A c.1892+19 with C18:2 ($p<0.05$) and SLC2A4 ss62538460 with ΣSFA ($p<0.01$), ΣMUFA and MUFA/SFA ($p<0.05$). In the set of genes analyzed that participate in the anabolic pathway of AMPK, the three markers in SCD were associated with different FAs. The marker c.702 was associated with C14:0 and C16:0 ($p<0.05$), C762 with

ΣSFA ($p<0.05$) and c.878 with ΣSFA ($p<0.01$), C16:0, C17:1, C18:1, ΣMUFA and MUFA/SFA ($p<0.05$). The marker PLTP ss77832104 was the only one of the genes analyzed in the cholesterol pathway that had a significant association, and this marker had an effect on C16:0 ($p<0.05$). Finally, IGF2R ss77831885 was associated with C16:0 ($p<0.05$), and MYOZ1 ss77832104 was associated with C17:1 ($p<0.05$).

Table 3. Least squares means for the effect of genetic group on the fatty acid deposition in Wagyu beef and its crosses.

FA	WAN	WBM	WBR	WHF	WAG	P value
	n= 18	n= 12	n= 23	n=12	n= 31	
C12:0	0.217±0.046	0.161±0.071	0.249±0.045	0.183±0.046	0.190 ±0.040	0.1312
C14:0	3.402±0.918	3.012±1.408	2.857±0.888	3.352±0.901	2.971±0.802	0.8433
C14:1	0.492±0.311	0.602±0.477	0.329±0.301	0.654±0.305	0.510±0.271	0.7473
C15:0	0.402±0.128	0.730±0.197	0.379±0.124	0.364±0.126	0.367±0.112	0.4437
C16:0	28.380±3.506	31.604±5.376	26.279±3.391	28.056±3.442	28.363±3.061	0.7128
C16:1	2.706±0.911	1.895±1.398	3.075±0.881	2.591±0.895	2.623±0.796	0.8519
C17:0*	0.810±0.225^{b}	1.890±0.345^{a}	0.819±0.218^{b}	0.534±0.221^{b}	0.689±0.196^{b}	0.0026
C17:1	0.490±0.121	0.601±0.186	0.522±0.117	0.379±0.119	0.457±0.106	0.4559
C18:0	18.621±4.185	24.081±6.418	17.928±4.048	19.157±4.109	18.519±3.655	0.9395
C18:1	39.849±4.810	31.543±7.377	41.756±4.653	41.095±4.722	40.281±4.201	0.6626
C18:2	4.376±1.286	2.848±1.973	5.602±1.244	3.502±1.263	4.741±1.123	0.3482
ΣSFA	51.834±4.239	61.481±6.501	48.514±4.100	51.648±4.162	51.101±4.162	0.3352
ΣMUFA	43.538±4.620	34.643±7.085	45.682±4.469	44.721±4.536	43.873±4.034	0.5524
MUFA/SFA	0.826±0.164	0.528±0.251	0.935±0.158	0.843±0.162	0.862±0.143	0.5406
PUFA/MUFA	0.084±0.0322	0.052±0.049	0.118±0.310	0.064±0.031	0.095±0.028	0.3101

FA: Fatty acids. *P<0.05. WAN: Wagyu x Angus. WBM: Wagyu x Beefmaster. WBR: Wagyu x Brangus. WHF: Wagyu x Hereford. WAG: Wagyu. SFA: Saturated fatty acid. MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

Table 4. Effects of polymorphisms in candidate genes on the fatty acid concentration in beef cattle.

Gene and locus	**A/B**	**AAF**	**FA**	**LSM ± SE**			**P value**
				AA	**AB**	**BB**	
MEF2C ss38329156	G/T	0.784	C14:0	-2.783±1.761^{a}	-1.367±1.614^{b}	-3.010±2.030^{ab}	0.0367
PPARGC1A c.1892+19	T/C	0.194	C18:2	-2.003±3.258^{ab}	-1.751±2.645^{a}	0.204±2.413^{b}	0.0353
SLC2A4 ss62538460	G/A	0.969	ΣSFA	-4.864±2.413^{b}	-14.537±8.975^{a}	-	0.0090
			ΣMUFA	5.276±7.686^{b}	14.922±8.975^{a}	-	0.0350
			AGM/AGS	0.204±0.266^{b}	0.570±0.311^{a}	-	0.0224
SCD c.702	A/G	0.662	C14:0	-1.844±1.606^{ab}	-0.587±1.564^{b}	-4.730±1.564^{a}	0.0140
			C16:0	-0.004±5.072^{b}	-0.263±4.941^{b}	-12.002±7.366^{a}	0.0322
SCD c.762	T/C	0.680	ΣSFA	-6.477±6775^{ab}	-6.913±6.590^{b}	-15.711±6.778^{a}	0.0202
SCD c.878	C/T	0.0.593	C16:0	-7.358±5.995^{a}	-5.891±6.04^{a}	0.978±5.052^{b}	0.0270
			C17:1	-0.104±0.229^{b}	0.2151±0.23^{a}	0.018±0.193^{ab}	0.0154
			C18:1	16.620±9.800^{ab}	15.001±9.876^{a}	3.450±8.259^{b}	0.0255
			ΣSFA	-14.598±6.950^{a}	-11.859±7.003^{a}	-2.643±5.857^{b}	0.0087
			ΣMUFA	14.325±8.897^{ab}	13.212±8.966^{a}	2.759±7.498^{b}	0.0272
			AGM/AGS	0.545±0.308^{ab}	0.484±0.311^{a}	0.133±0.260^{b}	0.0302
PLTP ss77832104	G/A	0.785	C16:0	-8.638±5.856^{a}	-6.060±5.679^{a}	2.427±5.8052^{b}	0.0394
IGF2R ss77831885	G/A	0.829	C16:0	-5.477±5.834^{ab}	-7.730±5.795^{a}	0.936±5.652^{b}	0.0423
MYOZ1 ss77832104	T/C	0.396	C17:1	-0.123±0.202^{b}	0.061±0.218^{ab}	0.181±0.226^{a}	0.0427

A/B: Allelic relationship of locus. AAF: A allele frequency, FA: Fatty acid

DISCUSSION

Wagyu beef is recognized worldwide as a meat with a high IMF content, which is one of the most important characteristics because it improves sensory perceptions such as taste, juiciness and softness (18,19); in addition, the content and proportion of FAs in red meat has been linked to health effects.

SFAs are generally the most abundant SFAs in beef and are negatively associated with health; 16:0 and C18:0 are the most abundant SFAs in beef. In the population analyzed, the sum of these is equivalent to 89.5%, of which C12:0, C16:0 and C18:0 have been associated with the risk of type II diabetes, coronary heart disease, and cardiovascular disease (7,20). On the other hand, C16:1 and C18:1 are the most abundant MUFAs, and both have been associated with improving sensory perception in beef (8,21). Together, these MUFAs make up 97.3% of ΣMUFAs. PUFAs have been linked to multiple positive health effects (22,23). Therefore, we sought to improve the MUFA/SFA and PUFA/SFA proportions in meat. There is no consensus on the ideal MUFA/SFA ratio; however, this ratio allows the fat composition to be compared. In the Wagyu breed, values of 1.17 (9,10) are usually reported; in this study, a value of 0.95 was found. The PUFA/SFA ratio is one of the main parameters used to measure the nutritional quality of the lipid ratio of foods. Nutritional guidelines recommend a ratio higher than 0.4, and the proportion for beef is close to 0.1 (24). In the present study, a value of 0.097 was found.

Differences in the deposition of fatty acids in Wagyu cattle and their crosses. The genetic differences among breeds influence the metabolism of FAs from dietary sources and de novo synthesis, both of which greatly influence the contents of SFAs and MUFAs (22,25,26). Table 3 presents the least squares means of the FA concentration in the *longissimus dorsi* samples of the different Wagyu crosses studied. Of the groups analyzed, WBR obtained the best proportion of FAs, with increases in either the MUFA/AFS ratio or the PUFA/AFS ratio; however, there were no significant differences ($p \geq 0.05$). In the results obtained, only C17:0 had a significant difference, where WBM produced up to 3.5 times more C17:0 than WHF. Large-scale population studies have shown effects of C17:0 on reducing the risks of mortality, obesity and numerous cardio-metabolic and liver diseases (7,27).

Previous studies have clarified the difference between Angus and Wagyu breeds. In Wagyu and Angus steers fed a special diet (e.g., Japanese), significant differences were found where the Wagyu *longissimus dorsi* muscle had a higher concentration of C14:1 and lower concentration of C18:0 with a higher MUFA/SFA ratio, in addition to higher amounts of IMF and subcutaneous fat, and differences were found in four FAs that favorably influenced the MUFA/SFA ratio of Wagyu (9). In Wagyu and Angus steers, significant differences were found in six FAs in the longissimus muscle; three favored the MUFA/SFA ratio of Wagyu, and two favored the Angus ratio; however, no significant differences were observed in the MUFA/SFA ratio. Angus had a higher concentration of C18:2, which significantly improved the PUFA/SFA ratio. In addition, differences in six subcutaneous fat FAs were found (28). In another study where different finishing systems were evaluated between Angus and Wagyu cattle, differences were found due to the effect of the breed on C14:0, C16:0 and three subcutaneous fat PUFAs, where Wagyu animals had lower concentrations of C14:0 and C16:0 and higher concentrations of PUFAs in most fattening systems (29).

There are few comparisons of FAs in meat of Wagyu crosses with respect to other breeds. In Angus animals and their crosses with Brangus and Brahman (25, 50 and 75%), multiple FA differences were reported in the longissimus. The Angus 25%, Angus 50% and Brahman 75% groups exhibited the lowest concentrations of SFAs because they also had low concentrations of C16:0; additionally, the Brahman group showed high concentrations of 9 PUFAs, reflecting a difference in ΣPUFAs (25). In Hereford crosses with Angus and Limousin, a difference in C16:0 was found, as well as ΣSFA, ΣMUFA, and 7 PUFAs (30). Another study of Hereford crosses with Belgian Blue, Limousin and Galician Blonde revealed differences between these breeds with respect to FAs C10:0 and C18:0 (31).

Analysis of the effect of markers in candidate genes on the concentration of beef fatty acids. The promise of genetic improvement through the implementation of genomics has encouraged the discovery and validation of genetic markers that include studies in different cattle breeds. In the present study, nine genetic markers were found to have an effect on the composition of FAs in Wagyu and Wagyu-Cross meat (Table 4).

Some of the markers that showed significant effects are part of the AMPK pathway, which participates as a sensor of cellular energy status activated by increases in the AMP/ATP cell ratio caused by metabolic stress that interferes with ATP production. Once activated, the AMPK pathway induces catabolic pathways such as FA oxidation and glycolysis for ATP production (32). In the AMPK signaling pathway, PPARG is regulated by PPARGC1A, which induces SLC2A4, thereby activating mitochondrial biogenesis; on the other hand, MEF2 interacts specifically with the MEF2C response element of the SLC2A4 gene, which is essential for the expression of GLUT4 in skeletal muscle (12).

The PPARGC1A gene encodes peroxisome proliferator-activated receptor gamma coactivator 1-alpha, and SNP c.1892 + 19 is found in intron 9 of this gene, where the T allele of this marker is associated with increased fat in milk from Holstein cattle (15, 33). In this analysis, it was found that the T allele reduced the concentration of C18:2. CC homozygotes produced 1,955 g/100 g more C18:2 than TC homozygotes ($p<0.05$). PPARGC1A regulates the expression of genes involved in adipogenesis, gluconeogenesis, oxidative metabolism, adaptive thermogenesis, and energy homeostasis and activates multiple nuclear hormone receptors (15). Conjugated linoleic acids (CLAs) make up a family of isomers of 18 carbons and two double bonds (C18:2), which have been associated with anticancer, anti-obesity and antidiabetic effects (34). Moreover, linoleic acid (C18:2c9,12) is an essential ω-6 because it cannot be synthesized by humans; it is the most abundant PUFA in meat and has been positively correlated with taste, juiciness and tenderness (21).

The MEF2C gene encodes myocyte enhancing factor 2C, and in the transversion ss38329156 in intron 10, homozygous GG was associated with a 50% lower concentration of C14:0 with respect to heterozygous GT ($p<0.05$). These data support what was previously found (11), where GG was associated with lower concentrations of C14:0, C15:0, C16:0, and 17:0 in Mexican commercial beef, including Wagyu-Cross. PPARGC1A and MEF2C are active skeletal muscle genes involved in fat metabolism and muscle development. However, MEF2C is selectively expressed in differentiated myocytes and activates almost all skeletal and cardiac muscle genes, thereby controlling morphogenesis, cardiac myogenesis, and vascular development (12).

The SNP ss62538460 is found in exon 11 of SLC2A4. In the present results, the GA genotype of this SNP was associated with a 66% lower content of SFAs and 64% higher MUFAs and MUFA/SFA ratio ($p<0.001$, $p<0.05$ and $p<0.05$, respectively) with respect to those of GG. Previously, this marker was associated with C17:0 and total FAs (11). The proportion of FAs influences the total FAs, so the previous association can be validated by the results obtained here. It is possible that the A allele has an additive effect; however, no homozygotes were found. As in this population, low segregation of the A allele was found in Spanish breeds (15). Solute transporter family member 4 (GLUT4) is a protein produced by SLC2A4 that captures insulin-mediated glucose in muscle and fat cells (12). Differences in the physiological impact of SLC2A4 expression on ruminant adipose development could be because glucose is a minor substrate for FA synthesis (12).

In addition to activating catabolic pathways, the AMPK pathway inhibits anabolic processes such as protein, FA, and glycogen synthesis to restore energy balance (12). The AMPK pathway inhibits SREBF1, which regulates the ACACA and SCD genes, which participate in anabolic pathways such as FA synthesis and the addition of double bonds to FAs (16). The SCD gene encodes the enzyme stearoyl Co-A desaturase l, which catalyzes the insertion of a double bond cis at position Δ-9 in the substrate. Taniguchi et al. (16) discovered three SNPs of exon 5, which were associated with different FAs in this study (Table 4). The present data showed that the A allele in c.702 increases the concentration of C14:0, and the GG homozygote was associated with an 87% lower concentration with respect to the AG heterozygote ($p<0.001$). In addition, the GG homozygote was associated with an 11,998 g/100 g lower concentration of C16:0 with respect to AA homozygotes and 11,739 g/100 g with respect to AG heterozygotes ($p<0.05$). For marker c.762, the presence of the T allele increased the SFA concentration, and the CC homozygote was associated with a 55% lower concentration than that of the TC heterozygote ($p<0.05$). For marker c.878, an additive effect of the C allele was observed, and the CC and CT genotypes were associated with lower C16:0 concentrations of 8,336 and 6,869 g/100 g with respect to the TT genotype ($p<0.05$) and 11,955 and 10,453/100 g for SFAs ($p<0.05$); in addition, TC was associated with lower concentrations of 11,551 g/100 g, 10,453 g/100

g and 0.351 of C18:1, MUFAs and the MUFA/SFA ratio, respectively, compared to CC genotype ($p<0.05$); on the other hand, CT was associated with a 0.3191 g/100 g higher concentration of C17:1 with respect to CC ($p<0.05$). Multiple studies agree on the association of the C allele of the marker c.878 with the increase in C18:1 and MUFAs of different cattle breeds (15,16,35,36); however, no effects of C16:0 and S17:1 have been reported. The effect of this marker on C16:0 is related to the enzyme stearoyl Co-A desaturase, which uses this FA as a substrate, giving rise to C16:1 (12).

Cholesterol forms part of the membranes in mammalian cells and serves as a precursor to bile acids, vitamin D and steroid hormones. Among the genes that interact in cholesterol metabolism, the phospholipid transfer protein synthesized by the PLTP gene transports a large number of different amphipathic molecules (such as phospholipids, diacylglycerol and lipopolysaccharides), playing a fundamental role in lipid and lipoprotein metabolism (12). The SNP ss77832104 located in the 3'UTR has been associated with the ratio of n6/n3 FAs in the muscle of Spanish breeds (37,38). In this study, an additive effect on the G allele was observed; the GG and GA genotypes were associated with lower contents (11.066 g/100 g and 8,487 g/100 g, respectively) of C16:0 compared to the AA genotype ($p<0.05$).

Within the group of genes unrelated to FA metabolism, IGF2R encodes insulin-like growth factor receptor 2, which has multiple cellular functions, such as differentiation, migration and apoptosis of multiple cells (37). In previous studies, the marker ss77831885 has been associated with five FAs and the intensity of flavor of Spanish cattle meat (37,38). In this study, GA heterozygotes presented an 8,666 g/100 g lower concentration of C16:0 with respect to AA homozygotes ($p<0.05$). Finally, the product of the MYOZ1 gene plays an important role as an intracellular binding protein and localizes calcineurin signals in the sarcomere (12). In Spanish breeds, it was found that the marker ss77832104 influences CLAs, the ratio of C18:2/C18:3 and C22:6 (37). In the present analysis, an additive effect of the C allele was observed, and the CC homozygote presented a 0.303 g/100 g higher content of C17:1 than the TT homozygote ($p<0.05$).

The present study evaluated the deposition of Wagyu and Wagyu-Cross meat, which are known to produce meat with a high IMF content. This trait and the composition of FAs influence the concepts of sensory and nutritional quality. In general, polyunsaturated FAs were identified to confirm the favorable deposition in the population studied; however, the distribution of samples allowed their comparison in some cases. The results showed that Wagyu and Wagyu-Cross beef had a very similar FA composition between the groups evaluated but the 3.5-fold higher concentration of C17:0 in WBM with respect to the other groups was highlighted. These findings allow us to conclude that there is no important effect on the deposition of FAs between Wagyu and its crosses but that specific aspects in the deposition such as C17:0 could be subjected to particular studies to confirm their effects.

Conclusively, 17 significant associations were found in 9 SNPs linked to the FA composition in Wagyu and Wagyu-Cross meat, and some associations confirm previous reports; however, most of the associations found in this study are new. Among the new associations, it is important to highlight the effect of the marker SLC2A4 ss62538460, where the GA heterozygote was associated with a considerable reduction in the MUFA and SFA concentrations and the MUFA/SFA ratio. In addition, the markers PLTP ss77832104 and IGF2R ss77831885 were associated with C16:0, the SNP MYOZ1 ss77832104 was associated with C17:1, and PPARGC1A c.1892+19 was associated with C18:2. On the other hand, the relationships between MEF2C ss38329156 and SCD c.878 support the relationships described above. This evidence confirms that SLC2A4, PLTP, MYOZ1, PPARGC1A and SCD are candidate genes and that they could serve as markers for potential use in assisted selection strategies. In this way, this information also allows us to expand the number of markers for understanding the genetic architecture of the FA composition of beef.

Finally, it is important to highlight that a larger sample size, which also implies increased financing costs, could validate these results, with a particular focus on evaluating the effects that are correlated with benefits in other carcass characteristics (e.g., intramuscular fat) with the deposition of FAs in the Wagyu-derived crosses and ensuring the best implementation of the information for commercial purposes and the markers studied in an integrated manner for marker assisted selection programs.

Conflict of interest

The authors state that they have no conflict of interest to disclose.

Acknowledgements

The authors thank the donation of the beef samples and information of the animals for the realization of this study to Eng. Paola Mares and the staff of Agroindustrias Coahuayana, as well as to M. en C. Williams Arellano Vera and Dr. Diana A. Vela Vásquez of the Laboratory of Animal Biotechnology CBG-IPN for the collections and training of the meat bank used in this study, funded by the CONACYT project 299055.

REFERENCES

1. Motoyama M, Sasaki K, Watanabe A. Wagyu and the factors contributing to its beef quality: A Japanese industry overview. Meat Sci. 2016; 120:10–18. http://dx.doi.org/10.1016/j.meatsci.2016.04.026

2. Gotoh T, Nishimura T, Kuchida K, Mannen H. The Japanese Wagyu beef industry: current situation and future prospects - A review. Asian-australas J Anim Sci. 2018; 31(7):933–950. http://dx.doi.org/10.5713/ajas.18.0333

3. Nguyen DV, Nguyen OC, Malau-Aduli AEO. Main regulatory factors of marbling level in beef cattle. Vet Anim Sci. 2021; 14(100219):100219. http://dx.doi.org/10.1016/j.vas.2021.100219

4. Frank D, Ball A, Hughes J, Krishnamurthy R, Piyasiri U, Stark J, et al. Sensory and flavor chemistry characteristics of Australian beef: Influence of intramuscular fat, feed, and breed. J Agric Food Chem. 2016; 64(21):4299–4311. http://dx.doi.org/10.1021/acs.jafc.6b00160

5. Jung E-Y, Hwang Y-H, Joo S-T. The relationship between chemical compositions, meat quality, and palatability of the 10 primal cuts from Hanwoo steer. Korean J Food Sci Anim Resour. 2016; 36(2):145–151. http://dx.doi.org/10.5851/kosfa.2016.36.2.145

6. Troy DJ, Tiwari BK, Joo S-T. Health implications of beef intramuscular fat consumption. Korean J Food Sci Anim Resour. 2016; 36(5):577–582. http://dx.doi.org/10.5851/kosfa.2016.36.5.577

7. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kröger J, Schulze MB, et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. Lancet Diabetes Endocrinol. 2014; 2(10):810–818. http://dx.doi.org/10.1016/S2213-8587(14)70146-9

8. O'Quinn TG, Woerner DR, Engle TE, Chapman PL, Legako JF, Brooks JC, et al. Identifying consumer preferences for specific beef flavor characteristics in relation to cattle production and postmortem processing parameters. Meat Sci. 2016; 112:90–102. http://dx.doi.org/10.1016/j.meatsci.2015.11.001

9. May SG, Sturdivant CA, Lunt DK, Miller RK, Smith SB. Comparison of sensory characteristics and fatty acid composition between Wagyu crossbred and Angus steers. Meat Sci. 1993; 35(3):289–298. http://dx.doi.org/10.1016/0309-1740(93)90034-F

10. Shirouchi B, Albrecht E, Nuernberg G, Maak S, Olavanh S, Nakamura Y, et al. Fatty acid profiles and adipogenic gene expression of various fat depots in Japanese Black and Holstein steers. Meat Sci. 2014; 96(1):157–164. https://doi.org/10.1016/j.meatsci.2013.06.027

11. Vela DAV. Caracterización genética y fenotípica de la carne de res comercializada en el note de Mexico, con énfasis en la composición de los ácidos grasos. Instituto Politécnico Nacional: Mexico; 2018. http://tesis.ipn.mx/handle/123456789/26866

12. Sevane N, Armstrong E, Cortés O, Wiener P, Wong RP, Dunner S, et al. Association of bovine meat quality traits with genes included in the PPARG and PPARGC1A networks. Meat Sci. 2013; 94(3):328–335. http://dx.doi.org/10.1016/j.meatsci.2013.02.014

13. Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, et al. Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res. 2002; 12(2):222-231. http://dx.doi.org/10.1101/gr.224202

14. Medrano JF, Rincon G. SNPs associated with fatty acid composition of bovine meat and milk. U.S. Patent 8,551,703. 2013.

15. Weikard R, Kühn C, Goldammer T, Freyer G, Schwerin M. The bovine PPARGC1A gene: molecular characterization and association of an SNP with variation of milk fat synthesis. Physiol Genomics. 2005; 21(1):1–13. http://dx.doi.org/10.1152/physiolgenomics.00103.2004

16. Taniguchi M, Utsugi T, Oyama K, Mannen H, Kobayashi M, Tanabe Y, et al. Genotype of stearoyl-coA desaturase is associated with fatty acid composition in Japanese Black cattle. Mamm Genome. 2004; 15(2):142–148. http://dx.doi.org/10.1007/s00335-003-2286-8

17. Rousset F. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour. 2008; 8(1):103–106. http://dx.doi.org/10.1111/j.1471-8286.2007.01931.x

18. Corbin CH, O'Quinn TG, Garmyn AJ, Legako JF, Hunt MR, Dinh TTN, et al. Sensory evaluation of tender beef strip loin steaks of varying marbling levels and quality treatments. Meat Sci. 2015; 100:24–31. http://dx.doi.org/10.1016/j.meatsci.2014.09.009

19. Stewart SM, Gardner GE, McGilchrist P, Pethick DW, Polkinghorne R, Thompson JM, et al. Prediction of consumer palatability in beef using visual marbling scores and chemical intramuscular fat percentage. Meat Sci. 2021; 181(108322):108322. http://dx.doi.org/10.1016/j.meatsci.2020.108322

20. Khaw K-T, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. PLoS Med. 2012; 9(7):e1001255. http://dx.doi.org/10.1371/journal.pmed.1001255

21. Hwang Y-H, Joo S-T. Fatty acid profiles, meat quality, and sensory palatability of grain-fed and grass-fed beef from Hanwoo, American, and Australian crossbred cattle. Korean J Food Sci Anim Resour. 2017; 37(2):153–161. http://dx.doi.org/10.5851/kosfa.2017.37.2.153

22. Barendse W. Should animal fats be back on the table? A critical review of the human health effects of animal fat. Anim Prod Sci. 2014; 54(7):831-855. http://dx.doi.org/10.1071/an13536

23. Wood JD, Enser M. Manipulating the fatty acid composition of meat to improve nutritional value and meat quality. En: New Aspects of Meat Quality. Elsevier; 2017. https://doi.org/10.1016/B978-0-08-100593-4.00023-0

24. Romero MC, Romero AM, Doval MM, Judis MA. Nutritional value and fatty acid composition of some traditional Argentinean meat sausages. Food Sci Technol. 2013; 33(1):161–166. http://dx.doi.org/10.1590/s0101-20612013005000007

25. Flowers S, Hamblen H, Leal-Gutiérrez JD, Elzo MA, Johnson DD, Mateescu RG. Fatty acid profile, mineral content, and palatability of beef from a multibreed Angus-Brahman population. J Anim Sci. 2018; 96(10):4264–4275. http://dx.doi.org/10.1093/jas/sky300

26. Scollan ND, Dannenberger D, Nuernberg K, Richardson I, MacKintosh S, Hocquette J-F, et al. Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. Meat Sci. 2014; 97(3):384–394. http://dx.doi.org/10.1016/j.meatsci.2014.02.015

27. Venn-Watson S, Lumpkin R, Dennis EA. Efficacy of dietary odd-chain saturated fatty acid pentadecanoic acid parallels broad associated health benefits in humans: could it be essential? Sci Rep. 2020;10(1):8161. http://dx.doi.org/10.1038/s41598-020-64960-y

28. Xie YR, Busboom JR, Gaskins CT, Johnson KA, Reeves JJ, Wright RW, et al. Effects of breed and sire on carcass characteristics and fatty acid profiles of crossbred wagyu and angus steers. Meat Sci. 1996; 43(2):167–177. http://dx.doi.org/10.1016/0309-1740(96)84588-8

29. Chung KY, Lunt DK, Choi CB, Chae SH, Rhoades RD, Adams TH, et al. Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to US and Japanese endpoints. Meat Sci. 2006; 73(3):432–441. http://dx.doi.org/10.1016/j.meatsci.2006.01.002

30. Papaleo Mazzucco J, Goszczynski DE, Ripoli MV, Melucci LM, Pardo AM, Colatto E, et al. Growth, carcass and meat quality traits in beef from Angus, Hereford and cross-breed grazing steers, and their association with SNPs in genes related to fat deposition metabolism. Meat Sci. 2016; 114:121–129. http://dx.doi.org/10.1016/j.meatsci.2015.12.018

31. Domingo G, Iglesias A, Monserrat L, Sanchez L, Cantalapiedra J, Lorenzo JM. Effect of crossbreeding with Limousine, Rubia Gallega and Belgium Blue on meat quality and fatty acid profile of Holstein calves: Effect of Crossbreeding on Meat Quality. Anim Sci J. 2015; 86(11):913–921. http://dx.doi.org/10.1111/asj.12373

32. Garcia D, Shaw RJ. AMPK: Mechanisms of cellular energy sensing and restoration of metabolic balance. Mol Cell. 2017; 66(6):789–800. http://dx.doi.org/10.1016/j.molcel.2017.05.032

33. Pasandideh M, Mohammadabadi MR, Esmailizadeh AK, Tarang A. Association of bovine PPARGC1A and OPN genes with milk production and composition in Holstein cattle. Czech J Anim Sci. 2016; 60(3):97–104. http://dx.doi.org/10.17221/8074-cjas

34. Koba K, Yanagita T. Health benefits of conjugated linoleic acid (CLA). Obes Res Clin Pract. 2014; 8(6):e525-e532. http://dx.doi.org/10.1016/j.orcp.2013.10.001

35. Li C, Aldai N, Vinsky M, Dugan MER, McAllister TA. Association analyses of single nucleotide polymorphisms in bovine stearoyl-CoA desaturase and fatty acid synthase genes with fatty acid composition in commercial cross-bred beef steers: SCD and FASN SNPs and fatty acids. Anim Genet. 2012; 43(1):93–107. http://dx.doi.org/10.1111/j.1365-2052.2011.02217.x

36. Oh D-Y, Lee Y-S, Yeo J-S. Identification of the SNP (single necleotide polymorphism) of the stearoyl-CoA desaturase (SCD) associated with unsaturated fatty acid in Hanwoo (Korean cattle). Asian-australas J Anim Sci. 2011; 24(6):757–765. http://dx.doi.org/10.5713/ajas.2011.10410

37. Dunner S, Sevane N, Garcia D, Levéziel H, Williams JL, Mangin B, et al. Genes involved in muscle lipid composition in 15 European Bos taurus breeds. Anim Genet. 2013; 44(5):493–501. http://dx.doi.org/10.1111/age.12044

38. Sevane N, Levéziel H, Nute GR, Sañudo C, Valentini A, Williams J, et al. Phenotypic and genotypic background underlying variations in fatty acid composition and sensory parameters in European bovine breeds. J Anim Sci Biotechnol. 2014; 5(1):20. http://dx.doi.org/10.1186/2049-1891-5-20