Journal MVZ Cordoba
2021; January-April. 26(1):e1995.
https://doi.org/10.21897/rmvz.1995

Original

Effect of corn supplementation on the expression of intramuscular fat genes

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Received: April 2020; Accepted: October 2020; Published: November 2020.

ABSTRACT

Objective. The aim of the present study was to determine the effect of supplementing pasture-finished Braford steers with corn silage on the expression level of genes associated with intramuscular fat in *longissimus dorsi* muscle. **Materials and methods**. Thirty Braford steers grazing on summer pasture were used for the study. For 120 days fifteen animals were supplemented with corn silage at 1% of body weight per head per day (Supl) whereas the remaining 15 steers only received pasture (Cont). Animals were slaughtered at 26 month of age with 464±17 Kg. Gene expression of glucose transporter type 4 (*glut4),* insulin-like growth factor 1 (*igf1)* and myostatin were measured using real-time polymerase chain reaction (RT-qPCR). **Results.** Supplementation produced increased expression of *glut4* and *igf1* genes. The expression of the genes studied was correlated with hot carcass weight, fat score of carcass and intramuscular fat content. **Conclusions.** Results suggest a gene expression-diet interaction in *glut4* and *igf1* genes, which impact on carcass fattening and intramuscular fat content in *longissimus dorsi* muscle of Braford steers, suggesting that these meat variables could be modulated through differential gene expression.

Keywords. Adipose tissue; Bovine; Corn; Food Supplements; Steers (Source: AGROVOC).

RESUMEN

Objetivo. Determinar el efecto de la suplementación con silo de maíz en la expresión de genes asociados al contenido de grasa intramuscular en el músculo *longissimus dorsi* de novillos Braford. **Materiales y Métodos.** Se utilizaron 30 novillos Braford. Durante 120 días 15 animales fueron suplementados con silo de maíz al 1% de su peso vivo (Supl), y 15 animales fueron alimentados solamente en pastura tropical (Cont). La faena se realizó a los 26 meses de edad con 464±17 Kg. Mediante la reacción en cadena de la polimerasa en tiempo real (RT-qPCR), se determinó la expresión de los genes transportador de glucosa 4 (*glut4)*, factor de crecimiento tipo insulínico 1

How to cite (Vancouver).
Coria MS, Álvarez-Gutiérrez MA, Reineri PS, Palma GA. Effect of corn supplementation on the expression of intramuscular fat genes. Rev MVZ Cordoba. 2021; 26(1):e1995. https://doi.org/10.21897/rmvz.1995

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ISSNL: 0122-0268

(*igf1)* y miostatina. La expresión de genes fue asociada con parámetros de calidad de la canal y de la carne. **Resultados**. La suplementación con silo de maíz produjo mayor expresión de los genes *glut4* e *igf1*. La expresión de los genes estudiados fue correlacionada con el peso de la carcasa caliente, el engrasamiento de la canal y el contenido de grasa de la carne. **Conclusiones.** Estos resultados sugieren interacción expresión genética-dieta en los genes *glut4* y *igf1,* la cual impactan en el engrasamiento de la canal y el contenido de grasa intramuscular en músculo *longissimus dorsi* de los novillos Braford, sugiriendo que estas variables podrían ser moduladas a través de la expresión diferencial de los genes.

Palabras clave. Bovinos; Maíz; Novillo; Suplementos Alimentarios; Tejido Adiposo (*Fuente: AGROVOC).*

INTRODUCTION

Meat quality is mainly perceived by sensory attributes such as appearance, juiciness, flavor and texture (1). The texture depends on many factors: on the zootechnical characteristics of the animal (breed, age and gender), on anatomical characteristics (type of muscle), on environmental factors (feeding), on post-mortem processing (ageing) or the cooking method (2,3).

The intramuscular fat or "marbling" of the meat has become an element that defines the quality of the carcass in the beef industry (4).

From a development and growth perspective, the intramuscular fat deposit is the last step of the growth process and occurs in the finishing stage of the animals. In this sense, it has been determined that, during the growth of the animals, there is an interaction between adipogenesis and myogenesis; in this sense, increased muscle fiber content results in lower intramuscular fat content (5).

Glucose transporter 4 (*glut4*) is a high-affinity glucose transporter, which is highly expressed in adipose and muscle tissue and is sensitive to insulin. It plays an important role in the energy / metabolic functions of adipocytes by allowing the transport of glucose to the cell (6).

Previous studies in rats show that diets with high-energy content increase the expression of the *glut4* gene (7). Furthermore, the expression of the *glut4* gene was increased in the skeletal muscle of cattle with the "double musculature" phenotype, which contains the inactivated myostatin gene, compared to animals without this mutation (8). These authors suggest that the myostatin gene, (mstn, or growth and differentiation factor 8), plays an important role in glucose metabolism and fat accumulation, acting as a direct inhibitor of *glut4* gene expression (8).

On the other hand, the insulin-like growth factor type 1 (*igf1*) gene is part of the family of proteins that mediate growth and development, participating in cell differentiation, embryogenesis, growth, and regulation of metabolism. Previous studies carried out on Angus and Charolais breeds showed an association between polymorphisms present in *igf1* with the deposition of muscle fat and other attributes of meat quality (9,10,11). Likewise, it has been determined that the *igf1* gene modifies its expression based on diet. Increased energy intake increases cellular energy status and thereby lipogenic gene expression by promoting *igf1* secretion, generating increased intramuscular fat content (12).

It has been determined that the production system of the beef cattle used (grass, grass plus supplementation, confinement) has an impact on the chemical composition of the meat and the fat content, aspects related to the quality of the meat (13,14). Likewise, it was suggested that the production systems of meat breeds under extensive conditions allow the production of meats with an excellent fatty acid profile (13). In this context, the present work aimed to evaluate the effect of supplementation with corn silage, on the expression of the *igf1, glut4* and *mstn* genes in the muscle *longissimus dorsi* of Braford steers finished in tropical pastures and its association with carcass and meat characteristics.

MATERIALS AND METHODS

Animals and sampling. The study was conducted with animals from a commercial breeding herd in Santiago del Estero, in the northwest of Argentina (coordinates 27°17'34.3"S - 62°15'14.1"W). Animal handling and experimental procedures were following Handbook of Procedures for Animal Welfare of the National Service of Animal Health of

Argentina (Servicio Nacional de Sanidad Animal, SENASA). Samples used in the current study were procured from animals fed diets as described by Coria et al (15). Briefly, thirty Braford steers, with 22 months of age, grazing on summer pasture (*Megathyrsus maximus*) were used. The experimental trial started in November (spring) and lasted 120 days. Animals were divided randomly into 2 experimental groups: 15 animals were fed ad libitum pasture and supplemented with corn silage (1% of body weight per head per day [DM basis] during 120 days) (Supl) whereas the other 15 steers were fed ad libitum similar pasture in different paddocks without corn supplement (Cont), generating average daily gain increases of 0.85 kg/day and 1.01 kg/day, respectively. Both groups had ad libitum access to water and were weighed at the end of the trial reaching weights of 455±16 Kg in the Cont group and 473±18 Kg in the Supl group (p=0.05).

Animals were slaughtered when they were on average 26 months of age. Steers were transported to a licensed commercial abattoir which was approximately 210 km from the establishment where the animals were reared. Animals were stunned using a captive bolt pistol and slaughtered according to standard commercial procedures.

After slaughter, carcasses were weighed (HCW: Hot Carcass Weight). The HCW for the Cont group was 264±15 Kg, and for Supl group 298±17 Kg (p<0.05). The fattening degree was determined according to the Argentine Bovine Carcass Typification System (Junta Nacional de Carne, Resolucion J-378/73 de la SAGPSyA). Based on a visual appreciation of the amount and distribution of subcutaneous fat, the fattening could take values between 1 and 4. High values indicate a greater thickness of this deposit. Control group carcass presented fattening degree values of 1±0.0 and those of the group Supl 1.7±0.5 (p<0.05).

Samples of approximately 500 mg of the *longissimus dorsi* muscle were obtained within 20 min of exsanguination, frozen in liquid N2 and stored at –70°C until molecular analysis.

RNA Extraction. Total RNA was extracted from muscle with TRI Reagent® (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions. The concentration, purity, and integrity of the extracted total RNA were evaluated with NanoDrop 2000c UV-Vis spectrophotometer (Thermo Scientific, USA) and by agarose gel electrophoresis with Syber Green (Biotium).

cDNA Synthesis. Previous cDNA synthesis, genomic DNA was eliminated using the amplification grade deoxyribonuclease I (AMPD1, Sigma, USA) following manufacturer protocol. After DNAse treatment, reverse transcription of 1,200 ng was performed using SuperScript III Reverse transcriptase (Invitrogen).

Gene expression analysis. Levels of gene expression were determined by reverse transcription and quantitative polymerase chain reaction (RT-qPCR) by triplicate using specific primers (Table 1). Primers efficiency and specificity were validated using PCR and 1.5% agarose gel electrophoresis. Every qPCR reaction, was performed with 10 µL as final volume and contained 3 µL of cDNA template [diluted 1:3], 0.25 mM of forward and reverse primers and 5 µL of iTaq Universal SYBR Green Supermix (Bio-Rad, USA) in CFX96 Real-Time thermocycler (Bio-Rad, Hercules, CA, USA).

Tabla 1. Primers used in RT-qPCR.

Gene	Primers Forward (F) and Reverse (R), Product Size (A, pb) Accession Number (AN)	Ref
glut4	F: CCACCAGGCACACTTACCACA R: CTCTTCCTTCCCAGCCACTGA A: 113 AN: AB005286	(16)
mstn	F: GGCCATGATCTTGCTGTAACCT R: GCATCGAGATTCTGTGGAGTG A: 144 AN: NM_001001525.2	(17)
igf1	F: AGTTGGTGGATGCTCTCCAGT R: CACTCATCCACGATTCCTGTC A: 115 AN: NM_001077828	(18)
rplp0	F: CAACCCTGAAGTGCTTGACAT R: AGGCAGATGGATCAGCCA A: 226 AN: NM_001012682	(19)
gapdh	F: AGATGGTGAAGGTCGGAGTG R: GAAGGTCAATGAAGGGGTCA A: 117 AN: NM_001034034	(20)

Gene: *glut4:* glucose transporter 4; *mstn:* myostatin; *igf1:* insulin-like growth factor type 1; *rplp0:* ribosomal protein large protein 0; *gapdh:* glyceraldehyde-3-phosphate dehydrogenase.

The PCR program consisted of an initial step of 5 minutes at 95°C, followed by 50 cycles of 30 sec at 95°C, hybridization of 30 sec at annealing temperature (58°C) and extension of 30 sec at 60°C. Negative controls were performed to exclude genomic DNA contamination.

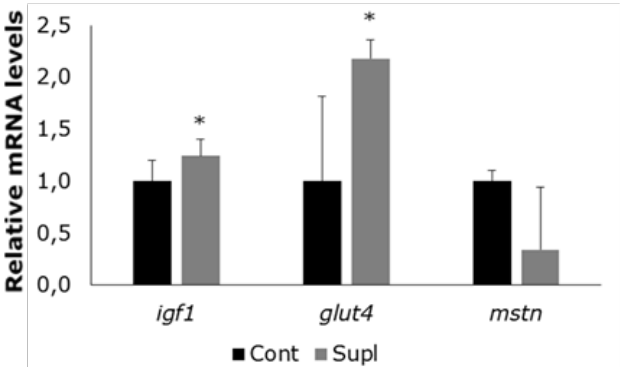
Statistical Analysis. Messenger RNA levels were analyzed according to ΔΔCq method using CFX Manager Version 3.0 software (Bio-Rad Laboratories). Fifteen biological replicates were made, and the average Cq of the technical

triplicates was reported. The Cq value obtained for each sample was normalized by dividing with the geometric mean of the expression of *gapdh* y *rplp0* reference genes in the given sample. The entire study was carried out following the MIQUE guidelines (Minimum Information for Publication of Quantitative Real-Time PCR).

Correlation analysis was performed between gene expression and quality characteristics of carcass (HCW and fattening degree) and meat (intramuscular fat content) evaluated in a previous study (15). Briefly, meat quality determinations were made in steaks 2.5 cm thick with 48 hours of maturation. The intramuscular fat content was determined in duplicate according to the Soxlhet AOAC method, by continuous distillation with hexane from 5g of meat. The results were expressed as a percentage of fat (grams of fat per 100 grams of meat), obtaining values of 3.47 ± 1.24 for the Supl group and 2.22 ± 01.44 for the Cont group ($p<0.05$). For all tests, the level of significance was $p<0.05$.

RESULTS

Figure 1 shows the results of the relative expression of the genes evaluated. In this sense, corn silage supplementation produces an increase in the relative levels expression of *igf1* and *glut4* genes (1.24, p=0.049, and 2.18 p=0.049, respectively) compared to pasture-finished steers. However, no differences were observed in myostatin gene expression (0.34, p=0.46).



*p<0.05 between nutritional treatments. The bars indicate the standard deviation of the mean for each group. Fold change was calculated as the relative expression of Suppl group vs Contr group. Fold change greater than 1 denotes increased expression in supplemented group.

Figure 1. Relative expression of *igf1, glut4* and *mstn* genes in *longissimus dorsi* muscle from steers finished on tropical pastures (Cont) or with corn silage supplementation (Supl). The results of the supplemented treatment were normalized with the pasture treatment.

Table 2 shows the statistically significant correlation coefficients obtained between *igf1, glut4,* and *mstn* genes and the quality characteristics of carcass and meat. It should be noted, that the expression of the *igf1* gene was directly associated with the expression of *glut4* gene (r=0.98, p<0.001) and inversely associated with the expression of the *mstn* gene (r=0.97, p<0.001). Likewise, the expression of these genes was correlated with the weight of hot carcass (p<0.001), the fattening degree of carcass (p<0.001), and intramuscular fat content (p<0.05).

Table 2. Correlation coefficients between meat quality attributes and *igf1, glut4* and *mstn* gene expression.

	ARNm ‡		
Variable†	***igf1***	***glut4***	***Mstn***
HCW	0.740***	0.738***	-0.742***
FD	0.709***	0.712***	-0.713***
IMF	0.439*	0.441*	-0.438*

† HCW: Hot carcass weight, FD: fattening degree of carcass according to the Argentine Bovine Carcass Typification System (Junta Nacional de Carne, Resolución J-378/73 de la SAGPSyA), IMF: Intramuscular fat content (g/100 g of mear). * p<0.05, *** p<0.001. ‡ Gene expression: *igf1:* insulin-like growth factor type 1; *glut4:* glucose transporter 4; *mstn:* myostatin.

DISCUSSION

Corn silage supplementation produces a differential expression of the *igf1* and *glut4* genes. In this sense, it has been previously determined that the increase of energy in diets increases the state of cellular energy and this could generate an increase in lipogenic gene expression through the secretion of *igf1* and *glut4* gene, generating an increase in fat content (7,12). In agreement with the results obtained, Pfaffl et al (21) obtained higher intramuscular fat content and expression of the *igf1* gene in Simmental steers fed with corn silage, compared to steers fed with pasture and hay. Likewise, Ladeira et al (22) demonstrated that the expression of the *glut4* gene in skeletal muscle could be altered by incorporating supplements in diets. In this sense, Hocquette et al (5) suggest that grain-based diets increase the conversion of glucose into intramuscular fat, compared to grass-based diets, explaining the results obtained.

On the other hand, the expression of *mstn* gene was not affected by supplementation. Previous

work with "double musculature" cattle, whose *mstn* gene is mutated, reported an increase in muscle mass and a decrease in the amount of intramuscular fat (23). Likewise, it has been proposed that low concentrations or myostatin inactivation produce an increase in *glut4* gene expression (8). These authors suggest that myostatin is a direct inhibitor of glut4 gene expression. In agreement with results obtained in the present work, other authors have determined that the *mstn* expression decreases while the *igf1* expression remains constant in "double muscled" cattle (18). The increased expression of g*lut4* and *igf1* genes, together with weight increase, fattening degree of carcasses, and intramuscular fat content in the corn silage supplemented group, could explain the relationship between adipogenesis and myogenesis processes, detailed in the bibliography (5.24).

In conclusion, meat intramuscular fat content is important in the meat industry, determining the quality of the product, but is difficult to measure and select by classical methods. Results of the present work indicate that differences obtained in carcass and meat, and hot carcass weight could be partially explained by the variations in the expression of *igf1, mstn,* and *glut4* genes. Likewise, supplementation with corn silage produced an increase in the expression of the *igf1* and *glut4* genes in the *longissimus dorsi* muscle of Braford steers, which could explain the differences obtained in the intramuscular fat content of this group. In this sense, the results obtained contribute to the knowledge of the biological processes of adipogenesis and fat deposition in cattle.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

The authors would like to thank PhD. Eduardo Alberto Parellada for her technical assistance in molecular analysis and Programa de Intercambio y Movilidad Académica (PIMA) that allowed the assistance of Eng. Zoot Álvarez-Gutiérrez . The present study was supported by Consejo Nacional de Investigaciones Científicas y Técnicas [PUE 2018 0035], Instituto Nacional de Tecnología Agropecuaria [PD-E5-I107-001] and Universidad Nacional de Santiago del Estero [UNSE 23/A262].

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