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PhD THESIS

TESIS DOCTORAL

EFFECT OF PRODUCTION FACTORS ON FOAL CARCASS AND MEAT QUALITY. CONSUMERS PREFERENCES.

EFECTO DE LOS FACTORES PRODUCTIVOS SOBRE LA CALIDAD DE LA CANAL Y LA CARNE DE POTRO. PREFERENCIAS DE LOS CONSUMIDORES.

Memoria para optar al grado de Doctora con Mención Internacional del programa de doctorado en Agroalimentación presentada por

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CERTIFICAN QUE:

la presente memoria de Tesis Doctoral titulada "*Effect of production factors on foal carcass and meat quality. Consumers preferences*" elaborada por Dña. MARTA RUIZ DARBONNENS ha sido realizada bajo nuestra dirección, y que cumple las condiciones exigidas por la legislación vigente para ser presentada bajo la modalidad de "**Compendio de Publicaciones**" (Acuerdo A3/2015 del comité de dirección de la EDONA de la Universidad Pública de Navarra) y para optar al grado de **Doctora con Mención Internacional** (Resolución 2307/2017 del Boletín Oficial de Navarra, Nº30, 12 de Febrero de 2018).

Y para que así conste, firman la presente en Pamplona a 16 de Abril de 2018.

Fdo.: Mª José Beriain Apesteguía Mª Victoria Sarriés Martínez

The PhD thesis entitled "*Effect of production factors on foal carcass and meat quality. Consumers preferences*", carried out by the Agronomic Engineer MARTA RUIZ DARBONNENS, has been presented in the "Compendium of publications" modality. The complete references of the articles that are part of the thesis' body are as follows:

1. Ruiz, M., Sarriés, M.V., Beriain, M.J., Crecente, S., Domínguez, R. and Lorenzo, J.M. 2017. Relationship between carcass traits, prime cuts and carcass grading from foals slaughtered at the age of 13 and 26 months and supplemented with standard and linseed-rich feed. Animal 12 (5), 1084-1092. 2017. DOI: org/10.1017/S1751731117002555.

2. Ruiz, M., Beriain, M.J., Insausti, K., Lorenzo, J.M., Cantalejo, M.J. and Sarriés, M.V. 2018. Efecto de la maduración en la conservación de la carne de potro. ITEA. Información Técnica Económica Agraria, 114 (1), 45-60. DOI: org/10.12706/itea.2018.004.

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A David...

"El secreto de la motivación personal se puede resumir en las cuatro ces:

curiosidad, confianza, coraje y constancia."

Walt Disney

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ABBREBIATIONS

%	Percentage	Porcentaje
12:2c-6	Oleic acid	Ácido oleico
18:1n-9c	Linoleic acid	Ácido linoleico
18:3n-3	α-linolenic acid	Ácido α-linoleico
n3	Omega-3 fatty acids	Ácidos grasos omega 3
n6	Omega-6 fatty acids	Ácidos grasos omega 6
a*	Redness	Coordenada a*
A	Age	Edad
ADF	Acid Detergent Fibre	Fibra ácido detergente
ADG	Average daily gain	Ganancia media diaria
AS	Artificial suckling	Lactación artificial
b*	Yellowness	Coordenada b*
BF	*Biceps femoris*	*Bíceps femoris*
BP	Brute protein	Proteína bruta
BW	Body weight	Peso del cuerpo
C*	Chromaticity	Cromaticidad
Ca	Calcium	Calcio
CCW	Cold carcass weight	Peso de la canal en frío
CO2	Carbon dioxide	Dióxido de carbono
Cu	Cupper	Cobre
DE	Digestible energy	Energía digestible
DM	Dry matter	Materia seca
DP	Dressing percentage	Rendimiento cárnico
EU	European Union	Unión Europea
F	Female	Hembra
FD	Finishing diet	Dieta de acabado
Fe	Iron	Hierro

FES	Free extensive system	Sistema extensivo en libertad
GM	Galician Mountain	Gallego de Monte
GMxB	Galician Mountain x Burguete	Gallego de Monte x Burguete
h*	Hue	Tonalidad
HB	Hispano-Bretón	Hispano-Bretón
HCW	Hot carcass weight	Peso de la canal en caliente
IHDH	Italian heavy draft horses	Caballos italianos “pesados”
IMF	Intramuscular fat	Grasa intramuscular
K	Potassium	Potasio
L*	Lightness	Luminosidad
LC	Linseed concentrate	Concentrado de lino
LD	*Longissimus dorsi*	*Longissimus dorsi*
LW	Life weight	Peso vivo
M	Male	Macho
MAP	Modified Atmosphere Packaging	Envasado en atmósfera modificada
MDA	Malonaldehyde	Malonaldehído
M/B	Meat/Bone	Carne/Hueso
MF	Martina Franca	Martina Franca
Mg	Magnesia	Magnesio
MIR	Medium infrared	Infrarrojo medio
Mn	Manganese	Manganeso
MUFA	Monounsaturated fatty acids	Ácidos grasos monoinsaturados
N	Number of samples	Número de muestra
Na	Sodium	Sodio
NIR	Near infrared	Infrarrojo cercano
NL	Nutritional level	Nivel nutricional
NS	Natural suckling	Lactación natural
O2	Oxygen	Oxígeno
P	Sulphur	Azufre
PM	*Psoas major*	*Psoas major*
PUFA	Polyunsaturated fatty acids	Ácidos grasos poliinsaturados
RD		Real Decreto
RF	*Rectus femoris*	*Rectus femoris*

S	Supplementation	Suplementación
SA	Slaughter age	Edad de sacrificio
SC	Standard concentrate	Concentrado convencional
SES	Semi-free extensive system	Sistema extensivo en semi-libertad
SF	Shear Force	Fuerza de Corte
SFA	Saturated fatty acids	Ácidos grasos saturados
SM	*Semimembranosus*	*Semimembranosus*
ST	*Semitendinosus*	*Semitendinosus*
TB	*Triceps brachii*	*Tríceps bracchi*
TBAR's	Thiobarbituric acid reactive substances	Sustancias reactivas al ácido tiobarbitúrico
TPA	Texture Profile Analyses	Análisis de Perfil de Textura
WBSF	Warner-Bratzler Shear Force	Fuerza de Corte Warner-Bratzler
Zn	Zinc	Zinc

ABSTRACT

This PhD thesis is part of a research project (INIA, RTA 2012-00090-C03-01) entitled "*Influence of cross breeding with Burguete on the productive parameters and characteristics of the carcass and meat of the Galician foal. Ageing and commercial life of the meat*" focused on the preservation of the Galician Mountain breed by crossing with Burguete foals. The general objective of the present PhD thesis was to study the quality along the foal meat production chain by means of the use of the carcass and the meat from 46 Galician Mountain x Burguete crossbred foals (GMxB foals).

The animals were slaughtered at the ages of 13 and 26 months, with a slaughter weight of 339 ± 41 and 444 ± 37 kg, for young and adult foals, respectively. They were supplemented with a standard finishing diet and a diet enriched with 5% linen. In order to meet the overall objective, four specific objectives have been defined which study carcass quality (1), meat quality (2), consumer preferences (3) and the application of medium infrared spectroscopy (MIR) as a sustainable technique in the determination of meat quality (4).

First of all, the effect of the slaughter age and the finishing diet on the production parameters, carcass dimensions and primary cuts obtained from the GMxB foals was studied. The slaughter age showed a significant effect on almost all parameters ($P < 0.05$). The dressing percentage was improved (52 vs. 54%, respectively) ($P < 0.001$) as well as the yield (% carcass weight) of the highest value commercial pieces. Most of the carcasses of adult foals were classified in higher conformation (E: Extra) and fat cover (5: Total fat cover) categories (79% and 54%, respectively). However, the aptitude for meat production (meat:bone ratio) did not improve despite the fact that the slaughter age was doubled. The main cause was the subcutaneous fat percentage in the carcasses of adult foals, almost 3 times higher than that of

young foals (19.5 vs. 7.2%, respectively). On the other hand, the effect of the finishing diet did not produce significant differences between the carcasses of foals supplemented with conventional and linseed-enriched concentrate. From these results, it can be appreciate the need to study the slaughter age in order to improve the aptitude of the animals for meat production by maximising the number of carcasses classified in the best quality categories.

Secondly, the foal meat quality was studied under different conditions of ageing and storage. To reach this goal, 2 works were carried out. In the first study, the Burguete breed was used, as it is a breed of commercial prestige and the genetic basis of the crossing of the animals studied (GMxB foals). Eight foals reared under extensive production and slaughtered at 281 ± 14.87 kg (15 ± 0.5 months of age) were used. The effect of ageing the *Longissimus dorsi* (LD) muscle in whole piece was studied once it was extracted from the carcass after 24 hours and after keeping the LD muscle 7 days in refrigeration at 4ºC (0 vs. 7 days). After these periods (0 and 7 days), the meat was sliced and packaged in trays covered with an oxygen permeable film (the traditional way of preserving the meat at the point of sale) and the effect of the storage time (0, 3, 6 and 9 days) was studied. The parameters studied were chemical composition (moisture, protein, intramuscular fat, ash, myoglobin and total iron content), colour (L*, a*, b*, C*, h*), compression and shear force as texture parameters, lipid oxidation (mg MDA/ kg flesh) and colour and odour assessed with a sensory trained panel. In the meat samples analysed, the high content of myoglobin (5.67 ± 0.93 mg/g muscle) and total iron (2.00 ± 0.19 mg/ 100 g meat) were noted. Furthermore, the meat could be defined, practically, “very tender” (SF < 31.4 N) because of its initial tenderness (33.7 N) 24 hours *post-mortem*. The ageing of the whole loin favoured an increase in the values of TBAR'S and the intensity of the sensory odour ($P < 0.001$) with respect to the non-aged meat, but in neither case, the TBA limit (2 mg MDA/ kg meat) for degradation was reached. With respect to the storage time, the lipid oxidation and the increase in the metmyoglobin content ($P < 0.001$) in both types of meat resulted in a decrease in the redness (a*: 12.27 vs. 8.44, for 0 and 9 days, respectively) and loss of the characteristic colour

(46.4 vs. 115 mm, for 0 and 9 days, respectively) ($P < 0.001$). The limit at which the colour became uncharacteristic (75 mm) was exceeded on the third day of storage. These results indicate, on the one hand that it would not be necessary to age the meat since at 24 hours *post-mortem* the meat was tender (33.7 N), and on the other hand that if the meat is aged inside the loin, it maintains its properties for 7 days.

In the second work, developed with GMxB foals, the effect of the slaughter age and finishing diet on the meat 24 hours *post-mortem* (without ageing) during 0, 4, 8 and 12 days of storage time in vacuum packaging conditions, were determined. The chemical composition, colour and texture and lipid and protein stability (carbonyl, nmol /mg protein content) were determined. The percentage of intramuscular fat in the meat of adult foals was more than 3 times higher than that of young foals ($P < 0.001$) (1.72 vs. 0.56, respectively). Both the slaughter age and the finishing diet influenced the colour coordinates (L*, a*, b*, b*, C*, h*) and the shear force of the meat (WBSF). The meat from adult foals was darker (L*: 28.90 vs. 32.49), redder (a*: 20.35 vs. 17.59) ($P < 0.001$) and harder (WBSF: 53.27 vs. 45.88 N) than that of young foals ($P < 0.05$). The most noteworthy thing was that the shear force of the meat from both groups decreased most pronouncedly during the first 4 days. On the other hand, the meat from young foals took a minimum of 8 days to become 'Very tender' (SF < 31.4 N), meat from adult foals took, at least, 12 days. With respect to the finishing diet, the redness increased in the meat of foals supplemented with conventional concentrate (16.8 to 0 days, 20.47 to 12 days) while it decreased in the meat of foals supplemented with linseed-enriched concentrate (18.3 to 0 days, 16.0 to 12 days). With regard to preservation conditions, vacuum packaging reduced the susceptibility of the meat to oxidation below the degradation values for lipid oxidation (2 mg MDA/ kg meat) and close to the limit for protein oxidation (3.75 nmol/mg protein) considering values between 2 and 14 nmol/mg protein of oxidized muscle.

Thirdly, the effect of slaughter age and finishing diet on the sensory quality of meat from GMxB foals was studied. A trained panel of 10 tasters was used to define the sensory attributes

of foal meat. On the other hand, a consumers test was carried out to determine its acceptability. This test was carried out in Pamplona and Ourense with a total of 474 consumers and two information levels were established (1: blind panel, 2: information on the origin, breeding and slaughter of the foals). The trained panel showed that both the slaughter age and the finishing diet did not show a significant effect on the sensory descriptors studied (characteristic flavour, fatty flavour/odour, tenderness, fibrousness...) ($P > 0.05$). Nevertheless, this panel showed that the meat of adult foals was juicier and had a more intense smell than that of young foals ($P < 0.05$).On the other hand, the consumer test showed that the meat of young foals was perceived more tender and juicier ($P < 0.01$) than that of adult animals, both attributes (juiciness and tenderness) being the determining parameters in the acceptability of the product. Regarding the level of information, it was shown that the acceptability of foal meat was higher only for consumers in Pamplona when they were provided with product information. The results obtained in this section show the need to develop new sensory studies since the objectivity indicated by the trained panel in the foal meat description is not in concordance with the subjectivity shown by consumers in the foal meat acceptability.

In the fourth and final part of this thesis, the application of mid-infrared spectroscopy (MIR) was investigated for the evaluation of the quality of the 24-hour *post-mortem* meat from the GMxB foals. This technique is fast, simple, does not require the use of chemicals and is environmentally sustainable. The spectra of all the samples were analysed and models were constructed to estimate the quality variables (chemical composition, colour, texture and sensory parameters). In overall, fatty acids showed the absorbance values that best differentiated the meat samples resulting in better prediction models. For the rest of the variables, the estimates showed R_v^2 values lower than 50%, with the exception of the grease, red, green and blue coordinates, which plus the fatty acids, showed R_v^2 values higher than 60%. Nevertheless, the meat could be classified both by slaughter age and by finishing diet with an accuracy of 78.3 and

71.8 %, respectively, from absorbencies between 2198-1118 cm^{-1}. Based on the results obtained, it is considered that this technique could have a great application and utility in the meat industry.

In general, the results obtained in this PhD thesis contribute significantly to improve the knowledge of the quality of foal meat within the equine sector by providing relevant data to improve the value chain in foal meat production. It is worth mentioning the study of acceptability and the sensory evaluation of foal meat by the consumer, which, being a pioneer in foal meat, provides a very useful knowledge for future work and future commercial strategies.

RESUMEN

Esta Tesis Doctoral se encuadra en el proyecto de investigación INIA (RTA 2012-00090-C03-01) titulado "*Influencia del cruce con Burguete en los parámetros productivos y características de la canal y carne del potro gallego. Maduración y vida comercial de la carne*", enfocado hacia la preservación de la raza equina Gallego de Monte mediante el cruce con potros de raza Burguete. El objetivo general de la presente Tesis Doctoral fue estudiar la calidad a lo largo de la cadena de producción de carne de potro mediante el empleo de las canales y de la carne procedente de 46 potros cruzados Gallego de Monte x Burguete (GMxB).

Los animales se sacrificaron a las edades de 13 y 26 meses, con un peso al sacrificio de 339 ± 41 y 444 ± 37 kg, para potros jóvenes y adultos, respectivamente. Fueron suplementados con una dieta de acabado convencional y otra enriquecida en lino al 5%. Para cumplir el objetivo global se definieron 4 objetivos específicos en los que se estudia la calidad de canal (1), la calidad de la carne (2), las preferencias de los consumidores (3) y la aplicación de la espectroscopía en el infrarrojo medio (MIR) como técnica sostenible, en la determinación de la calidad de la carne (4).

En primer lugar se estudió el efecto de la edad de sacrificio y de la dieta de acabado sobre los parámetros productivos, las dimensiones de la canal y los cortes primarios obtenidos, procedentes de los potros cruzados GMxB. La edad de sacrificio mostro un efecto significativo prácticamente en todos los parámetros ($P < 0,05$). El rendimiento cárnico fue mejorado con un aumento de la edad de sacrificio (52 vs. 54 %, respectivamente) ($P < 0,001$) así como el rendimiento (% peso canal) de las piezas comerciales de mayor valor. La mayoría de las canales de potros adultos fueron clasificadas en categorías de conformación (E: Extra) y grado de engrasamiento (5: Cobertura de grasa total) más altas (79% y 54%, respectivamente). Sin

embargo, la aptitud cárnica (ratio carne:hueso) no mejoró a pesar de duplicar la edad de sacrificio. La causa principal fue el porcentaje de grasa subcutánea de las canales de potros adultos, casi 3 veces superior a la de los jóvenes (19,5 vs. 7,2%, respectivamente). Por otro lado, el efecto de la dieta de acabado no produjo diferencias significativas entre las canales procedentes de potros suplementados con concentrado convencional y enriquecido en lino. En base a estos resultados, se aprecia la necesidad de incidir en el estudio de la edad de sacrificio con el fin de mejorar la aptitud cárnica de los animales maximizando el número de canales clasificadas en las mejores categorías de calidad.

En segundo lugar, se estudió la calidad de la carne de potro bajo diferentes condiciones de maduración y posterior conservación. Para ello, se llevaron a cabo 2 trabajos. En el primer estudio se empleó la raza Burguete, por ser una raza de prestigio comercial y base genética del cruce de los animales estudiados (GMxB). Se emplearon 8 potros criados bajo una producción extensiva y sacrificados con 281 ± 14,87 kg (15 ± 0,5 meses de edad). Se estudió el efecto de la maduración del músculo *Longissimus dorsi* (LD) en pieza entera una vez extraído de la canal a las 24 horas y tras 7 días de maduración en refrigeración a 4ºC (0 vs. 7 días). Tras ambos periodos (0 y 7 días), la carne fue fileteada y envasada en bandejas cubiertas con un film permeable al oxígeno (forma tradicional de conservación en los puntos de venta) y se estudió el efecto del tiempo de conservación (0, 3, 6 y 9 días). Los parámetros estudiados fueron la composición química (humedad, proteína, grasa intramuscular, cenizas, contenido de mioglobina y hierro total), color (L*, a*, b*, C*, h*), la comprensión y la fuerza máxima de corte como parámetros de textura, oxidación lipídica (mg MDA/ kg carne) y color y olor evaluado sensorialmente con un panel entrenado. En las muestras de carne analizadas, destacó el alto contenido de mioglobina (5,67 ± 0,93 mg/g músculo) y hierro total (2,00 ± 0,19 mg/ 100 g carne). Además, la carne podría definirse prácticamente como “muy tierna” (SF < 31,4 N) debido al valor de terneza inicial (33,7 N) 24 horas *post-mortem*. La maduración de la carne en la pieza entera (7días), favoreció el incremento de los valores de TBAR’S y la intensidad del olor sensorial ($P < 0.001$) con respecto a

la carne que no había sido madurada (0 días), pero en ninguno de los dos casos se alcanzó el limite de TBA (2 mg MDA/ kg carne) considerado de degradación oxidativa. Respecto al tiempo de conservación, la oxidación lipídica y el incremento del contenido de metamioglobina ($P < 0,001$), en ambos tipos de carne, dieron lugar a la disminución de la coordenada a* (12,27 vs. 8,44, para 0 y 9 días, respectivamente) y a la pérdida de color (46,4 vs. 115 mm, para 0 y 9 días, respectivamente) ($P < 0,001$). El límite en el que el color dejó de ser característico (75 mm) fue superado al tercer día de conservación. Estos resultados indican que no sería necesario madurar la carne puesto que a las 24 horas *post-mortem* la carne fue tierna (33.7 N), pero que si se madura en el interior del lomo, la carne mantiene sus propiedades durante 7 días.

En el segundo trabajo, se emplearos los potros GMxB y se determinó el efecto de la edad de sacrificio y dieta de acabado sobre la calidad de la carne a las 24 horas *post-mortem* (sin maduración) durante 0, 4, 8 y 12 días de tiempo de conservación y envasada en condiciones de vacío. Se determinó la composición química, color y textura y estabilidad oxidativa, lipídica y proteica (contenido en carbonilos, nmol /mg proteína). Cabe destacar el porcentaje de grasa intramuscular de la carne de potros adultos que fue más de 3 veces superior al de los potros jóvenes ($P < 0,001$) (1,72 vs. 0,56, respectivamente). Tanto la edad de sacrificio como la dieta de acabado influyeron sobre las coordenadas de color (L*, a*, b*, C*, h*) y la fuerza de corte de la carne (WBSF). La carne procedente de los potros adultos, fue más oscura (L*: 28.90 vs. 32.49), más roja (a*: 20.35 vs. 17.59) ($P < 0,001$) y más dura (WBSF: 53.27 vs. 45.88 N) que la de los jóvenes ($P < 0,05$). Lo más destacable fue que la fuerza al corte de la carne precedente de ambos grupos, disminuyó de forma más pronunciada durante los 4 primeros días ($P < 0,001$). Por otro lado, la carne procedente de potros jóvenes necesitó un mínimo de 8 días para ser “Muy tierna” ($SF < 31,4$ N), la carne de potros adultos necesitó, al menos, 12 días. Respecto a la dieta de acabado, la coordenada a* aumento en la carne de potros suplementados con concentrado convencional (16,8 a 0 días, 20,47 a 12 días) mientras que disminuyó en la carne de potros suplementados con concentrado enriquecido en lino (18,3 a 0 días, 16,0 a 12 días). Respecto a

las condiciones de conservación, el envasado al vacío redujo la susceptibilidad de la carne a la oxidación por debajo de valores propios de degradación en cuanto a oxidación lipídica (2 mg MDA/ kg carne) y próximos al límite respecto a la oxidación proteica (3,75 nmol/mg proteína) considerando valores entre 2 y 14 nmol/mg proteína propios de músculo oxidado.

En tercer lugar se estudió el efecto de la edad de sacrificio y de la dieta de acabado sobre la calidad sensorial de la carne, procedente de los potros GMxB. Se empleó un panel entrenado de 10 catadores para definir los atributos sensoriales de la carne de potro. Por otro lado, se realizó un test de consumidores con el fin de conocer la aceptabilidad de la misma. Dicho test se realizó en Pamplona y Ourense empleando 474 consumidores y se establecieron dos niveles de información (1: panel ciego, 2: información del origen, de la cría y sacrificio de los potros). El panel entrenado mostró que tanto la edad de sacrificio como la dieta de acabado no mostraron un efecto significativo sobre los descriptores sensoriales estudiados (sabor característico, sabor/olor graso, terneza, fibrosidad…) ($P > 0,05$). Sin embargo dicho panel, mostró que la carne de potros adultos fue más jugosa y mostró un olor más intenso que la de potros jóvenes ($P < 0,05$).Por otro lado, el test de consumidores mostró que la carne de potros jóvenes fue percibida más tierna y jugosa ($P < 0,01$) que la de los animales adultos, resultando ambos atributos (jugosidad y terneza) los parámetros determinantes en la aceptabilidad del producto. Respecto al nivel de información, se mostró que la aceptabilidad de la carne de potro fue mayor únicamente por parte de los consumidores de Pamplona cuando se les proporcionó información del producto. Los resultados obtenidos en este apartado ponen de manifiesto la necesidad de desarrollar nuevos estudios sensoriales ya que la objetividad indicada por el panel entrenado en la descriptiva de la carne de potro, no concuerda con la subjetividad mostrada por los consumidores en la aceptabilidad de la misma.

En la cuarta y última parte de esta tesis, se investigó la aplicación de la espectroscopía de infrarrojo medio (MIR) para la evaluación de la calidad de la carne de potro 24 horas *post-*

mortem procedente de los potros GMxB. Ésta técnica es rápida, sencilla, no requiere el empleo de reactivos y es sostenible desde el punto de vista medioambiental. Se analizaron los espectros de todas las muestras y se construyeron modelos de estimación de las variables de calidad (composición química, color, textura y parámetros sensoriales). En general, los ácidos grasos mostraron los valores de absorbancia que mejor diferenciaron las muestras de carne resultando a su vez en mejores modelos de predicción. Para el resto de variables, las estimaciones mostraron valores de R_v^2 inferiores al 50%, a excepción de las coordenadas de color Gris, rojo, verde y azul que junto a los ácidos grasos mostraron valores de R_v^2 superiores al 60%. Sin embargo, la carne pudo ser clasificada tanto por edad de sacrificio como por la dieta de acabado con una precisión del 78,3 y 71,8 %, respectivamente a partir de las absorbancias comprendidas entre 2198-1118 cm^{-1}. A raíz de los resultados obtenidos, se considera que esta técnica podría tener una gran aplicación y utilidad en la industria cárnica.

En general los resultados obtenidos en esta Tesis Doctoral, contribuyen de forma significativa para mejorar el conocimiento de la calidad de la carne de potro dentro el sector equino proporcionando datos relevantes para mejorar la cadena de valor en la producción de carne de potro. Cabe destacar el estudio de aceptabilidad y la valoración sensorial de la carne de potro por el consumidor, que al ser pionero en carne de potro, aporta un conocimiento muy útil para futuros trabajos y futuras estrategias comerciales.

CHAPTER 1. INTRODUCTION

Foal meat production is based on extensive livestock systems that help preserve ecosystems, natural habitats, lands and landscapes. This supports the principle of sustainability. In addition equine livestock could be very beneficial in mitigating some of the existing environmental problems. For instance, equines, due to their digestive physiology, have an environmental advantage over other livestock species such as ruminants (bovine, ovine and caprine cattle), in terms of their contribution to the total greenhouse gases produced by livestock farming. In general, energetic losses in horse due to CH_4 production average 3.5% of digestible energy of feeds compared to 10-13% in adult ruminants (Vermorel, 1997). In addition, they could help to mitigate the damage to natural resources caused by deforestation and serious forest fires that are recently affecting much of northern Spain.

The Burguete breed is an autochthonous and commercial breed from Navarre (Northern Spain) with great prestige that, since many years ago has become one of the most important breeds for meat production. This equine breed presents a great potential and aptitude for meat production due to its particular characteristics such as good conformation of the carcass or high dressing percentages (Sarriés et al., 2006; Sarriés and Beriain, 2006, 2005). On the other hand, the Galician Mountain is a local breed original from Galicia that shows a lower aptitude for meat production due of the early slaughter of foals (6-8 months of age). For many years, several researches developed with this breed have characterized the production of this meat (Franco et al., 2013, 2011a, 2011b, 2011c, Lorenzo et al., 2014c, 2013c, 2010). Some of them suggested different ways to improve Galician Mountain breed, as increasing the slaughter age or being crossed with heavier breeds to obtain animals with better characteristics (better carcass weight, conformation, prime cuts...). These crossings would result in (1) the preservation of the local

breeds and consequently and in (2) the sustainable maintenance of the environment. Moreover, in recent years, Galicia (Northwest Spain) has become one of the regions most affected by fires and deforestation and the production of horsemeat could be beneficial to promote the environment and landscapes preservation.

Sustainability makes the difference between foal meat and other types of meat production, which are currently highly intensified. Apart from this particularity, the quality of foal meat has been widely studied and, in general, shows a healthy profile of fatty acids, high amounts of protein and bioavailable iron and low fat and cholesterol content (Lorenzo et al., 2010; Lorenzo and Pateiro, 2013a; Sarriés et al., 2006; Sarriés and Beriain, 2005). In addition, its high content of myoglobin and polyunsaturated fatty acids make it susceptible to oxidation reactions, the conservation storage being an important factor in preserving the shelf life of foal meat (Gómez and Lorenzo, 2012; Lorenzo and Gómez, 2012).

The equine livestock is characterized by a great diversity of breeds, linked to the geographical location of origin, not only in Spain, but also in Europe. This fact results in the lack of standardised guidelines for the animal management and the subsequent meat processing, what makes it difficult to offer the consumer a product with defined quality. It should be also mentioned the lack of consumption of this type of meat. Although horse meat is associated with a healthy product, its consumption is limited by cultural issues as it is considered a companion animal or for leisure purposes (Iwobi et al., 2017). In addition, the traditional consumption of horse meat in Spain until the middle of the last century was associated with very low quality meat from old and without aptitude for meat production; nothing to do with the horsemeat offered today, from young animals and with an adequate fattening that allows us to obtain meat of excellent quality. Moreover, it is worth highlighting that the information disclosed of the scandals that have occurred in recent years regarding the contamination of meat products with horsemeat was transmitted in a confusing manner. It was not clarified that its inclusion did not affect the consumers' health, as it was a fraud in the labelling and not a food contamination.

Because of this fact, sensory analysis with consumers must be developed to understand their liking, preferences and opinions about foal meat. In the same line, a positive promotion of foal meat and foal meat products and their properties is needed to help change society's perception.

The present PhD thesis, which is part of a national research Project (INIA-RTA 2012-00090-C03-01), aims to characterize and define the quality of the foal carcass and meat in order to improve the value chain of the foal meat production in the equine sector and make foal meat another chioce for meat consumption, based on the principle of sustainability. The development of a consumer study will provide evidence on the social perception and on the current consumer's assessment of a less popular meat. Finally, the present thesis also proposes the use of sustainable techniques for the quality evaluation of meat that is produced under sustainable conditions.

CHAPTER 2. FOAL MEAT PRODUCTION: A REVIEW

2.1. Horse meat sector

At present, the equine meat sector is in absolute minority compared to the pork, chicken or beef sectors. Figure 1 shows the percentage of production of the main types of meat. In 2016, almost 315 million tonnes (Tn) of meat were produced, of which only 738,000 Tn were horse meat (FAOSTAT, 2018). Asia is the main producing region (Figure 2. a) where China, Kazakhstan and Mongolia lead the production of equine meat (196,070; 107,773; 41,734 Tn, respectively). Figure 2. b shows the main European producers of equine meat, Spain being the third producer behind Russia and Italy.

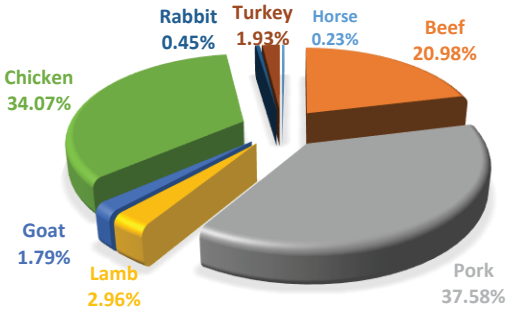

Figure 1. Meat production in the world in 2016 (FAOSTAT, 2018).

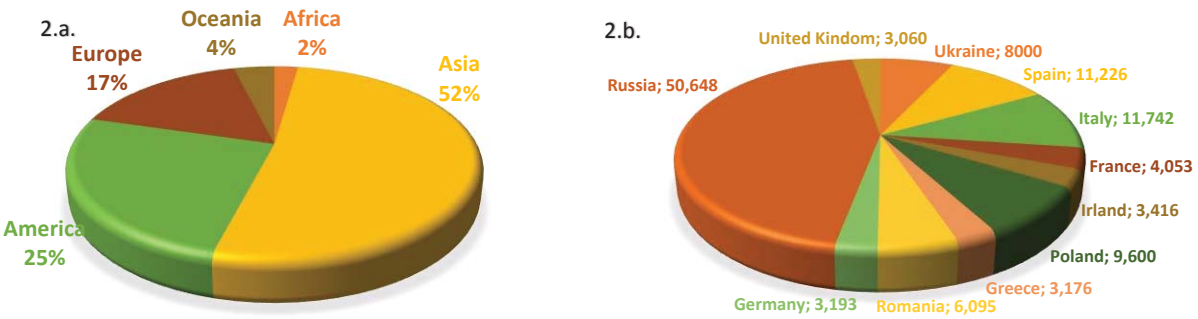

Figure 2. Horsemeat production (2.a.) in the world and the main European producers of horsemeat (Tn) (2.b.) in 2016 (FAOSTAT, 2018).

In Spain, 9.8% of the total equine livestock is destined to meat production (MAPAMA, 2018). Within this percentage, Asturias has the largest number of farms dedicated to meat production (42.6%), followed by Cantabria (33.2%), Navarra (10%) and Catalonia (6.6%). Equine meat production has increased notably in the last decade, from 5,275 tons in 2006 to 13,103 tons in 2016. It should be noted that more than 98 % of total equine meat production comes from horses, while less than 1.5 % comes from donkeys and mules. The most important equine meat producing communities are Navarra (26.5%), Aragon (21.2%), Valencia (12.8%), Castilla y León (12%), Catalonia (11.5%) and Cantabria (8.8%). Notably, Navarra is the region that has increased its production the most, going from 109 tons in 2006 to 3476 Tn in 2016 (MAPAMA, 2018).

According to foreign trade, Spain is a major importer of live animals (112,000 head of horses) mainly from France (84%). In contrast, it exports only 29,000 head of live animals to Hungary (49%), France (21%) or Bulgaria (12%). With regard to meat, Spain has become one of the largest exporting countries in the EU with more than 9,000 Tn exported (MAPAMA, 2018).

In terms of consumption, total meat consumption was 50.13 kg of meat/person/year in 2016. The species at the top of the list is chicken (13.87) followed by pork (10.68) and beef (5.61) (kg meat/person/year) (MAPAMA, 2018). Of this total quantity, 37.11 kg corresponds to "Consumption of fresh meat", which in turn includes the group "Other meat" (horsemeat, turkey, ostrich and rabbit) and reaches 3.11 kg of meat/person/year. Fàbregas (2002) indicated that the consumption of horsemeat in Spain was around 0.2 kg/person/year. More recent studies estimate the world-wide supply of horse meat to be 0.1 kg/person/year (on average) (Belaunzaran et al., 2015). Nevertheless, several countries have substantially higher consumption levels, such as Mongolia (5.81 kg), Kazakhstan (4.92 kg), Kyrgyzstan (3.50 kg), Iceland (2.19 kg), Switzerland (0.73 kg), Italy (0.70 kg), Croatia (0.69 kg), Belgium (0.58 kg), Russia and Finland (0.51 kg each) or France (0.27 kg).

2.2. Spanish horse breeds for meat production

The equine sector is characterised by a great breed diversity, linked to its distribution to the geographical location of origin. It can be found heavy breeds (>650 kg) such as the Hispano-Breton (Castilla y León, Cantabria and Huesca), Burguete (Navarra), Caballo de Monte del País Vasco (Basque Country) and the Cavall Pirinenc Català (Catalonia), small-sized breeds (<350 kg) such as Pura Raza Gallega (Galicia), Asturcón (Asturias), Losino (Castilla y León), Monchino (Cantabria) and Pottoka (País Vasco) and also medium-sized breeds (351-650 kg) such as Jaca Navarra (Navarra). The most common breeds of horses for meat production are shown in Table 1 and Figure 3. All of them are autochthonous breeds in danger of extinction according to RD 2129/2008 ("*National Program for the conservation, improvement and promotion of livestock breeds"*) and are registered in the *Official Catalogue of Breeds in danger of extinction* (MAPAMA, 2018).

Table 1. Census data of the most common horse breeds for meat production (2017).

Breed	Region	Total reproducers		Total animals		Total	Livestock farms
		Female	Male	Female	Male		
Burguete	Navarra	3367	367	5232	1424	6656	216
Jaca Navarra	Navarra	852	65	1303	202	1505	40
Caballo de Monte del País Vasco	País Vasco	3208	194	4054	331	4385	248
Caballo de Pura Raza Gallega	Galicia	867	125	1402	280	1682	302
Cavall Pirinenc Català	Catalonia	4340	412	2039	1561	3600	377
Hispano Breton	Andalucía	29	3	55	12	67	4
	Aragón	382	17	665	235	900	38
	Cantabria	2507	154	3629	329	3958	267
	Castilla y León	3834	303	6358	1584	7942	380
	Galicia	4	0	9	3	12	5
	Madrid	5	1	12	1	13	1
	Asturias	5	3	15	5	20	8

Source: MAPAMA (2018)

Figure 3.a. Jaca Navarra mare

Figure 3.b. Burguete stallion

Figure 3.c. Caballo de Monte del País Vasco male

Figure 3.e. Caballo de Pura Raza Gallega mare with the offspring

Figure 3.e. Caballo Pirenáico Catalán

Figure 3.f. Hispano-Bretón male

Figure 3. Spanish horse breeds destined to meat production

Sources: Fig. 3.a and b.: Asociación de criadores de raza equino Jaca Navarra (JACANA) and Asociación de criadores de ganado equino Burguete (ASCANA) (2004). Fig. 3.c.: Asociación de criadores de Cabalo de Pura raza Galega (PURAGA) (2013). Fig. 3.d and e. Federación Española de Ganado Selecto (FEAGAS). Fig. 3.f.: Asociación de criadores de ganado equino de raza hispano-bretona en Cantabria. MANADAS (2013).

The Jaca Navarra is of Navarre origin, although its specific origin is unknown. The Burguete horse is the result of crossing Jaca Navarra mares with non-native breeds, most of them from France

(Trait, Postier, Breton, Percherón, and more recently, Ardanes and Contois). The Hispano-Breton has its origin in the improvement of local semi-heavy mares with Breton stallions (French breed). They are located mainly in Castilla y León, Cantabria and Huesca. As for Caballo de Monte del País Vasco, there is a notable lack of data. It is only known to be original from the Basque Country. The Caballo de Pura Raza Gallega is native to Galicia, about which there are contrasted references since the fifteenth century and is spread throughout the region.

2.3. Livestock production system

Equine breeding is based on extensive systems and has been previously described by Sarriés (2005). In general, the most common farming system is based on the use of native breeds that have redirected their productive end of work or transport with a fundamental collaboration in agricultural activities towards meat production due to the shift towards agricultural mechanization in the last century. This production system is normally carried out on family farms where horses intended for meat production are a complement to the usual income normally obtained from other livestock production. For these reasons, this type of livestock play an important environmental and socio-cultural role, as animals contribute to the maintenance of ecosystems and rural areas, keeping mountains and forests clean and reducing the risk of fires. This happens because of the fact that their diet is based on the use of natural resources, without complementary supplies, just some exceptions in very hard seasons or mares at certain reproductive times (Lorenzo et al., 2013c). Equine meat production thus meets the criteria of a sustainable food production chain (Belaunzaran et al., 2015).

Generally, foals are kept in pasture with their mothers for the first 6-7 months. Then, they are weaned and continue freely in the pastures following a rotational grazing system until slaughter. Before slaughter, a fattening period is carried out based on concentrates to cover the growth and development needs of the animals. This period is necessary in most cases due to extreme weather conditions, especially in winter. For example, in Navarra, Burguete foals are

usually slaughtered at the age of 16 months ("Milk-fed foals") or 24 months ("Fifteen-fed foals"). The name designation depends on the time spent with their mothers (Sarriés, 2005). Nevertheless, there is great variability in the management of animals depending on the region where they are reared. This lack of homogenisation of production factors (breed and crossbreed, sex, livestock production system, age of slaughter and final diet) gives rise to a high degree of heterogeneity in the quality of the carcass of foals and meat.

2.4. Foal carcass quality

There is a great diversity of carcass characteristics due to the high variability observed between breeds, slaughter age, finishing diets and management of animals, among other factors. This heterogeneity makes it very difficult to establish a general description and define the quality of the foals' carcasses. Several authors have studied the dimensions of the foal carcass and the prime cuts (Franco et al., 2013, 2011b; Juárez et al., 2009; Sarriés and Beriain, 2005). In most cases, the best results were obtained for animals slaughtered at a later age. They have a greater length of the carcass or loin, or greater leg circumference (cm), and consequently, a greater percentage of the most valuable prime cuts. In addition, later slaughter ages give animals a better carcass conformation. Table 2 provides a summary of the studies carried out in recent years according to the different production factors.

In contrast to beef cattle, foal carcases cannot be defined correctly because the existing quality classification system (ONIBEV, 1979) is not used in slaughterhouses. Animals are classified according to a criteria based on the slaughter age (sucking foals, foals 15/24 months old or adult foals) leaving a substantial gap and lack of knowledge about the definition of carcasses. The methodology followed for equine conformation is the ''Catalogue de Classement des Équidés'' classification from the Office National Interprofessionnel du Bétail et des Viandes (ONIBEV, 1979). The scores have a three-point scale (E-Extra, M-Moyen, B-Bon). The fat degree is assessed using the scale for the classification of fat cover in adult bovine carcases (EU

Regulation No 1208/81). Sarriés and Beriain (2005) carried out an investigation with 16 and 24 months old Burguete foal and classified the conformation of carcasses and fat cover. Apparently, the classification systems were useful.

Updating the equine classification systems could be very beneficial for progress in the equine sector. Three grading levels are not sufficient to give an accurate description of the carcasses. According to the degree of fattening, and although it could be an approximation, the classification system for beef carcases' is not representative for foal carcasses and the development of quality grading systems for horses would be essential as an important step in defining horsemeat production.

Table 2. Equine carcass characteristics.

Breed	N	Slaughter age (months)	Livestock	LW (kg)	DP (%) (HCW/BW)	Meat (%)	Fat (%)	Bone (%)	M/B	Reference
IHDH	12	11	SES		68.9	72.1	11.7	11.9	6.1	Tateo et al. (2005)
Poland	107	72–144				68.4	7.85	22.7	3.0	Znamirowska (2005)
Burguete	56	24	SES	395	67.1					Sarriés and Beriain (2005)
Burguete		16		411	63.6					
MF donkey	15	15	NR	181	53.3					Polidori et al. (2008)
Burguete	15	24	SES	395	65.0*					Juárez et al. (2009)
Hispano-Bretón		24		406	68.0*					
Sanfratellano	15	18	SES	411	59.3					Lanza et al. (2009)
Haflinger		18		349	59.6					
GM	31	11	FES	199	53.3*	73.0	6.7	20.2	3.6	Franco et al. (2011c)
GM	12	15	SES	256	50.2*	69.7	6.4	23.8	2.9	Franco et al. (2013)
GM x HB	9		SES	312	52.8*	70.1	7.1	22.7	3.1	
GM/ GM x HB	21		SES (1.5kg S)	273	49.9	69.7	5.6	24.7	2.8	
			SES (3kg S)	287	52.9	70.1	7.7	22.2	3.2	
GM x HB	21	18	FES	217	46.9					Lorenzo et al. (2014b)
	14		SES (1.5kg S)	258	51.2					
	14		SES (3kg S)	276	54.2					
IHDH	18	11	150% NL	448	73.7	78.96	11.82	9.25		de Palo et al. (2014)
			180% NL	457	72.6	74.48	15.95	9.55		
			200% NL	473	73	74.73	15.52	9.77		
GM x HB	10	8	SES	248	53.8					Domínguez et al. (2015)
	11	11		275	54.1					
MF donkey	8	8	SES	101 ± 18	49.2 ± 0.88					Polidori et al. (2015)
	8	12		122 ± 13	53.9 ± 3.31					

HCW/BW: hot carcass weight/ bodyweight; * = CCW/BW: cold carcass weight/body weight; LW = Live weight; DP = dressing percentage; M/B = meat/bone ratio.
Breeds: IHDH = Italian heavy drafts horses; MF = Martina Franca; GM = Galician Mountain; HB = Hispano-Bretón.
Livestock: SES = Semi-extensive system; FES = free extensive system; 1.5/3kg S = 1.5/ 3Kg of supplementation in the finishing period; % NL = percentage of the nutritive level.

2.5. Foal meat quality

2.5.1. Physic-chemical quality

2.5.1.1. Chemical composition

In recent decades, interest in foal meat has increased notably. Many studies characterize foal meat for being low in fat and high in protein and heam iron (Badiani et al., 1997). Despite these statements, there is general variability for all chemical parameters according to production factors (Table 3). For example, moisture content ranges from 68.34 to 77.40%, protein content from 19.54 to 22.31% and intramuscular fat from 0.15 to 4.76%. This makes it difficult to provide a clear description of the composition of the foal meat.

Table 3. Chemical composition of equine meat.

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Moisture (%)	Protein (%)	Intramuscular Fat (%)	Cholesterol (mg/100g)	Reference
NR	5	72-120	M F	NR	Thigh	70.9 ± 0.67	19.8 ± 0.50	6.63 ± 0.96	61 ± 4	Badiani et al. (1997)
Burguete	12	16	M	SES	LD	68.34 ± 4.20	19.91 ± 1.59	3.01 ± 0.80	NR	Sarriés and Beriain (2005)
	13		F			70.70 ± 2.43	19.90 ± 1.84	3.32 ± 1.18		
	23	24	M			68.98 ± 10.03	20.59 ± 1.29	5.21 ± 3.19		
	8		F			71.37 ± 1.72	20.50 ± 1.55	4.76 ± 1.26		
IHDH	24	11	M	Indoors	BF	68.39	20.68	4.23	NR	Tateo et al. (2008)
					LD	69.51	21.67	4.28		
					RF	69.15	21.08	4.31		
					SM	73.59	19.57	4.47		
					ST	71.08	21.8	3.91		
Burguete	15	24	M	SES	LD	72.32 ± 0.71	20.64 ± 0.73	2.08 ± 0.11	NR	Juárez et al. (2009)
Hispano-Bretón					LD	70.58 ± 0.91	21.81 ± 0.68	2.22 ± 0.15		
Sanfratellano	15	18	M	SES	LD	73.23	NR	2.29	NR	Lanza et al. (2009)
Haflinger						72.8		2.44		
GM	31	9	M F		LD	75.43	20.61	0.31	NR	Franco et al. (2011c)
	11	12				75.93	20.44	0.16		
GM	12	15	M F	SES	LD	74.78	20.98	0.36	NR	Franco et al. (2013)
GM x HB	9			SES		74.79	20.65	0.40		
				SES (1.5 kg						
GM/ GM x HB	21			S)		75.06	20.67	0.15		
				SES (3kg S)		74.54	20.99	0.58		
GM	12	15	M F	FES	LD	76.49 ± 0.66	22.31 ± 0.74	0.22 ± 0.08	62 ± 6	Lorenzo et al. (2013b)
					SM	76.69 ± 0.77	21.98 ± 1.17	0.15 ± 0.07	59 ± 9	
					ST	77.40 ± 0.48	21.39 ± 1.05	0.31 ± 0.18	60 ± 11	
					BF	76.83 ± 0.73	21.67 ± 1.04	0.37 ± 0.10	57 ± 16	
					TB	77.40 ± 0.73	21.44 ± 0.72	0.28 ± 0.16	62 ± 8	
					PM	77.24 ± 0.78	21.17 ± 1.17	0.67 ± 0.13	61 ± 15	

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Moisture (%)	Protein (%)	Fat (%)	Cholesterol (mg/100g)	Reference
GM	6	15	M	FES	LD	76.63 ± 0.54	22.30 ± 0.51	0.12 ± 0.03	64 ± 8.0	Lorenzo et al. (2013c)
	6		F			76.28 ± 0.80	22.31 ± 1.06	0.16 ± 0.09	60 ± 3.0	
GM x HB	21	18	M F	FES	LD	76.09	21.62	0.23	0.6	Lorenzo et al. (2014b)
	14			SES (1.5 kg S)		75.78	20.45	0.96	0.51	
	14			SES (3kg S)		74.79	20.70	1.38	0.50	
IHDH	18	11	M	150% NL	LD	71.26	21.90	2.96		de Palo et al. (2014)
				180% NL		71.32	21.24	3.11		
				200% NL		68.98	20.81	3.15		
GM x HB	10	8	M F	SES	LD	74.54	20.41	1.27	0.47	Domínguez et al. (2015)
	11	11	M F			74.78	21.05	1.29	0.28	
MF donkey	8	8	M	SES	LD	77.3 ± 2.26	19.8 ± 0.24	1.76 ± 0.23	62.4 ± 2.3	Polidori et al. (2015)
	8	12				75.8 ± 1.64	21.0 ± 2.32	1.87 ± 0.18	63.9 ± 3.1	
MF donkey	20	12		AS (SES)	LTL	74.32	20.45	1.67		De Palo et al. (2017)
				NS (SES)		75.44	19.83	1.13		
		18		AS (SES)		74.72	19.54	1.84		
				NS (SES)		72.64	21.35	1.94		

Sex: M = Male; F = Female.
Breeds: IHDH = Italian heavy draft horse; GM = Galician Mountain; HB = Hispano-Bretón; MF = Martina Franca.
Livestock: SES = Semi-extensive system; FES = free extensive system; 1.5/3kg S = 1.5/ 3Kg of supplementation in the finishing period; % NL = percentage of the nutritive level; AS = Artificial suckling; NS = Natural suckling
Muscle: LD = *longissimus dorsi*; BF = *biceps femoris*; RF = *rectus femoris*; SM = *semimembranosus*; ST = *semitendinosus*; BF = *biceps femoris*; TB = *triceps brachii*; PM = *psoas major & minor*; LTL = *longissimus thoracis et lumborum*
NR: not reported.

Foal meat follows the general pattern of meat that shows a high nutritional value. Nevertheless, it is worth highlighting its high PUFA content. In general, the fatty acid profile of horsemeat is described as "healthy" due to its high content of unsaturated fatty acids (more than 55%) (Franco et al., 2013; Sarriés et al., 2006) (Table 4); mainly, essential n-6 (LA, 18:2n-6) and n-3 (ALA 18:3n-3, polyunsaturated fatty acid) or oleic acid (18:1n-9c, monounsaturated fatty acid). It therefore contains a substantial proportion of components of the family of alpha-linolenic fatty acids (Lorenzo, 2013). Some researchers even claimed that horse meat is a "dietetic meat" as it could be an alternative to red beef (Badiani et al., 1997; Lorenzo et al., 2010).

Nevertheless, it should be also positive to describe the absolute contribution according to the nutritional recommendations, for instance, of linoleic acid (LA) and α-linoleic acid (ALA), being 17 and 12 g/d for men and women, respectively for LA, and 1.6 and 1.1 g/d for men and women, respectively for ALA (Institute of Medicine Food and Nutrition, 2002).

Table 4. Intramuscular fatty acid composition of equine meat.

Breed	N	Slaughter Age (months)	Sex	WS	Livestock	IMF	SFA	MUFA	PUFA	Σ n3	Σ n6	n6/n3	PUFA/SFA	Reference
Burguete	12	16	M	412	SES	3.01	43.4	28.4	28.1	1.78	26.16	15.56	0.65	Sarriés et al. (2006)
	13		F	411		3.32	43.5	29.2	27.3	1.99	25.11	15.43	0.62	
	23	24	M	393	SES	5.21	41.5	31.9	26.6	3.12	23.26	9.04	0.64	
	8		F	397		4.76	41.1	36.6	22.3	2.81	19.40	8.00	0.54	
IHDH	12	11	M	497	SES	4.52	44.2	31.1	24.8	NR	NR	0.53	0.56	Tateo et al. (2008)
			F			4.01	44.7	33.9	21.4	NR	NR	0.57	0.48	
MF donkey	12	14	M	169	NR	NR	41.1	33.8	25.7	NR	NR	NR	0.62	Polidori et al. (2009)
	12					NR	40.8	34.3	24.9	NR	NR	NR	0.62	
Burguete	15	24	M	400	SES	2.08	38.0	34.4	27.3	NR	NR	NR	0.72	Juárez et al. (2009)
Hispano-Bretón						2.22	38.7	37.6	23.8	NR	NR	NR	0.61	
Sanfratellano	15	18	M	411	SES	2.29	36.9	33.3	29.9	4.18	25.08	6.70	0.82	Lanza et al. (2009)
Haflinger				350		2.44	36.3	32.9	30.8	6.29	23.91	4.09	0.85	
GM	9	9-12	M F	NR	SES	0.31	38.6	36.1	25.2	9.07	15.88	1.86	0.66	Lorenzo et al. (2010)
	22	9			FES	0.15	36.2	22.7	40.7	24.39	16.16	0.74	1.15	
GM	12	15	M	195	FES	0.10	40.2	18.1	41.5	14.70	26.40	1.80	1.00	Lorenzo et al. (2013c)
			F	185		0.10	39.1	20.3	40.5	17.10	23.00	1.30	1.00	
GM x HB	21	15	M F	273	SES (1.5 kg S)	0.15	35.0	31.6	32.6	13.10	19.30	1.47	0.91	Franco et al. (2013)
				287	SES (3kg S)	0.58	35.8	41.4	22.9	7.40	15.30	2.08	0.65	
GM x HB	21	18	M F	217	FES	0.23	37.8	21.6	40.0	20.68	19.33	1.03	1.06	Lorenzo et al. (2014b)
	14			258	SES (1.5 kg S)	0.96	37.5	45.9	16.4	4.86	11.55	2.96	0.44	
	14			276	SES (3kg S)	1.38	37.8	45.0	16.9	5.44	11.48	2.34	0.45	
IHDH	18	11	M	448	150% NL	2.96	47.8	23.8	28.4	NR	NR	4.35	0.50	de Palo et al. (2014)
				457	180% NL	3.11	45.4	28.5	25.8	NR	NR	4.35	0.58	
				473	200% NL	3.15	46.1	25.3	27.9	NR	NR	4.55	0.54	
GM x HB	10	8	M F	248	SES	1.27	37.7	39.5	22.8	10.99	11.76	1.14	0.61	Domínguez et al. (2015)
	11	11	M F	275		1.29	36.5	40.8	22.8	8.03	14.66	1.89	0.63	

Breed	N	Slaughter Age (months)	Sex	WS	Livestock	IMF	SFA	MUFA	PUFA	Σ n3	Σ n6	n6/n3	PUFA/SFA	Reference
MF donkey	8	8	M	101	SES	1.76	40.2	33.2	26.4	4.47	22.43	5.02	0.66	Polidori et al. (2015)
	8	12		122		1.87	40.0	33.5	26.4	4.09	22.68	5.55	0.66	
MF donkey	20	12	NR		AS (SES)	1.67	40.8	32.5	27.3	3.46	22.06	5.81	0.71	De Palo et al. (2017)
					NS (SES)	1.13	48.6	31.3	21.0	3.57	17.29	5.83	0.40	
		18	NR		AS (SES)	1.84	43.4	35.1	19.8	3.50	15.80	4.41	0.42	
					NS (SES)	1.94	46.9	36.2	19.3	4.17	16.81	4.59	0.71	
GM x Burguete	11	13	M F	NR	SC (FES)	0.37	34.7	32.7	32.5	14.20	18.30	1.28	0.94	Domínguez et al. (2018)
	12	26		NR		1.82	33.8	35.8	30.5	14.40	16.10	1.13	0.90	
	11	13			LC (FES)	0.74	35.2	28.8	36.0	14.90	21.10	1.43	1.02	
	12	26				1.71	33.4	39.3	27.3	13.80	13.60	1.00	0.82	

Sex: M = Male; F = Female.
WS: Weight at slaughter (Kg).
Breeds: IHDH = Italian heavy draft horse; MF = Martina Franca; HB = Hispano-Bretón; GM = Galician Mountain.
Livestock: SES = Semi-extensive system; FES = free extensive system; 1.5/3kg S = 1.5/ 3Kg of supplementation in the finishing period; % NL = percentage of the nutritive level; AS = Artificial suckling; NS = Natural suckling; SC = Standard concentrate supplemented in the finishing period; LC = Linseed concentrate supplemented in the finishing period.
IMF = Intramuscular fat (%)
SFA = total saturated fatty acids; MUFA = Total monounsaturated fatty acids; PUFA = Total polyunsaturated fatty acids; Σ n3: Total n-3; Σ n6: total n-6. Results expressed as g/100 g of total fatty acids.
NR: not reported.

Dealing with the essential amino acids, foal meat data are in line with the characteristic pattern of meat. Lorenzo and Pateiro (2013a) stated that foal meat appears to be an excellent source of proteins of high biological value because it contains essential amino acids in an appropriate proportion according to the needs of adults (arginine, histidine, phenylalanine, threonine, isoleucine, methionine and valine) (Institure of Medicine, Food and Nutrition, 2002). The requirement for essential amino acids for an adult male weighing 70 kg is about 12.90 g/day (FAO/WHO/UNU, 2007). Lorenzo et al. (2013c) indicated that 100 g of foal meat covered 15.5% of the daily requirement for essential amino acids.

Table 5. Amino acid composition (g/100 g meat) of equine meat.

Reference	Badiani et al. (1997)	Polidori et al. (2009)	Franco et al. (2013)	Lorenzo and Pateiro (2013a)	Lorenzo et al. (2014b)	Domínguez et al. (2015)	Polidori et al. (2015)	Domínguez et al. (2018)
N	5	12	42	12	49	21	16	46
Slaughter Age (months)	6–10 years	14	15	15	18	8 and 11	8 and 12	13 and 26
Breed	NR	MF	GM	GM	GM x HB	GM x HB	MF	GM x B
Essential								
Arginine	1.08–1.26	1.38–1.44	1.23–1.48	1.15–1.44	1.53-1.95	1.49-1.58	1.22-1.33	1.75-1.87
Histidine	0.86–0.92	0.86–0.93	0.96–0.99	0.89–1.04	0.84-1.14	0.91-0.99	0.74-0.81	1.08-1.14
Isoleucine	0.81–0.98	0.99–1.05	1.13–1.19	1.07–1.16	0.86-1.24	0.84-0.85	0.85-0.90	1.11-1.15
Leucine	1.37–1.62	1.51–1.60	1.85–1.88	1.81–1.93	1.72-2.09	0.16-0.17	1.44-1.55	1.94-1.99
Lysine	1.45–1.67	1.63–1.77	1.91–2.01	1.95–2.04	1.61-2.28	0.17-0.18	1.53-1.59	2.06-2.14
Methionine	0.44–0.51	0.65–0.74	0.27–0.28	0.31–0.36	0.35-0.39	0.10-0.13	0.51-0.57	0.17-0.21
Phenylalanine	0.76–0.92	0.76–0.83	0.95–0.98	0.93–0.98	0.83-1.01	0.77-0.78	0.85-0.92	0.92-1.04
Threonine	0.77–0.91	0.88–0.91	1.05–1.10	1.12–1.16	1.01-1.23	0.94-0.99	0.86-0.99	0.99-1.05
Valine	0.88–1.02	1.01–1.09	1.11–1.16	1.12–1.18	0.99-1.28	0.90-0.92	1.03-1.11	1.11-1.17
Non-essential								
Alanine	1.12–1.25	1.09–1.22	1.24–1.30	1.28–1.33	1.14-1.45	1.06-1.13	1.08-1.22	1.26-1.34
Aspartic acid	1.66–1.90	1.79–1.92	1.93–2.10	2.03–2.11	1.75-2.28	1.76-1.79	1.65-1.81	2.08-2.18
Glutamic acid	2.62–3.02	3.09–3.26	3.23–3.38	3.27–3.41	2.85-3.76	2.83-2.89	2.53-2.99	3.38-3.57
Glycine	0.99–1.08	0.84–0.97	0.88–0.96	0.97–1.00	0.88-1.05	0.82-0.85	0.88-0.93	0.93-1.02
Proline	0.81–0.99	0.95–1.00	0.83–0.91	0.87–0.91	0.77-1.12	0.79-0.82	0.84-0.91	0.85-1.01
Serine	0.63–0.76	0.64–0.75	0.85–0.91	0.97–0.90	0.83-0.99	0.82-0.83	0.74-0.82	0.77-0.83
Tyrosine	0.62–0.71	0.59–0.70	0.71–0.74	0.71–0.77	0.72-0.92	0.68-0.70	0.48-0.66	0.63-0.71

Breed: GM = Galician Mountain; MF = Martina Franca donkey; HB = Hispano Bretón; B = Burguete
NR: Non reported

The presence of animal proteins in the diet favours the absorption of minerals by our metabolism. Foal meat appears to be a very good nutritional source of major minerals (Ca, K, Mg, Na, P) and minor minerals (Cu, Mn, Zn) (Table 6), especially iron (Fe), magnesium (Mg) and copper (Cu) (Lorenzo et al., 2014c). As for total and heam Fe, the content of which is highly relevant to the bioavailability of human metabolism, it appears to be higher (4.04 and 2.44 mg/100 g) (Lorenzo and Pateiro, 2013a) than in other species such as beef (2.07 and 1.72 mg/100 g) or lamb (2.23 and 1.68 mg/100 g) (Lombardi-Boccia et al., 2002).

Table 6. Mineral composition (mg/100 g meat) of equine meat.

Reference	Badiani et al. (1997)	Lee et al. (2007)	Polidori et al. (2008)	Lorenzo and Pateiro (2013a)*
N	5	20	12	12
Slaughter Age (months)	6–10 years	30-36	14	15
Breed	NR	Jeju	MF	GM
Major elements				
Ca	3.77 ± 0.1	6.30 ± 0.5	8.65 ± 2.1	4.11-4.51
K	331 ± 8.0	315.50 ± 17.6	343.70 ± 65.9	198.32-202.61
Mg	28.90 ± 0.01	21.00 ± 1.3	24.80 ± 6.7	40.07-43.31
Na	74.20 ± 2.7	38.10 ± 3.3	52.50 ± 13.3	52.56-68.00
P	231.00 ± 3.0	168.70 ± 6.7	212.90 ± 56.7	186.2-196.5
Minor elements				
Fe	3.89 ± 0.20	2.10 ± 0.40	3.80 ± 1.01	2.56-4.04
Cu	0.200 ± 0.01	NR	NR	0.135-0.213
Mn	NR	0.022 ± 0.004	NR	0.010-0.016
Zn	3.72 ± 0.21	2.30 ± 0.50	3.67 ± 0.78	1.82-2.74

*Range of values (minimum - maximum) reported for *longissimus dorsi*, *biceps femoris*, *semitendinosus*, *semimembranosus*, *triceps brachii* and *psoas major & minor*.
Breed: GM = Galician Mountain; MF = Martina Franca donkey
NR: Non reported

2.5.1.2. Texture

If colour is the main attribute at the time of purchase, texture is the most important factor in the repurchase decision (Bindon and Jones, 2001). The texture is defined as an organoleptic property of food, resulting from the mechanical and geometric characteristics and

related to the amount of moisture and fat in the product and the way in which each of them is detected when chewing (Dumont, 1986). The high heterogeneity of this attribute became a major challenge in the meat sector (Koohmaraie, 1996). This fact has implied the development of numerous studies on this subject (Belew et al., 2003; Christensen et al., 2011; della Malva et al., 2017; Maltin et al., 2003; Sierra et al., 2010; Torrescano et al., 2003).

Many research studies studied the influence of breed, slaughter age or finishing diet on the texture of foal meat. The slaughter age showed the most important differences in the texture of the meat. Regarding the feeding regime, Franco et al. (2011a) and Lorenzo et al. (2014b) found no difference. Currently, there is no texture classification available for foal meat. The most common method used comes from the classification of tenderness in beef (Shackelford et al., 1991) (Very tender: SF < 3.2 kg; Tender: 3.9 kg < SF < 3.2 kg; Intermediate 4.6 kg < SF < 3.9 kg; Tough: 4.6 kg < SF; SF: Shear force). Sarriés and Beriain (2006) used this classification for 4 and 8 days aged meat from Burguete foals slaughtered at 16 and 24 months of age. They concluded that ageing favoured tenderness, but tenderisation took place at different times because of the difference in livestock.

The values reported of WBSF vary from 21.0 N to 61.5 N (Table 7). Therefore, it can range from "very tender" to "tough". In this respect, the description of the texture of foal meat cannot be provided due to the high variation reported in the different researches due to breed, slaughter age/weight, muscle... Other studies (Domínguez et al., 2015; Franco et al., 2013; Franco and Lorenzo, 2014; Lorenzo et al., 2014b) discussed attributes measured instrumentally to determine texture profile using the TPA (Texture Profile Analyses) test. The TPA test determines hardness (Kg), chewiness (Kg x mm), elasticity (mm), gumming (Kg) or cohesion. None of the authors mentioned above found effects due to breed, finishing diet or livestock system in the parameters TPA (Table 8).

Table 7. Texture parameters of equine meat. Warner-Bratzler Shear force (WBSF).

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Ageing	WBSF	Reference
Burguete	12	16	M	SES	LTD	24 h		Sarriés and Beriain (2005)
					RA			
	13		F		LTD			
					RA			
	23	24	M		LTD			
					RA			
	8		F		LTD			
					RA			
Burguete	12	16	M	SES	LD	4 days	48.7	Sarriés and Beriain (2006)
						8 days	34.6	
	13		F			4 days	47.2	
						8 days	35.8	
	23	24	M			4 days	44.8	
						8 days	42.3	
	8		F			4 days	48.8	
						8 days	44.4	
NR	131	10 years	M F		LL	24 h	62.8	Litwińczuk et al. (2008)
					ST		77.5	
IHDH	24	11	M F	Indoors	BF	4 days	58.3	Tateo et al. (2008)
					LD		56.6	
					RF		56.0	
					SM		52.0	
					ST		52.3	
Burguete	15	24	M	SES	RA	24 h		Juárez et al. (2009)
HB					RA			
Sanfratellano	15	18	M	SES	LD	4-6 days	58.4	Lanza et al. (2009)
Haflinger							55.8	
GM	31	9	M F		LD	4 days	26.2	Franco et al. (2011a)
	11	12					27.3	
GM	12	15	M F	FES	LD	4 days	34.2	Lorenzo et al. (2013b)
					SM		39.3	
					ST		39.8	
					BF		44.8	
					TB		44.6	
					PM		36.6	
GM	12	15	M F	SES	LD	4 days	27.3	Franco et al. (2013)
GM x HB	9						34.1	
GM/ GM x HB	21			SES (1.5 kg S)			28.6	
				SES (3kg S)			31.7	
GM x HB	21	18	M F	FES	LD	4 days	49.6	Lorenzo et al. (2014b)
	14			SES (1.5 kg S)			21.0	
	14			SES (3kg S)			23.6	
IHDH	18	11	M	150% NL	LD	2 days	46.9	de Palo et al. (2014)
				180% NL			52.1	
				200% NL			52.8	

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Ageing	WBSF	Reference
GM x HB	10	8	M F	SES	LD	4 days	37.3	Domínguez et al. (2015)
	11	11	M F				34.5	
MF donkey	8	8	M	SES	LD	4 days	53.0	Polidori et al. (2015)
	8	12					61.5	
MF donkey	20	12		AS (SES)	LTL	24 h	51.9	De Palo et al. (2017)
				NS (SES)			54.9	
		18		AS (SES)			52.0	
				NS (SES)			51.3	

Sex: M = Male; F = Female.
Breeds: IHDH = Italian heavy draft horse; HB = Hispano-Bretón; GM = Galician Mountain; MF = Martina Franca.
Livestock: SES = Semi-extensive system; FES = free extensive system; 1.5/3kg S = 1.5/ 3Kg of supplementation in the finishing period; % NL = percentage of the nutritive level; AS = Artificial suckling; NS = Natural suckling; SC = Standard concentrate in the finishing period; LC = Linseed concentrate in the finishing period.
Muscle: LTD = *latissimus dorsi*; RA = *rectus abdominis*; LD = *longissimus dorsi*; LL = *longissimus lumborum*; ST = *semitendinosus*; BF = *biceps femoris*; RF = *rectus femoris*; SM = *semimembranosus*; TB = *triceps brachii*; PM = *psoas major & minor*; LTL = *longissimus thoracis et lumborum*.
NR: not reported.

Table 8. Texture Profile Analyses obtained for horsemeat

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Ageing	Hardness (Kg)	Springiness (mm)	Cohesiveness	Chewiness (Kg · mm)	Gumminess (Kg)	Reference
GM	12	15	M F	FES	LD	4 days	4.11 ± 1.11	0.43 ± 0.03	0.53 ± 0.05	0.99 ± 0.36		Lorenzo et al. (2013b)
					SM		6.34 ± 1.64	0.47 ± 0.03	0.57 ± 0.03	1.78 ± 0.61		
					ST		4.23 ± 1.32	0.49 ± 0.04	0.55 ± 0.05	1.17 ± 0.52		
					BF		5.63 ± 1.74	0.52 ± 0.03	0.54 ± 0.05	1.69 ± 0.62		
					TB		5.54 ± 2.34	0.48 ± 0.05	0.57 ± 0.05	1.58 ± 0.91		
					PM		4.17 ± 1.21	0.48 ± 0.02	0.56 ± 0.03	1.13 ± 0.36		
GM	6	15	M	FES	LD	4 days	3.9	0.4	0.9	0.5	2.1	Lorenzo et al. (2013c)
	6		F				4.2	0.4	0.1	0.5	2.3	
GM	12	15	NR	SES	LD	4 days	4.66	0.47	0.53	1.17	2.48	Franco et al. (2013)
GM x HB	9			SES			5.24	0.45	0.56	1.37	2.94	
GM/ GM x HB	21			SES (1.5 kg S)			4.53	0.49	0.53	1.18	2.42	
				SES (3kg S)			5.24	0.45	0.56	1.33	2.91	
GM	10	15	NR	SES (1.5 kg S)	LD	7 days	4.53	0.48	0.52	1.18		Franco and Lorenzo (2014)
GM x HB					SM		6.20	0.50	0.52	1.68		
					ST		4.67	0.50	0.54	1.31		
					BF		6.12	0.52	0.53	1.76		
					TB		6.24	0.52	0.53	1.78		
					PM		4.56	0.51	0.52	1.25		
GM	11	15		SES (3kg S)	LD		5.24	0.44	0.55	1.32		
GM x HB					SM		6.75	0.49	0.56	1.91		
					ST		5.17	0.48	0.57	1.43		
					BF		6.51	0.50	0.54	1.83		
					TB		5.83	0.46	0.54	1.60		
					PM		4.40	0.49	0.55	1.21		

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Ageing	Hardness (Kg)	Springiness (mm)	Cohesiveness	Chewiness (Kg · mm)	Gumminess (Kg)	Reference
GM x HB	21	18	M F	FES	LD	4 days	3.74	0.05	0.06	0.99	2.13	(Lorenzo et al., 2014b)
				SES (1.5 kg S)			3.44	0.47	0.57	0.96	1.95	
				SES (3kg S)			3.96	0.48	0.56	1.05	2.19	
GM x HB	10	8	M F		LD	4 days	3.07	0.05	0.05	0.75	1.59	(Domínguez et al., 2015)
	11	11	M F				3.80	0.05	0.05	0.91	1.90	

Sex: M = Male; F = Female.
Breeds: GM = Galician Mountain; HB = Hispano-Bretón.
Livestock: FES = free extensive system; SES = Semi-extensive system; 1.5/3kg S = 1.5/ 3Kg of supplementation in the finishing period.
Muscle: LD = *longissimus dorsi*; SM = semimembranosus; ST = *semitendinosus*; BF = *biceps femoris*; TB = *triceps brachii*; PM = *psoas major & minor.*
NR: not reported.

2.5.1.3. Colour

Colour is the factor that most affects the appearance of meat during storage, and the one that most influences consumer preference (Pérez and Andújar, 2008). Therefore, colour alteration throughout the production-sale cycle is an important object of study.

Different researches on foal meat colour have been developed during the last decade (Table 9). The colour of the foal meat depends on the breed, sex, slaughter age and animal husbandry system among others (temperature, type of muscle...) (Lorenzo et al., 2014b; Sarriés et al., 2005). Sex is the only parameter that, in most cases, has no influence on colour (Franco et al., 2011c; Sarriés and Beriain, 2006; Tateo et al., 2008).

According to the large number of works (Table 9), the variability between the minimum and maximum value of brightness, redness and yellowness has been calculated, reaching values of 32%, 41% and 75% for brightness, redness and yellowness, respectively. This underlines the importance of establishing homogeneous husbandry practices in order to define the colour of foal meat or at least reduce its variability.

Table 9. Colour parameters of equine meat.

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Ageing	L*	a*	b*	C*	h*	Reference
Burguete	12	16	M	SES	LTD	24 h	49.54	12.28	14.32	18.86	49.38	Sarriés and Beriain (2005)
					RA		36.61	13.61	5.51	14.68	22.04	
	13		F		LTD		53.61	10.26	14.77	17.98	55.21	
					RA		34.12	15.47	5.71	16.49	20.26	
	23	24	M		LTD		49.56	13.28	14.75	19.85	48.00	
					RA		37.33	13.71	5.81	14.89	22.96	
	8		F		LTD		50.11	12.79	13.52	18.61	46.58	
					RA		34.59	17.71	8.12	19.48	24.63	
Burguete	12	16	M	SES	LD	4 days	34.09	15.13	8.41	17.31	29.06	Sarriés and Beriain (2006)
						8 days						
	13		F			4 days	34.17	13.79	7.33	15.62	27.99	
						8 days						
	23	24	M			4 days	35.23	16.84	16.90	23.86	45.10	
						8 days						
	8		F			4 days	35.70	10.40	10.58	14.84	45.49	
						8 days						
NR	131	10 years	M F		LL	24 h	44.90	15.60	19.60	25.05	51.48	Litwińczuk et al. (2008)
					ST		44.20	15.70	19.80	25.27	51.58	
IHDH	24	11	M F	Indoors	BF	4 days	34.13	9.38	−0.80	12.43	−4.87	Tateo et al. (2008)
					LD		36.58	11.34	−0.25	14.31	−1.26	
					RF		38.21	11.92	0.93	15.48	4.46	
					SM		35.32	11.62	−0.67	14.68	−3.30	
					ST		37.73	10.70	−0.87	13.53	−4.65	
Burguete	15	24	M	SES	RA	24 h	34.60	24.20	9.60	26.03	21.64	Juárez et al. (2009)
HB					RA		28.40	19.80	7.00	21.00	19.47	

Breed	N	Slaughter Age (months)	Sex	Livestock	Muscle	Ageing	L*	a*	b*	C*	h*	Reference
Sanfratellano	15	18	M	SES	LD	4-6 days	38.80	15.46	8.28	17.56	28.13	Lanza et al. (2009)
Haflinger							40.83	17.02	9.67	19.58	29.48	
GM	31	9	M F		LD	4 days	41.19	10.78	4.87	11.86	21.56	Franco et al. (2011a)
	11	12					41.07	9.89	4.32	10.81	21.55	
GM	12	15	M F	FES	LD	4 days	39.16	17.40	10.97	20.62	32.34	Lorenzo et al. (2013b)
					SM		38.68	18.74	11.58	22.03	31.75	
					ST		40.86	17.30	11.17	20.63	32.68	
					BF		38.76	18.58	10.90	21.55	30.38	
					TB		37.16	19.15	11.05	22.11	29.86	
					PM		38.11	19.15	10.62	21.91	28.86	
GM	12	15	M F	SES	LD	4 days	36.95	15.67	8.57			Franco et al. (2013)
GM x HB	9						37.35	15.19	8.60			
GM/ GM x HB	21			SES (1.5 kg S)			35.95	15.38	8.07			
				SES (3kg S)			38.20	15.53	9.05			
GM x HB	21	18	M F	FES	LD	4 days	41.78	15.22	10.80			Lorenzo et al. (2014b)
	14			SES (1.5 kg S)			38.89	11.62	10.94			
	14			SES (3kg S)			38.95	11.60	10.93			
IHDH	18	11	M	150% NL	LD	2 days						de Palo et al. (2014)
				180% NL								
				200% NL								
GM x HB	10	8	M F	SES	LD	4 days	39.66	12.25	11.64			Domínguez et al. (2015)
	11	11	M F				37.88	12.17	10.94			
MF donkey	8	8	M	SES	LD	4 days	33.57	12.24	8.76			Polidori et al. (2015)
	8	12					32.34	11.49	7.87			
MF donkey	20	12		AS (SES)	LTL	24 h	39.68	16.05	-2.16	15.40	0.39	De Palo et al. (2017)
				NS (SES)			36.64	16.96	-1.47	17.56	0.43	
		18		AS (SES)			38.50	16.88	-0.71	17.99	0.41	
				NS (SES)			35.95	18.23	-0.63	20.81	0.47	

Sex: M = Male; F = Female.
Breeds: IHDH = Italian heavy draft horse; HB = Hispano-Bretón; GM = Galician Mountain; MF = Martina Franca.
Livestock: SES = Semi-extensive system; FES = free extensive system; 1.5/3kg S = 1.5/ 3Kg of supplementation in the finishing period; % NL = percentage of the nutritive level; AS = Artificial suckling; NS = Natural suckling; SC = Standard concentrate in the finishing period; LC = Linseed concentrate in the finishing period.
Muscle: LTD = *latissimus dorsi*; RA = *rectus abdominis*; LD = *longissimus dorsi*; LL = *longissimus lumborum*; ST = *semitendinosus*; BF = *biceps femoris*; RF = *rectus femoris*; SM = *semimembranosus*; TB = *triceps brachii*; PM = *psoas major & minor*; LTL = *longissimus thoracis et lumborum*.
NR: not reported.

2.5.2. Sensory quality of foal meat

Today, consumers demand not only healthy but also desirable, attractive and pleasant food. At the same time, sensory quality comes into play. Currently, the UNE-EN ISO 5492:2010 norm defines it as "*Science related to the evaluation of organoleptic attributes of a product through the senses*".

In this sense, foal meat has not been widely studied, and the only sensory studies developed, have been carried out using trained panels in order to characterize cooked foal meat. Colour, smell, hardness, fibrousness, juiciness, flavour or sweetness are some of the attributes employed. Breed and finishing diet (Franco et al., 2013), livestock (Lorenzo et al., 2014b; Sarriés et al., 2004) or slaughter age (Domínguez et al., 2015; Sarriés and Beriain, 2005) are production factors whose effect has been studied in the aforementioned sensory parameters. Some studies (Franco et al., 2013; Lorenzo et al., 2013b), described foal meat with a weak sweetness, and medium intensity values of colour, odour, juiciness or hardness. In this regard, Sarriés and Beriain (2005) showed that meat from 16-month-old foal had an aftertaste, initial juiciness and less floweriness and a higher sweet taste than the meat of 24-month-old foals, maintaining similar fatty taste and characteristic odour and colour.

On the other hand, there are no references to sensory studies on fresh foal meat. This lack of knowledge makes it necessary to develop in-depth sensory studies and establish descriptive patterns of the main freshness attributes: characteristic colour and characteristic odour of fresh foal meat. Then, we can understand their sensory evolution and know when the meat becomes undesirable. Thus, Ruiz, Insausti, Beriain, Lorenzo, & Sarriés (2016) considered it essential an exhaustive formation of the trained panel, at the end of which the panellists were able to define the characteristic and uncharacteristic colour and odour of the fresh foal meat. This accurate description can be found in the Chapter 5. "*Results and discussion*" were it has been employed. These achievements will be very useful for future studies to help accurately describe the sensory properties of fresh foal meat.

2.5.3. Oxidation stability during ageing and storage time

Oxidation is one of the main causes of the loss of meat quality during preservation as it affects lipids, pigments, proteins, carbohydrates and vitamins in meat, and consequently the colour, texture and sensory properties. Apart from the ante-mortem factors mentioned above (breed, slaughter age, livestock systems, etc.), there are two important *post-mortem* factors directly related to oxidative stability, and therefore to the loss or improvement of meat quality: ageing and storage time. Oxidative processes begin when cells and muscles are damaged after slaughter and continue until the meat is unacceptable to the consumer.

It is known that ageing favours a series of changes in the sensory characteristics and technological aptitude of the meat. It consists of a progressive softening of the meat, a slight increase in water retention capacity and the development of flavours and aromas characteristic of fresh meat. All these changes are the result of degradation of proteins and lipids. Recent studies (Ruiz et al., 2017a, 2016a) indicated that the stability of lipids and myoglobin states, and consequently, the colour stability of meat decreases according to the days of ageing (14 days < 7 days < 0 days). It seems that this process, mandatory for other species, may not be positive for foal meat. However, there is neither a standardised ageing time nor knowledge of the optimal ageing time for foal meat, so further study would be necessary to address this issue.

Depending on storage time, the high relationship between lipid and protein oxidation in fresh meat and its influence on the deterioration of meat quality is known (Lorenzo and Gómez, 2012). These processes are favoured by long storage times in the presence of oxygen and produce, from the appearance of bad smells to discoloration of the meat surface (Zakrys et al., 2008). Protein oxidation involves the oxidation of myoglobin, which is the colour pigment in a muscle and responsible for oxygen binding. When meat is in contact with oxygen, oxymyoglobin (OMb) is the main haem pigment that gives meat a bright red colour. In the absence of oxygen, the meat surface is dark red or purple (deoxymyoglobin, DMb), and for a long time in the presence of air induces oxidation of myoglobin (metmyoglobin, MMb) which gives the meat a

brown or reddish-brown colour. Although this colour change is not harmful and does not denote deterioration, it is considered undesirable by customers (Brooks, 2007).

Colour changes in foal meat occur mainly due to the susceptibility of the myoglobin molecule, especially iron, to alterations in the chemical environment and to energy input (Brewer, 2004). Some studies state that MMb% on the surface of foal meat is higher than 20% after 3 days of storage for meat aged 4 days (Sarriés et al, 2011) and after 3 and 2 days for meat aged 7 and 14 days, respectively (Ruiz et al., 2016a), being unacceptable to 50% of consumers (MacDougall, 1982). On the other hand, tenderness is greatly affected by the oxidation of proteins. Changes in proteins are related to deterioration of the textural aspects of meat. Meat becomes harder and less juicy due to changes in protein cross-linking due to oxidation reactions (Zakrys-Waliwander et al., 2010).

Foal meat is not only high in myoglobin (Lorenzo et al., 2014c; Sarriés and Beriain, 2005) (3.27-3.75 mg/ g) but also high in PUFA (see Table 4). Therefore, storage time is one of the most important factors determining lipid and protein stability and, consequently, the quality of foal meat and its shelf life.

2.5.4. Packaging

The development of preservation studies arises from the need to maintain acceptable the freshness properties of meat (colour, texture and sensory attributes) by controlling degradation factors (lipids and protein oxidation). Few studies have been developed to find the optimal packaging system for foal meat. The packaging studied were vacuum, overwrapping and modified atmosphere (MAP) conditions using different gas mixtures: low O_2 % (30:70 O_2/CO_2) and high O_2 % (80:20 O_2/CO_2) (Gómez and Lorenzo, 2012; Lorenzo and Gómez, 2012).

From the sensory point of view, several investigations have determined that the useful life of foal meat is reached earlier by sensory perception than by microbiological growth (Gómez and Lorenzo, 2012; Lorenzo and Gómez, 2012). They conducted life studies with 15-month-old

GM and HBxGM foal meat, respectively. Both agreed that the colour and odour were acceptable for 10 days if the meat was kept in vacuum conditions, while they became undesirable within 7 days if the meat was kept wrapped in an over-wrapping or packaged in modified atmosphere.

As far as colour is concerned, it is very important to improve its stability because it will increase the shelf life of meat and meat products by increasing the time consumers spend visually accepting meat at the time of purchase (Font-i-Furnols and Guerrero, 2014). In this sense, Lorenzo and Gómez (2012) showed that the luminosity increased during storage time (0-14 days) and gradually increased with greater oxygen concentration. This same study described a high loss in redness stability (a*) between 4 and 7 days with 43%, 61% and 73% variation for overwrapping, low O2 and high O2 MAP, respectively, while the a* values remained constant under vacuum conditions. Similar results were found for (Gómez and Lorenzo, 2012). In terms of texture, there is no reference to its behaviour in foal meat throughout the shelf life. In any case, protein oxidation influences the physical and chemical properties of proteins, including their solubility, water retention capacity and therefore the meat tenderness (Zakrys-Waliwander et al., 2010).

Regarding lipid oxidation, Gómez and Lorenzo (2012) and Lorenzo and Gómez (2012) stablished that vacuum packaging was the most suitable to ensure the stability of lipids and therefore of colour for at least 14 days. The MAP system, especially the high oxygen level (80 %) and overwrapping packaging, led to a marked increase in the level of MDA from the 7th day onwards, resulting in values of more than 2 mg of MDA/kg of fresh meat (rancidity threshold established by Campo et al. (2006) for beef). Adding the protein oxidation, vacuum packaging reduced the oxidation process (lipid and protein) and increased colour stability during storage (Gómez and Lorenzo, 2012). Lorenzo & Gómez (2012) stated that the greatest protein oxidation occurred in the packaging with oxygen (overwrapping > MAP high O2 > MAP low O2), while vacuum packaging is the one that best preserved protein stability up to now.

These studies show that vacuum packing would currently be the ideal system for preserving foal meat and agree with the results obtained by Ruiz et al. (2017c) regarding foal meat preservation under vacuum conditions. Nevertheless, the lack of oxygen in this type of packaging causes an unattractive colour change due to the transformation of oxymyoglobin into deoxymyoglobin (the bright red colour turns into dark red and purple) (Li et al., 2012). Although the red colour recovers once the packaging has been opened, it is not as attractive to consumers at the moment of purchase. Lorenzo et al. (2014a) studied the shelf life of foal meat using an active film of essential oils containing 2% oregano and 1% green tea extract. Both were useful for preserving the quality properties of foal meat and oxidation stability. In the light of these results, further studies are needed to improve shelf life and extend shelf life, during which colt meat retains essential properties.

2.6. Added value of foal meat. Current sense of quality

The physical-chemical, microbiological, hygienic-sanitary and sensory quality are fundamental aspects of any food product. Nevertheless, the concept of quality is multidimensional and is based on a wide range of interacting components.

Nowadays, there are some important aspects that consumers take into account when purchasing a food product. A clear example is sustainability, the preservation of the atmosphere and ecosystems, the exploitation of natural resources... all these issues concern consumers who value them in a positive way at the purchase moment. All of them are entirely linked to equine production and confer on foal meat an added value that differentiates it from other meat products: "meat" or "sustainable meat"?

Therefore, it is very important to develop studies to promote this type of production in order to guarantee sustainable products. In fact, a recent European project (OPEN2PRESERVE) has been approved and is based on the use of autochthonous horse breeds with the aim of improving and ensuring environmental sustainability through the exploitation of natural resources.

2.7. Consumers' perception of foal meat and market trends

Traditionally, horses have coexisted with humans for centuries and have been used over the years as farm workers, companion animals or for sport and leisure purposes. These historical associations mean that horses are perceived as pets in many cultures, and their consumption is poorly viewed (Iwobi et al., 2017). These are some of the reasons why, at present, there are no consumer studies on foal meat.

In order to promote the consumption of foal meat, in-depth sensory studies are essential to know the liking and preferences of consumers and their behaviour, which depends on the regions (countries, cities). For example, there is a wide knowledge about these issues of beef: willingness to pay, perceptions by region (country, cities...) or level of product information (Baba et al., 2016; Beriain et al., 2016, 2014; Kallas et al., 2014; Realini et al., 2014, 2013; Sánchez et al., 2012). The development of these studies on foal meat would be very useful to better describe, define and commercialize foal meat and meat products.

Recent studies belonging to the INIA project in which this PhD thesis is developed have shown that the population gives much importance (%) to health (27.4%), denomination of origin (21.0%) and animal feed (20.5%), rather than to the price of food products (17.6%) (unpublished data). This trend could be very beneficial from a commercial point of view for foal meat if the above-mentioned characteristics are taken into account.

2.8. Development of new foal meat products

The increase in consumption of foal meat in recent years may be due to changes in attitudes and consumer interest in trying new meat products (Sarriés et al., 2006). Although the main consumption of this meat is as fresh meat, manufacturers can use it in the manufacture of meat products. Some dry-cured foal products studied are "Salchichón"(Lorenzo et al., 2012b), dry-cured sausages (Lorenzo et al., 2012a; Lorenzo and Franco, 2012), liver foal paté (Lorenzo and Pateiro, 2013b), "Chorizo"(Lorenzo et al., 2013a), "Cecina" (Lorenzo, 2014) or dry-cured loin

(Lorenzo and Carballo, 2015). All these studies were very useful because all the products mentioned are currently commercialised by the cooperative "Monte Cabalar" (Galicia). Nevertheless, much work remains to be done. More research on these products, their dissemination and promotion are needed to familiarise consumers and improve the perception of the foal's meat products. Recently, the high nutritional value and sensory properties of these products have been demonstrated, which are in line with consumer expectations for healthy foods with unique sensory characteristics (Lorenzo et al., 2017). With this latest study, the authors' aim was to improve the current perception of foal meat and meat products due to the fraud scandals in which foal meat has recently been involved.

2.9. Application of different techniques in the determination of meat quality

Commonly, the chemical composition of foods (moisture. protein, intramuscular fat, ash content...) is determined by destructive and, in many cases, long-lasting and environmentally unsustainable techniques such as the Kjeldahl (1883) method for protein content or Soxhlet (1879) for intramuscular fat content (Wu et al., 2008) the same is true for texture determination.

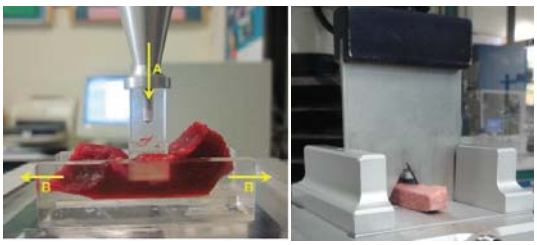

Figure 4. Compression and shear force determination for raw and cooked meat by the employment of a texturometer

2.9.1. Non-destructive techniques

At present, high levels of quality and safety are demanded throughout the food production chain. Strict controls are therefore required throughout the entire production and marketing process. This fact makes the necessary food analysis techniques quick, easy to use,

non-destructive, non-invasive, without preparation or minimal sample preparation and at low cost (Karoui et al., 2010). For this reason, alternative techniques for determining the chemical composition of meat, such as those shown in the following examples, have been studied for several years:

- Image analyse shows a wide variety of applications. This technique requires precise sample preparation for high quality images. Images are captured by a camera and digitized by an image analysis program. The colour coordinates (grease, red, green and blue) as well as the muscular surface and marbling percentages can be obtained. Many works have been developed such as for bull carcass grading (Oliver et al., 2010) and characterization of foal carcass (Tabar et al., 2017) and foal meat (Sarriés et al., 2017). And also for the determination of number and type of muscle fibres (Şirin et al., 2017) of for marbling and muscle area determination (Beriain et al., 2016; Mendizábal et al., 2005; Urrutia et al., 2015).

- Hyperspectral images is a powerful technique that combines space distribution and chemical composition information with image analysis. It allows the identification of compounds based on their chemical composition and subsequently, image analysis allows the detection of changes in foreign matter. Multiple studies have been developed to assess food quality (Baiano, 2017; Liu et al., 2017).

- Ultrasound involves mechanical waves at frequencies above 20 kHz, which are above the upper limit of the human auditory frequency range (viz 20-20 000 Hz). They are propagated by vibration of particles in the medium and can be reflected and transmitted as they pass from one medium to another (Cho and Irudayaraj, 2003). Detailed information on the different physical properties of the materials can be obtained through the amount of energy reflected or transmitted through the materials depending on their relative acoustic impedances.

- Infrared spectroscopy is being described as potential tools in this field. The infrared spectrum is divided into three regions: near (12.500 - 4.000 cm^{-1}), medium (4000 - 400 cm^{-1}) and

far infrared (400 - 5 cm^{-1}) (Figure 6). The most commonly used spectrum ranges are near and medium spectrum (NIR and MIR).

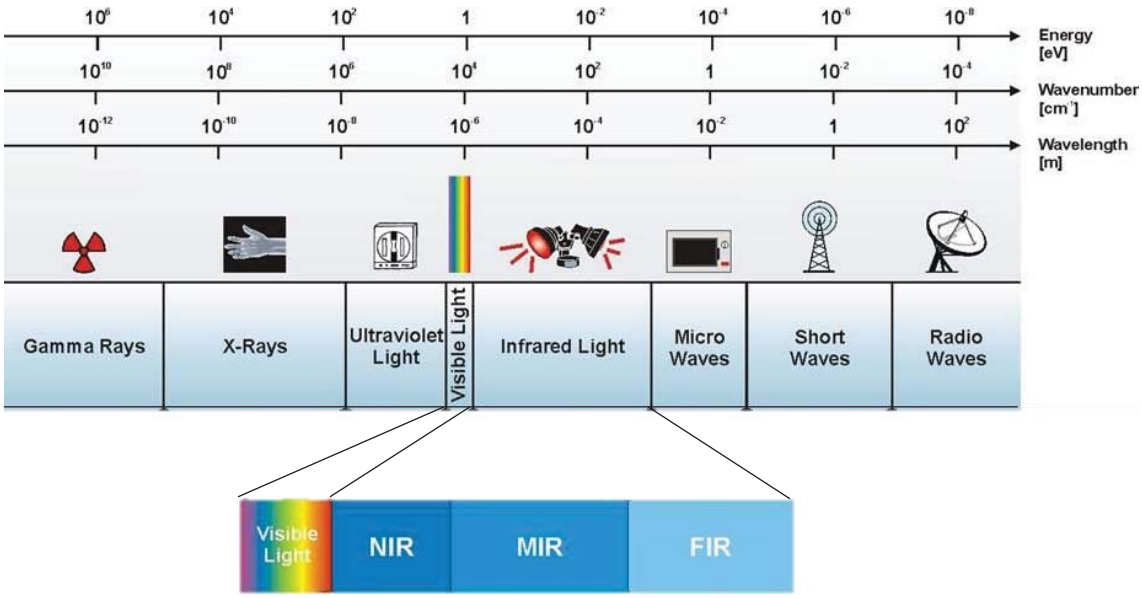

Figure 5. Electromagnetic spectrum. (Source: Bruker, 2007)

The spectra of the different molecular species in the infrared are the result of energetic changes produced in the transitions of molecules from one vibratory and rotational energy state to another. These transitions produce a net change in the dipole moment of the affected molecule causing the alternating electric radiation field to interact with the molecule and produce changes in the amplitude of some of its movements.

Interest in MIR stems from its high capacity to determine molecular structures (Sun, 2009). A large number of products have been studied. Some are honey (Minaei et al., 2017), wine (Genisheva et al., 2018), virgin oil (Özdemir et al., 2018) or tomato (Tilahun et al., 2018). Nevertheless, NIR spectroscopy does not give details about the types of molecules. In addition, MIR spectroscopy involves many of the general stretching, bending, and balancing movements of functional groups, such as C-C, C-H, O-H, and C=O and N-H (Workman, 2001). Some of the MIR's research includes the study of food component interactions, the quantification of

nutrients and other food-specific components, the characterization of the structure of food molecules, the determination of raw material and additive quality, or the detection of adulterations and food authentication.

The use of MIR spectroscopy in meat analysis remains very limited (Table 13). The content of proteins and lipids are parameters used to measure the quality level of various food products. Therefore, the MIR application has been studied by several authors such as Wu et al. (2008) in milk, Shiroma & Rodriguez-Saona (2009) in chips, Shi & Yu (2017) in wheat, Lozano et al. (2017) in different types of meat or Lucarini, Durazzo, Sánchez del Pulgar, Gabrielli, & Lombardi-Boccia (2017) in meat and meat-products. This technique may also be useful for distinguishing apparently similar products. In this case, two wines (one conventional, the other organically produced) could be classified due to spectrum differentiation (Cozzolino et al., 2009). Similarly, Arce et al. (2009) were able to distinguish three types of subcutaneous fat samples: (1) from pigs fed extensively, fattened pigs (2) with commercial feed and (3) with supplements enriched with oleic acid.

NIR spectroscopy is widely used for prediction of chemical composition, colour and texture and even sensory properties of different types of meat (Andrés et al., 2007; Prieto et al., 2014, 2009, 2008; Ripoll et al., 2008). It is worthwhile to develop similar studies with MIR spectroscopy as it is more accurate and gives more information.

Table 10. MIR spectroscopy studies developed in meat and meat products during the last two decades.

Researches	Pre-treatment	Measurement	Wavelength range (cm^{-1})	Reference
Differentiation among minced pork, chicken and turkey. Differentiation between fresh and thawed meat.	Minced meat	Reflectance	4000-800	Al-Jowder et al. (1997)
Differentiation between muscle and visceral tissue.	None	Reflectance	4000-800	Al-Jowder et al. (1999)
Determination of lamb content in minced beef and lamb.	Minced meat	Reflectance	4000-640	McElhinney et al. (1999)
Differences among raw minced pork, chicken, turkey, veal and lamb.	Minced meat	Reflectance	4000-640	Downey et al. (2000)
Bacterial growth in chicken meat.	None	Reflectance	4000-600	Ellis et al. (2002)
Differences between raw veal and veal meat with a content of 20% adulterants (visceral tissue).	None	Reflectance	4000-800	Al-Jowder et al. (2002)
Bacterial growth in beef meat.	None	Reflectance	4000-600	Ellis et al. (2004)
Amino acids composition of animal diets.	None	Reflectance	4000-650	Qiao and Van Kempen (2004)
Veal stored in the air, in a modified atmosphere and in active containers.	None	Reflectance	4000-400	Ammor et al. (2009)
Adulterations of minced beef with horsemeat, leftovers of fat or vegetable protein.	None	Reflectance	4000-650	Meza-Márquez et al. (2010)
Adulteration with pork fat from "Meatball" (traditional Indonesian food).	Previous IMF extraction	Reflectance	4000-650	Rohman et al. (2011)
Determination of adulteration in minced meat of veal with turkey.	Mincing and homogenization	Reflectance	4000-700	Alamprese et al. (2013)
Bacterial growth in chicken breasts.	None	Reflectance	4000-700	Sahar and Dufour (2014)
Detection and characterization of frauds in bovine meat *in natura* by non-meat ingredient additions.	None	Reflectance	4000-525	Nunes et al. (2016)
Determination of fat and protein content in meat of beef, chicken, cow, fighting bull, foal, hen, lamb, pork, rabbit, and turkey.	Mincing and homogenization	Reflectance	4000-400	Lozano et al. (2017)
Determination of fatty acid content in (beef, pork, chicken) and meat products (ham, salami, bacon, coppa).	Fat extraction	Reflectance	4000-650	Lucarini et al. (2017)
Characterization of deterioration of fallow deer and goat meat.	Mini-burgers (minced meat)	Reflectance	2000-900	Moreira et al. (2018)

CHAPTER 3. OBJETIVES

The overall objective of this PhD thesis was to characterize the foal carcass and meat quality by determining the effect of slaughter age and finishing diet on the physic-chemical and sensory characteristics and consumers' preferences. To achieve the general objective, 13 and 26-month old Galician Mountain x Burguete crossbred foals (GMxB foals), supplemented with standard and linseed-enriched concentrate (5%) were used.

The global objective comprises the following specific objectives:

1) Study the effect of slaughter age and finishing diet on carcass quality of GMxB foals and the carcasses classification in a grading system.

2) Characterise foal meat and study its meat quality evolution under different experimental conditions of ageing and storage time.
   a) The first experiment dealt with the traditional procedure in which the foal meat is managed once it is distributed to the points of sale. For this study, Burguete breed, native to Navarre, was used because (1) it is currently one of the most important breeds for meat production in Spain and (2) it was the breed selected to be the genetic basis of the GMxB foals employed throughout this PhD thesis. The meat was aged 24 hours and 7 days and preserved in trays overwrapped in PVC during 0, 3, 6 and 9 days of storage time.
   b) The second experiment goes on with the study of meat quality from GMxB foals. In this case, the meat was aged 24 hours and preserved under vacuum conditions during 0, 4, 8 and 12 days of storage time.

3) Study the effect of slaughter age and finishing diet on the sensory quality of the meat from the GMxB foals, assessed by a trained panel and a consumer test. The consumers test was carried out in two different regions (Orense and Pamplona) under two information scenarios (blind and full-information).

4) Study the application of MIR spectroscopy, as an environmentally sustainable alternative to the destructive techniques, on the evaluation of foal meat quality from GMxB foals.

CHAPTER 4. MATERIALS AND EXPERIMENTAL DESIGN

4.1. Animal management

- *Galician Mountain x Burguete crossbred foals*

Forty-six Galician Mountain x Burguete crossbred foals were used. In the North of Spain, horsemeat production is based on extensive systems. The foals were kept with their mothers and allowed to suck freely on pasture from the birth to the weaning at the age of 6-7 months (1st period). Next, foals were randomly divided in two groups to be slaughtered at two different ages (SA): 22 foals were slaughtered at 13 months (403 ± 30 days) (13M) and 24 foals were slaughtered at 26 months of age (784 ± 37 days) (26M). Both groups of animals were fed on the same pasture following a rotational grazing (2nd period). This period lasted 3 months (± 15 days) and 16 months (± 15 days) for 13M and 26M, respectively. Finally, foals were supplemented for 104 days (± 10 days) with a finishing diet (FD) based on concentrate (3rd period). The vegetation during the three periods aforementioned was composed by seeded (*Lolium perenne* and *Trifolium repens*) and natural fields (*Agrostis* spp., *Lotus corniculatus*, Holcus lanatus, *Bromus mollis*, *Pseudoarrenatherum longifolium*, etc.). During the last period (3rd period), 11 foals from 13M group and 12 from 26M group were randomly chosen and supplemented with standard concentrate (SC). Then again, the others 11 foals from 13M and 12 from 26M group were randomly chosen and supplemented with linseed-rich concentrate (5%) (LC), both concentrates were isoenergetic and isoproteic (Table 11). Thus, 4 experimental groups were obtained: 13M-SC, 13M-LC, 26M-SC, 26M-LC. Foals were supplemented with 2 kg of the mentioned concentrates to carry out an optimal animals' fattening and to improve the meat lipid profile (linseed supplementation). These supplementations were increased from 0.3 kg to 2 kg per foal/day for the first 10 days (adaptation term).

Table 11. Concentrates composition design

	Standard concentrate			Linseed concentrate		
	Ration (%)	Digestible energy (Mcal/ kg DM)	% Brute protein/ kg DM	Ration (%)	Digestible energy (Mcal/ kg DM)	% Brute protein/ kg DM
Ingredients						
Oat flour	30.03	2.5	7.4	45.95	2.5	7.4
Barley flour	30.10	2.9	8.3	13.07	2.9	8.3
Cornmeal	15.47	3.0	6.5	14.54	3.0	6.5
Wheat Bran	7.95	2.3	13.0	5.92	2.3	13.0
Soybean meal	9.99	3.0	38.2	9.06	3.0	38.2
Glycerol	3.96	3.2		3.96	3.2	
Linseed				5.01	3.9	19.1
TOTAL	97.50[1]	2.69	10.57	97.51[1]	2.70	10.61

[1] 2.5 % added to the total ration addition (%) (vitamins + salt + calcium carbonate + calcium phosphate).

. The intakes were estimated according to the methodology and equations proposed by NRC (1989):

- *DE (Mcal/ kgDM)* = 4.22 - (0.11 x BP) + (0.0332 x ADF) + (0.00112 x ADF^2)
- *Intake* $\overline{DE}$ *(Mcal/ foal-day)* = (1.4+0.03 x $\overline{\mathrm{LW}}$) + (4.81 + 1.17 x $\overline{\mathrm{A}}$ – 0.023 x $\overline{\mathrm{A}}^2$) x $\overline{\mathrm{ADG}}$
- *Intake* $\overline{DM}$ *(kg/ foal-day)* = [Intake $\overline{\mathrm{DE}}$ (Mcal/ foal-day)]/ [DE (Mcal/ kgDM)]
- *Intake* $\overline{DM}$ *(%LW)* = [Intake $\overline{\mathrm{DM}}$ (kg/ foal-day)/ $\overline{\mathrm{LW}}$ (kg)] x 100

where DE = Digestible energy; BP = Brute protein, ADF = Acid detergent fibre; DM = Dry matter; LW = Live weight, A = Age, ADG = Average daily gain. $\overline{DE}$,$\overline{\mathrm{LW}}$, $\overline{\mathrm{A}}$, $\overline{\mathrm{ADG}}$, $\overline{DM}$, average values for 13M and 26M foals.

The estimated $\overline{\mathrm{DM}}$ intakes during the 2nd period were 2.18 and 2.37 %LW for 13M and 26M foals, respectively, whereas the estimated $\overline{\mathrm{DE}}$ intake was 13.16 and 16.51 Mcal/ foal-day for 13M and 26M foals, respectively. For the finishing period (3rd period), the estimated $\overline{\mathrm{DM}}$ intakes (pasture plus concentrate) were 2.80 and 2.59 %LW for 13M and 26M foals, respectively, whereas the estimated $\overline{\mathrm{DE}}$ intake were 20.67 and 26.09 and Mcal/ foal-day for 13M and 26M

foals, respectively. All estimated intakes fell within the range 1.8-3.5 %LW, reported by Dulphy et al. (1997).

Foals were transported 50 km to the abattoir the day before slaughter in compliance with current European regulations (Council Regulation 1/2005EC, 2005), and were stunned with a captive bolt, slaughtered and dressed according to the specifications outlined in the European legislation (Council Directive 93/119/EC, 1993).

- *Burguete foals*

Eight Burguete foals slaughtered at 16 months of age were used. All foals were reared with their mothers, at pasture, and weaned at 7 or 8 months of age. Then, they were fattened indoors with commercial concentrates for 7–8 months. Animal management was similar to that indicated in previous studies with this same breed (Sarriés and Beriain, 2005). Finally, animals were slaughtered in the official slaughterhouse "La Protectora" S.A. of Pamplona (Navarra) (Directive of the Council of the European Union 95 / 221EC). The carcass weight was 281 ± 14.87 kg (on average).

4.2. Experimental design

The experimental approach was carried out in line with the overall objective pursued. In order to achieve the general objective of this PhD thesis, 5 research works have been developed and are presented in Chapter 5. “Results and discussion”. Figure 6 shows the experimental design for the work corresponding to GMxB foals. Figure 7 shows the experimental design for Burguete foals.

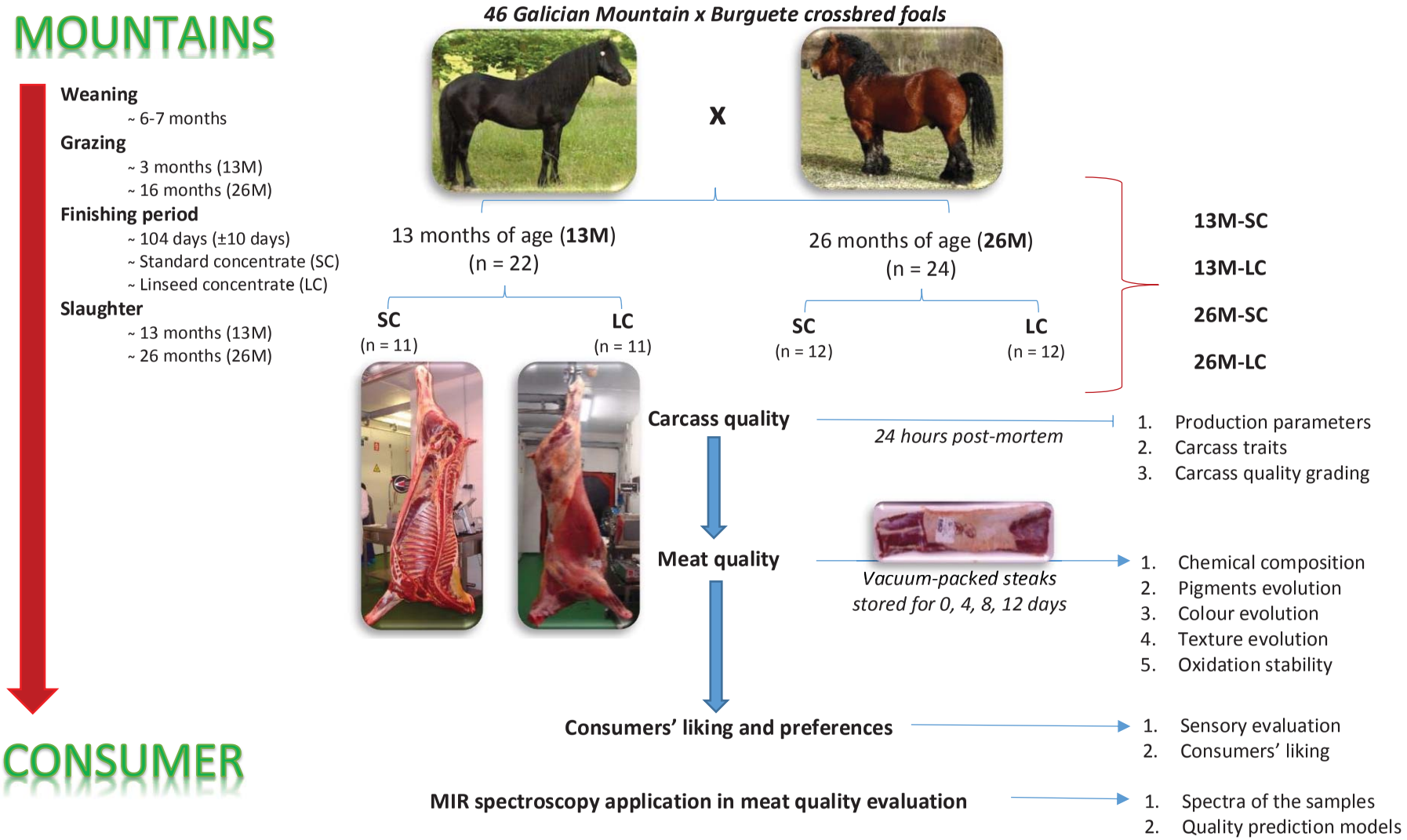

Figure 6. Experimental design of foal meat quality throughout the production chain from Galician Mountain x Burguete crossbred foals

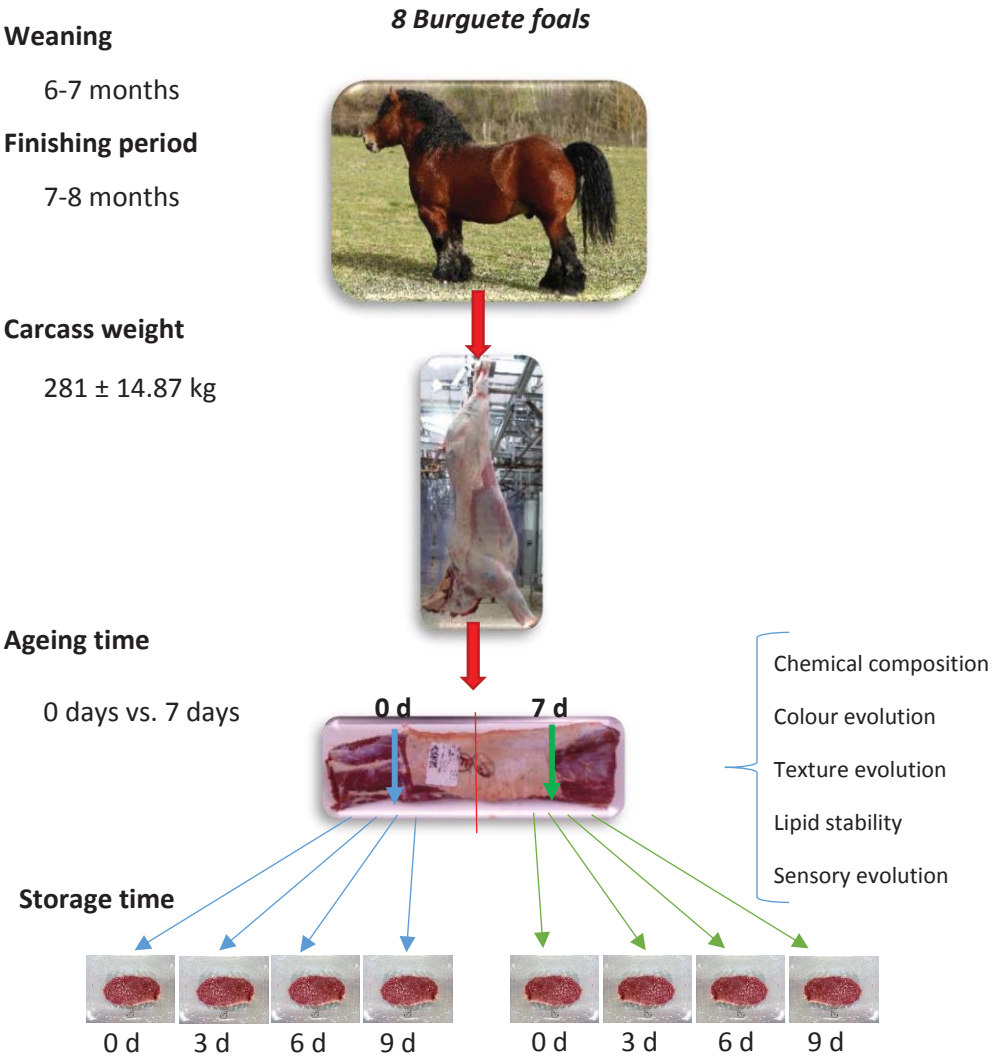

Figure 7. Experimental design of ageing and storage effect study from Burguete foals

CHAPTER 5. RESULTS AND DISCUSSION

The Chapter 5, "Results and Discussion" of this Doctoral Thesis are presented in five documents, two published and three prepared and submitted for publication in international journals in the field of animal production and meat science and technology.

PUBLICATION I

Relationship between carcass traits, prime cuts and carcass grading from foals slaughtered at the age of 13 and 26 months and supplemented with standard and linseed-rich feed.

Ruiz, M., Sarriés, M.V., Beriain, M.J., Crecente, S., Domínguez, R. and Lorenzo, J.M.

Animal. 12 (5), 1084-1092. 2017. DOI: org/10.1017/S1751731117002555.

PUBLICATION II

Efecto de la maduración en la conservación de la carne de potro.

Ruiz, M., Beriain, M.J., Insausti, K., Lorenzo, J.M., Cantalejo, M.J. and Sarriés, M.V.

ITEA. Información Técnica Económica Agraria, 2018. 114 (1), 45-60. DOI:

org/10.12706/itea.2018.004.

Efecto de la maduración en la conservación de la carne de potro

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Resumen

Para el desarrollo del presente estudio, se investigó el efecto de la maduración del músculo *Longissimus dorsi* (LD), por un lado como pieza entera y por otro lado tras el fileteado, sobre los parámetros de calidad de la carne de potro. Se emplearon músculos madurados 24 horas en la canal procedentes de ocho potros quincenos de raza Burguete. Cada lomo se dividió en dos partes iguales. Una parte no fue madurada, y la segunda fue madurada 7 días. Posteriormente, ambas partes fueron fileteadas y los filetes fueron conservados durante 9 días en bandejas cubiertas con film permeable al oxígeno en un expositor. No hubo interacción entre la maduración en el lomo y el tiempo de conservación posterior del filete. Los valores de textura fueron bajos desde el comienzo siendo considerada "tierna". Debido a la maduración, el enrojecimiento (a*) fue intenso y el contenido de metamioglobina fue bajo, pero la oxidación de los lípidos y la degradación del olor aumentaron. Cuando la maduración se llevó a cabo en filete, el tiempo de conservación y la atmósfera rica en oxígeno hicieron que la carne de potro se deteriorara rápidamente. La oxidación de los lípidos y la mioglobina y la degradación del color aumentaron día a día. Además, cuanto más tiempo estuvo expuesta la carne al oxígeno, peores fueron las valoraciones de color y olor sensorial de la carne de potro. El tiempo de conservación de la carne de potro fue inferior a 3 días, siendo el color característico el factor limitante.

Palabras clave: Potro, maduración, textura, degradación de color, oxidación, análisis sensorial.

Abstract
Ageing effect on foal meat preservation

Ageing effect in loin and steaks was investigated to study quality parameters on foal meat. Eight *Longissimus dorsi* muscles (LD) aged 24 hours inside the carcass from 15-month-old foals were used. One equal part of the loin was not aged, the second part was aged 7 days and both of them were filleted in steaks, aged for 9 days in trays and preserved in an expositor chamber. There was no interaction between loin ageing time and steak conservation time. Texture values were low at the beginning, the foal meat being described as "tender". Redness (a*) was intense and metmyoglobin content was low, but lipid oxidation and odor degradation increased. When ageing was carried out on steaks, oxygen exposure time and the free oxygen atmosphere made foal meat deteriorate rapidly. Lipid and myoglobin oxidation and color degradation increased day by day. In addition, the longer the meat was exposed to free oxygen, the worse were the scores for sensory color and odor of foal meat. The preservation time of foal meat was less than 3 days, the characteristic color being the limiting factor.

Keywords: Foal, ageing, texture, color degradation, TBAR'S, sensory analysis.

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Introducción

El color y la terneza son dos de las características más importantes de la carne en el momento de compra y de consumo (Koohmaraie, 1996; Rentfrow *et al*., 2004). La maduración es un proceso muy importante, cuyo principal objetivo es el ablandamiento de la carne. Pero, en este proceso no sólo ocurren cambios en la textura, sino que también tienen lugar alteraciones en el color, aroma y en la apariencia. Debido a la importancia de este proceso, muchos estudios han tratado de definirlo en diferentes especies; principalmente en vacuno y especialmente en diferentes razas de la misma, con el fin de conocer su punto óptimo de maduración (Campo *et al*., 2000). Sin embargo, este tipo de estudios no se ha extendido a razas equinas. La carne de potro destaca especialmente por su terneza a las 24 horas *post mortem*. Según la clasificación descrita por Belew *et al*. (2003) de acuerdo a la fuerza de corte (WBSF, Warner Bratzler Shear Force), la carne de potro, únicamente madurada 24 horas en la canal podría considerarse como "tierna" (3,2 < WBSF < 3,9 kg) o incluso "muy tierna" (WBSF < 3,2 kg) según los valores descritos por varios autores (Sarriés y Beriain, 2006; Franco *et al*., 2011a; Lorenzo *et al*., 2013). La falta de valores de referencia para este proceso ha dado lugar al desarrollo de diferentes estudios. Sarriés y Beriain (2006) estudiaron el músculo LD proveniente de potros de raza Burguete, madurado 24 horas dentro de la canal y 4 y 8 días fuera de la misma. Lanza *et al*. (2009) desarrollaron su estudio en el músculo LD de potros de raza Sanfratellano y Haflinger con una maduración de 4 y 6 días dentro de la canal. Otros autores como Gómez y Lorenzo (2012), Lorenzo y Gómez (2012) y Lorenzo *et al*., (2013) llevaron a cabo su estudio de maduración con el músculo LD proveniente de potros de raza Gallego de Monte y de potros cruzados Hispano-Bretón x Gallego de Monte, madurado 24 horas dentro de la canal y tras un posterior fileteado. Sin embargo, no hay suficientes estudios sobre el sistema más apropiado de maduración y sobre el manejo de la carne durante este periodo. Además, la falta de normas y directrices a seguir durante este proceso, hace que no sea posible ofrecer al consumidor un producto bien definido.

Por tanto, el objetivo de este estudio fue determinar el efecto de la maduración sobre los parámetros de calidad de la carne de potro y saber si existen diferencias entre la maduración de toda la pieza de lomo fuera de la canal y la maduración después de filetear el lomo.

Material y métodos

Material animal y diseño experimental

Para llevar a cabo este estudio, se emplearon 8 potros de raza Burguete de 15 a 16 meses de edad. El manejo animal fue similar al indicado en previos estudios en los cuales se empleó esta misma raza (Sarriés y Beriain, 2005). Los animales fueron sacrificados en el matadero oficial "La Protectora" S.A. de Pamplona (Navarra) (Directiva del Consejo de la Unión Europea 95/221EC). El peso de las canales fue de 281 ± 14,87 kg. La media canal izquierda de cada animal fue conservada en refrigeración durante 24 horas a 0ºC, con una humedad relativa del 98% y con una corriente de aire de 13.600 m^3/h. Tras 24 horas, el músculo LD fue extraído de la media canal izquierda y llevado en refrigeración a la Universidad Pública de Navarra.

El músculo LD fue dividido en dos partes iguales, entre la 6ª y la 7ª costilla. La zona craneal fue destinada a estudiar la carne 24 horas *post mortem* (M0) y la zona lumbar fue madurada 7 días envuelta en una doble película de PVC permeable al oxígeno y conservada en refrigeración durante 7 días (M7) a 4 ± 1ºC de temperatura, oscuridad total y

90-95% de humedad relativa. Transcurridos ambos tiempos de maduración (M0, M7), las muestras fueron fileteadas obteniendo 4 filetes de 1,5-2 cm de espesor de cada mitad. Dicho filetes fueron conservados durante 0, 3, 6 y 9 días (tiempo de conservación: TC) en bandejas de polietileno de 0,75 litros (0750-639015) cubiertas con una película de PVC permeable al oxígeno (transmisión de oxígeno: 1762 cm^3/254 cm^2/24 h/atm). Estas bandejas se almacenaron a 2 ± 1°C, simulando las condiciones de venta al por menor en los supermercados en una cámara de refrigeración iluminada por una luz fluorescente blanca durante 10 h / día.

Composición química

La composición química de la carne de potro se analizó 24 horas después del sacrificio. El contenido de humedad (%), proteína (%), grasa intramuscular (%) y ceniza (%) se determinó siguiendo las Normas Internacionales ISO R-1442: 1978, ISO R-937: 1978, ISO R-1443: 1978 e ISO R-936-1998, respectivamente. Se cuantificó el contenido de hierro total (mg/ 100 g de carne fresca) (Norma UNE-EN-14082: 2003), de hierro hemínico (mg / 100 g de carne fresca) y de mioglobina (mg/ g de músculo) (Boccard *et al*., 1981). Además, se analizó el contenido de colágeno total (g/ 100 g de carne) (CT) y soluble (% CT) (CS) de acuerdo con Bonnet y Kopp (1986).

Textura instrumental

Los parámetros de textura se determinaron tanto en el músculo LD no maduro (M0) como madurado 7 días (M7). El análisis de compresión se desarrolló en carne cruda utilizando un dispositivo de compresión modificado que evita el alargamiento transversal de la muestra (Lepetit y Culioli, 1994). Las variables consideradas fueron la compresión al 20% (kg / cm^2) (C20) y compresión al 80% (kg / cm^2) (C80). La compresión al 20% es una medida de la fuerza de las miofibrillas y la compresión al 80% está relacionada con el contenido de colágeno y el número de reticulaciones entre las moléculas de colágeno (Lepetit y Culioli, 1994). El análisis de fuerza al corte se realizó cocinando la carne envasada al vacío en un baño de agua a 75 ± 1°C hasta una temperatura interna de 70 ± 1°C durante 45 minutos. Esta temperatura fue medida con una sonda Digitron 3246 (Hereford, RU). La fuerza de cizallamiento máxima se evaluó utilizando un dispositivo de fuerza de cizalla Warner-Bratzler (Kg) hasta que las muestras se cortaron completamente. En ambos casos, se analizaron un mínimo de ocho muestras de 1 cm^2 (sección transversal cuadrada), con las fibras musculares paralelas al eje longitudinal de la muestra. Se utilizó un texturómetro TA-XT2i Stable Micro Systems conectado a un ordenador con IBM Foxen, microprocesador AutenticAMD-K6 (tm) y procesador 3D. Para el control del dispositivo y el procesamiento de los datos, se utilizó el programa informático "Texture Expert" versión 1.22 a Windows (Stable Micro Systems, Surrey, UK).

Oxidación lipídica

La oxidación lipídica se determinó en los filetes del músculo LD no madurado (M0) y madurado 7 días (M7) en los días 0 y 9 de tiempo de conservación (TC). El valor del ácido tiobarbitúrico (TBA) se calculó según lo descrito por Tarladgis *et al*. (1960). Los resultados se expresaron como mg de malonaldehído / kg de carne.

Color instrumental

El color de la carne se analizó en los filetes de M0 y M7 en los días 0, 3, 6 y 9 de TC. Los valores de color de CIE (1976) Luminosidad (L*), índice de rojo (a*) e índice de amarillo

(b*) fueron medidos con un espectrofotómetro Minolta CM2002, iluminante D65 y un 10 Observador estándar. Se realizaron 5 medidas en cinco zonas diferentes de cada filete, cambiando la orientación del instrumento cada vez, y se trabajó con la media obtenida de estas 5 repeticiones. Posteriormente, la cromaticidad (C*) y los valores de tonalidad (h*) fueron calculados a partir de las siguientes ecuaciones: $C^* = (a^{*2} + b^{*2})^{0.5}$ y $h^* = \arctan(b^*/a^*)$.

Por otro lado, se determinó la estabilidad del color en cada momento de TC (0d, 3d, 6d y 9d) y se basó en la cuantificación de la metamioglobina sobre la superficie de la carne (MMb%) (American Meat Science Association (AMSA), 2012). Los valores de reflectancia empleados fueron los correspondientes a los puntos isobésticos 525nm y 572nm, característicos de la curva de metamioglobina. A partir de estas reflectancias se obtuvieron los valores K/S de las curvas de referencia según la ecuación:

$$k/S = \frac{(1-R)^2}{2R}$$

El porcentaje de MMb se calculó mediante la fórmula:

$$\%MMb = \frac{K/S_{572}/K/S_{525\ 0\%MMb} - K/S_{572}/K/S_{525\ 0\%MMb\ de\ la\ muestra}}{K/S_{572}/K/S_{525\ 0\%MMb} - K/S_{572}/K/S_{525\ 100\%MMb}}$$

Análisis sensorial

Estos análisis se realizaron en todos los filetes (M0, M7) y en los días 0, 3, 6 y 9 de TC. Los atributos estudiados fueron el olor y el color característico de la carne de potro fresca. Se empleó la metodología utilizada por Gómez *et al*. (2014) en vacuno, pero adaptada para carne de potro (Ruiz *et al*., 2016). Se llevaron a cabo cinco sesiones de entrenamiento específico de carne de potro descrito por los mismos autores. Los panelistas fueron entrenados con muestras reales y cuya descriptiva aparece en la tabla 1. Un panel sensorial de 15 miembros evaluó ambos atributos. Se utilizó una escala lineal no estructurada de 150 mm para medir la evolución del olor y el color característico. Se indicó: en el extremo izquierdo (valor mínimo, 0 mm) "olor característico", "color característico" y en el extremo derecho (valor máximo 150 mm) "olor no característico", "color no característico", respectivamente. El punto medio (75 mm) marcó el límite entre "característico" y "no característico" ($0 < 75 \leq 150$ mm). Las sesiones se programaron en función de la maduración del músculo LD en pieza entera (M0, M7) y en filetes (0d, 3d, 6d y 9d). El olor fue el primer atributo evaluado con luz roja (100 lux) seguido del color, utilizando, en este caso, luz blanca (450 lux). Las muestras fueron identificadas por un número de tres dígitos en un orden aleatorio. La cata se realizó en la sala de análisis sensorial de la Universidad Pública de Navarra acreditada según la norma UNE EN ISO / IEC 17025 (Acreditación 300 / LE 584, 2001).

Análisis estadístico

Todos los análisis estadísticos se realizaron utilizando el paquete informático SPSS 22.0. Se realizaron análisis descriptivos estadísticos para caracterizar la carne de potro en su origen y se calcularon coeficientes de variación para explicar la heterogeneidad de este tipo de carne.

$$Y_{ijk} = \mu + \varepsilon_{ijk}$$

siendo: Y_{ijk}: La observación del parámetro analizado; μ: Media mínima cuadrada de la población; ε_{ijk}: Error vinculado a cada observación.

Además, para las variables en las que se estudió el efecto de la maduración del músculo LD en pieza entera y del tiempo de conservación en filetes, se realizó un análisis de varianza (ANOVA):

$$Y_{ijk} = \mu + P_i + F_j + L_i x\ F_j + \varepsilon_{ij}$$

Tabla 1. Descripción de la evolución del olor y el color visual relacionado con los diferentes puntos de la escala de valoración empleada y desarrollada de acuerdo a los resultados del entrenamiento de catadores sobre carne de potro
Table 1. Description of odour and visual colour evolution related to the scores on the scale developed according to the panellists training on foal meat

Atributo		Descripción visual		Valoración (mm)
Color	Característico	Rojo no intenso y brillo moderado (carne fresca)	Rojo Rojo no intenso	0
		Decoloración gradual y pérdida de brillo	Menos rojo	$0 < x \leq 37$
		Ligera tendencia a marrón y pérdida total de brillo	Ligeramente marrón	$37 < x \leq 75$
	Anómalo	Primeras apariciones de zonas ligeramente verdosas	Ligeramente verdoso	$75 < x \leq 112$
		Aumento importante de las zonas marrones y verdes sobre la superficie	Verde y marrón oscuro	$112 < x \leq 150$
Olor	Característico	Ausencia de olor o suave olor a carne fresca		0
		Incremento gradual en la intensidad de olor pero manteniendo la frescura		$0 < x \leq 37$
	Anómalo	Toque picante apreciado con la nariz o la garganta		
		Olor ligeramente ácido		$37 < x \leq 75$
		Incremento gradual del olor picante y ácido (no demasiado intenso)		$75 < x \leq 112$
		Percepción intensa de olor picante y ácido (similar a pan de molde)		$112 < x \leq 150$

Fuente: Ruiz *et al*. (2016). Description of the training methodology employed to describe foal meat attributes.

siendo: Y_{ijk}: La observación del parámetro analizado; μ: Media mínima cuadrada de la población; P_i: Efecto de la maduración en pieza entera. i = 1: 0 días; i = 2: 7 días; F_j: Efecto del tiempo de conservación en filete. j = 1: 0 días; j = 2: 3 días; j = 3: 6 días; j = 4: 9 días; PxF: Efecto de la interacción de los efectos principales; ε_{ij}: Error vinculado a cada observación. Las diferencias significativas (P > 0,05) para el tiempo de conservación fueron comprobadas con el Test de Tukey para un nivel de significación de P > 0,05.

Asimismo, se utilizaron correlaciones de Pearson para conocer la relación entre el conjunto de variables.

Resultados y discusión

Composición química

La composición química del músculo LD se muestra en la tabla 2. En general, los valores medios del contenido de humedad, cenizas y proteína se encuentran dentro del rango de valores descritos previamente por otros autores como Lorenzo *et al.* (2013) en potros de raza Gallego de Monte sacrificados con 15 meses de edad, Polidori *et al.* (2015) en potros Martina Franca sacrificados con 12 meses de edad, o Sarriés y Beriain (2005) en potros de raza Burguete de 16 meses de edad. Destaca el contenido de grasa intramuscular, el cual se aproximó al descrito por Sarriés y Beriain (2005) (3,17 %) y sin embargo, fue muy superior al obtenido por otros autores (Lorenzo *et al*., 2013; Polidori *et al*., 2015) (0,22 % en Gallego de Monte; 1,87 % en Martina Franca, respectivamente). Estas diferencias en grasa intramuscular podrían deberse a la raza (Lorenzo *et al*., 2014). Por otro lado, también es destacable el contenido de mioglobina (mg/g músculo). Dichos valores fueron muy superiores a los obtenidos por Tateo *et al*. (2008) (3,20) y Sarriés y Beriain (2005) (3,51). Según Lorenzo *et al*. (2014) un sistema de producción extensivo ayuda a incrementar el contenido de hierro de la carne de potro. Los animales a estudio permanecieron libres hasta el día previo al sacrificio. Además, su cría en alta montaña (800 msnm), ejercicio y pastoreo, exigen al organismo una oxigenación más alta y dificultosa y por lo tanto da lugar a una mayor cantidad de pigmentos en el músculo (Sañudo, 1993).

Tabla 2. Composición química del músculo Longissimus dorsi procedente de potros de raza Burguete
Table 2. Chemical composition of Longissimus dorsi muscle from Burguete foals

	Valores*
Humedad (%)	71,40 ± 0,81
Ceniza (%)	1,14 ± 0,03
Proteína (%)	23,46 ± 0,91
Grasa intramuscular (%)	5,13 ± 1,15
Hierro hemínico (mg /100 g carne)	1,92 ± 0,31
Mioglobina (mg/ g músculo)	5,67 ± 0,93
Hierro total (mg/ 100 g carne)	2,00 ± 0,19

*Valores indicados como media ± desviación estándar (n = 8).

Efecto de la maduración en la carne de potro

El efecto de la maduración en el músculo LD en pieza entera (P) y el tiempo de conservación en los filetes (F) no mostró ninguna interacción (PxF) (tabla 3). La maduración del músculo LD (P) sólo presentó diferencias significativas en el contenido de colágeno total (P ≤ 0,01). Por el contrario, el tiempo de conservación en los filetes (F) mostró un efecto más evidente en la mayo-

Tabla 3. Efecto del tiempo de maduración del músculo *Longissimus dorsi* madurado en pieza entera 0 y 7 días (M0 *vs.* M7) y tras el fileteado durante 0, 3, 6 y 9 días de tiempo de conservación sobre los parámetros físico-químicos y sensoriales, procedente de potros de raza Burguete

Table 3. Effect of ageing time over the Longissimus dorsi *muscle aged 0 and 7 days (M0* vs. *M7) in whole piece, and after a slicing process, aged 0, 3, 6 and 9 days of storage time on the physic-chemical and sensory parameters, from Burguete foals*

	Pieza entera (P)		Filetes (F)				Pieza entera	Filetes	P x F
	M0	M7	0 días	3 días	6 días	9 días			
Colágeno soluble (%CT[1])	9,46 ± 1,02	10,91 ± 3,05					NS		
Colágeno total (g/100 g carne)	0,58 ± 0,05	0,33 ± 0,02					***		
TBAR'S (mg malonaldehído /kg carne)	0,18 ± 0,01	0,56 ± 0,01	0,37 ± 0,06			1,76 ± 0,24	***	***	NS
MMb%	11,50 ± 4,24	4,55 ± 2,65	8,03 ± 2,66^{a}	67,79 ± 2,12^{bc}	70,09 ± 4,02^{c}	52,35 ± 6,78^{b}	NS	***	NS
L*	35,25 ± 1,04	34,58 ± 0,73	34,92 ± 0,60^{a}	35,57 ± 1,10^{a}	35,17 ± 0,67^{a}	31,53 ± 0,98^{b}	NS	**	NS
a*	10,24 ± 0,42	14,29 ± 0,49	12,27 ± 0,82^{a}	9,09 ± 0,73^{b}	8,14 ± 0,32^{b}	8,44 ± 0,43^{b}	***	***	NS
b*	6,34 ± 0,89	6,52 ± 0,53	6,43 ± 0,48	5,28 ± 0,77	5,56 ± 0,64	8,95 ± 2,04	NS	NS	NS
C*	12,13 ± 0,62	15,74 ± 0,61	13,94 ± 0,79^{a}	10,79 ± 0,80^{ab}	10,03 ± 0,87^{b}	12,89 ± 1,56^{ab}	**	*	NS
h*	31,26 ± 3,74	24,44 ± 1,49	27,85 ± 2,26	38,81 ± 8,54	33,76 ± 3,80	42,41 ± 6,08	NS	NS	NS
Olor característico[2]	37,3 ± 0,27	59,7 ± 0,41	48,5 ± 0,41^{a}	55,5 ± 0,47^{a}	77,0 ± 0,32^{b}	101,5 ± 0,52^{c}	***	***	NS
Color característico[2]	47,9 ± 0,57	44,8 ± 0,26	46,4 ± 0,30^{a}	77,5 ± 0,29^{b}	93,2 ± 0,21^{c}	115,3 ± 0,45^{d}	NS	***	NS

TBAR'S: Thiobarbituric acid reactive substances; MMb%: Metamioglobina; L*: Luminosidad; a*: índice de rojo; b*: índice de amarillo; C*: cromaticidad; h*: valores de tonalidad.

Valores mostrados como media ± desviación estándar (n = 8).

* = P<0,05, ** = P≤0,01, *** = P≤0,001, NS = no significativo.

$^{a, b, c}$: Valores medios dentro de la misma fila con diferentes letras son estadísticamente diferentes (P < 0,05).

[1]%CT: Porcentaje de colágeno total. [2]Escala sensorial: 0-150 mm, desaparición gradual del olor/color característico y aparición de aromas/colores de degradación.

ría de las variables asociadas con los procesos de oxidación y degradación. Este resultado deja ver que la evolución de los parámetros durante el tiempo de conservación seguiría una misma tendencia independientemente del tiempo de maduración del lomo.

Efecto de la maduración del músculo LD en pieza entera

El efecto de la maduración en pieza entera se muestra en la tabla 3. Por un lado, el contenido de colágeno total difiere ($P \leq 0,001$) entre el músculo no madurado (M0) y madurado 7 días (M7). Sin embargo, la maduración no influyó en el contenido de colágeno soluble ($P > 0,05$), ya que se obtuvieron resultados similares. Los resultados de colágeno total mostrados por Badiani *et al*. (1997) se encontraban entre 0,98% y 1,53% en carne madurada 10 y 14 días y procedente de potros sacrificados con 6 y 10 meses de edad; valores muy superiores a los obtenidos en este estudio. Por otro lado, los resultados de colágeno soluble en el LD no maduro (M0) fueron similares a los descritos por Sarriés y Beriain (2005) (10,23 ± 2,06). Es importante indicar que el colágeno es una estructura muy compleja, cuyo estado depende de sus características bioquímicas, polimerización y grado de reticulación, y la naturaleza, tipo y número de uniones (Lepetit y Culioli, 1994). Durante la maduración, se produjo una disminución significativa del contenido total de colágeno, que varió de 0,58 a 0,33 (tabla 3). Esto implicaría un cambio en la disposición de las fibras y en los enlaces que mantienen la estructura de colágeno. Roncalés (2001) indicó que en la carne de vacuno, la maduración no influye sobre el tejido conectivo; y Lepetit (2007) sostuvo que el colágeno en carne de vacuno se degrada después de un proceso de maduración volviéndose más soluble. Por el contrario, en nuestro estudio, la degradación del colágeno en carne de potro durante la maduración podría ser debida a cambios en la fracción insoluble de colágeno. Esos cambios pueden ser causados por factores desconocidos que actúan de manera diferente a los de la carne de vacuno.

En cuanto a los valores de compresión, el músculo no madurado (M0) y madurado 7 días (M7) mostraron diferencias por efecto de la maduración en la compresión al 20% o al 80% con un nivel de significación de $P < 0,1$. La diferente tendencia encontrada entre M0 y M7 aparece representada en las figuras A.1. y A.2. La compresión al 20% está relacionada con la disposición y la fuerza de las miofibras musculares (actina y miosina) (Lepetit y Culioli, 1994). Sin embargo, en el presente estudio, las calpainas y las catepsinas podrían no haber mostrado actividad efectiva durante la maduración y no haber deteriorado las miofibrillas; o quizás, puesto que la carne se considera tierna, ya habrían actuado lo suficiente tras 24 h. Sin embargo, estudios desarrollados en la carne de vacuno (Koohmaraie, 1996) sostienen que la actina y la miosina no se degradan hasta los 14 ó 18 días de maduración, ya que estas proteínas son resistentes a la proteólisis causada por calpainas. Con respecto a la compresión al 80%, los valores obtenidos (2,15 ± 0,67, para M0; 2,86 ± 1,46, para M7) fueron inferiores a los descritos por Sarriés *et al*. (2006) en carne de potro madurada 4 días (3,52 ± 1,49). La compresión al 80% está relacionada con el contenido de colágeno y sus propiedades (Lepetit y Culioli, 1994). En este caso, como la fracción soluble no varía con la maduración, tal vez sea la fracción insoluble la implicada en los cambios de compresión obtenidos.

Hubo un efecto significativo de la maduración en los resultados obtenidos de WBSF con un nivel de $P < 0,1$, y destacan los bajos valores en el músculo LD madurado 7 días (Figura A.2). Estos resultados coinciden con los indicados por Sarriés y Beriain (2006) en

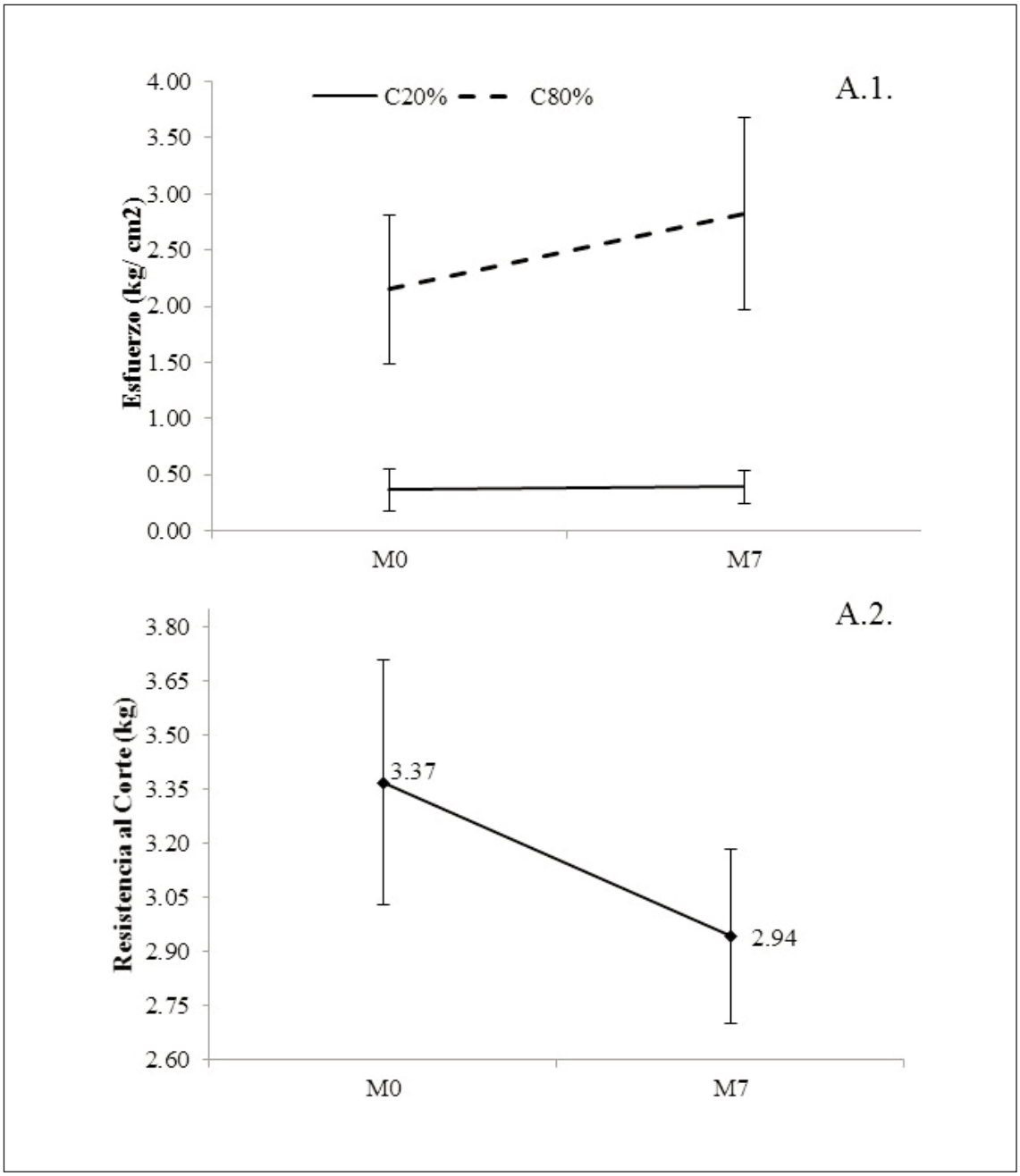

Figura A. Efecto de la maduración en la compresión (C) (A.1) y el la resistencia al corte (A.2) de muestras de carne de potro procedente del músculo *Longissimus dorsi* sin madurar (M0) y madurada 7 días en pieza entera (M7).

Figure A. Ageing effect at compression (C) (A.1) and shear force (A.2) of foal meat samples from the non-aged Longissimus dorsi *(M0) and aged 7 days (M7).*

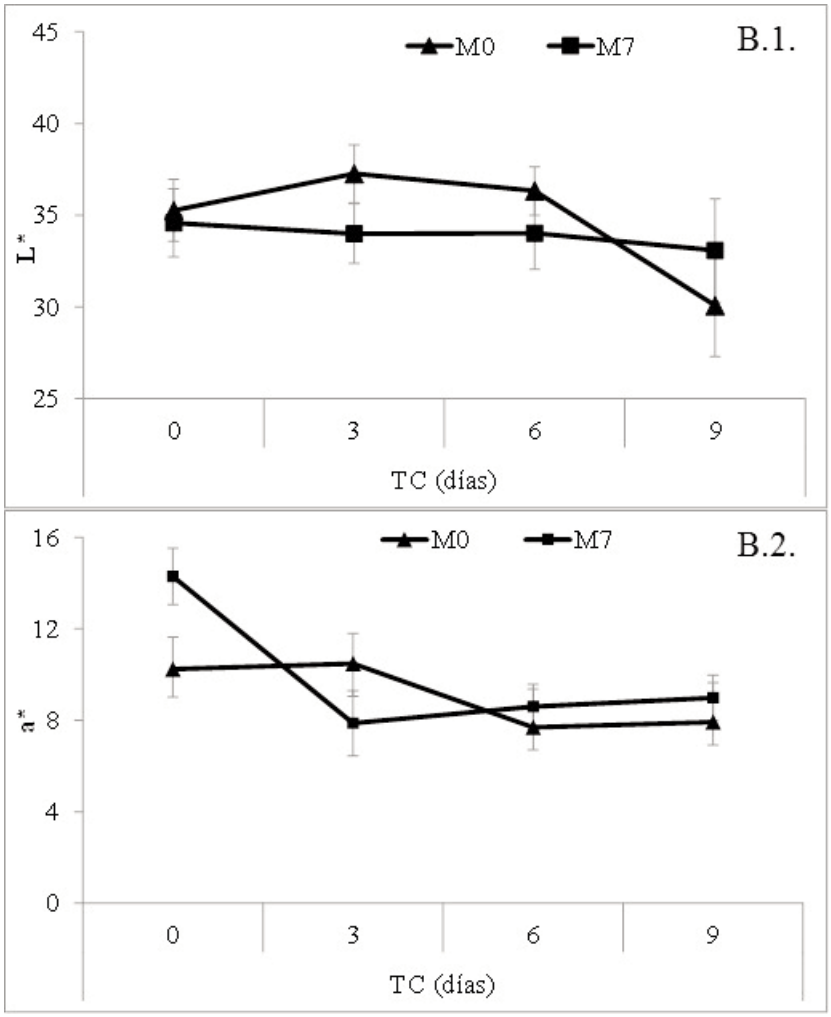

Figura B. Evolución de la luminosidad (L*) (B.1) e índice de rojo (a*) (B.2) en carne de potro fileteada y conservada durante 0, 3, 6 y 9 días, procedente de lomo *Longissimus dorsi* no madurado (M0) y madurado 7 días (M7).
Figure B. Brightness (L) and redness (a*) evolution in foal meat steaks during 0, 3, 6 and 9 days from the non-aged Longissimus dorsi (M0) and aged 7 days (M7).*

carne de potro madurada 4 (4,89 ± 0,88) y 8 días (3,59 ± 1,18), pero fue ligeramente inferior a los descritos por Tateo *et al*. (2008) (5,61) y Lorenzo *et al*. (2013) (3,49 ± 1,86). Bertola *et al*. (1994) sostienen que la terneza de la carne varía en el proceso de cocción, siendo el calor la razón de los cambios en la estructura del colágeno y de las proteínas miofibrilares. De acuerdo con la clasificación de la terneza y a las categorías establecidas por Belew *et al*. (2003), la carne podría considerarse entre "tierna" (3,2 kg < WBSF < 3,9 kg) (M0) y "muy tierna" (WBSF < 3,2 kg) (M7). Según los resultados de textu-

ra obtenidos, la carne de potro utilizada en este estudio ya fue tierna tras solamente 24 horas *post mortem*. Este hecho es de gran importancia a la hora de establecer tiempos de maduración para este tipo de carne.

Con respecto a la oxidación lipídica, la maduración mostró un efecto significativo ($P \leq 0,001$) (tabla 3). Los resultados indicaron un aumento evidente de la oxidación lipídica de 0 a 7 días de maduración. La carne de potro es rica en ácidos grasos poliinsaturados, que son altamente propensos a la oxidación (Tateo *et al*., 2008; Lorenzo y Gómez, 2012). Por lo tanto, el tiempo de maduración podría ser una causa directa de la oxidación de lípidos.

La oxidación de la mioglobina está representada por el contenido de MMb (MMb%) sobre la superficie de la carne (tabla 3). La maduración no mostró influencia sobre la misma ($P \geq 0,05$); aunque puede ser debido a la elevada variabilidad mostrada entre animales (mayor del 30%). Sin embargo, se puede apreciar una clara diferencia en el contenido entre ambos tiempos de maduración (11,50 ± 4,24 en M0 *vs.* 4,55 ± 2,65 en M7) (%). Este hecho podría explicar que un proceso de maduración mantiene la mioglobina en su estado oxigenado durante más tiempo (oximioglobina). Sarriés y Beriain (2006) afirmaron que con un proceso de maduración hay una reducción en la tasa de consumo de oxígeno en la carne; lo que provoca un aumento de la capa de oximioglobina, responsable del color rojo de la carne y su intensidad.

Los valores de las coordenadas de color aparecen en la tabla 3. Al contrario de la mayoría de los parámetros estudiados, hubo una variación muy baja entre muestras para L*, a* y C* (< 5%), pero alta en los parámetros b* y h* (20 - 30%). La maduración no mostró ningún efecto sobre L*, b* y h* ($P > 0,05$), pero sí sobre a* y C* ($P \leq 0,001$). El índice de rojo (a*) y la cromaticidad (C*) aumentaron con los días de maduración. Con respecto a la luminosidad (L*), algunos autores indican valores superiores (Gómez y Lorenzo, 2012; Lorenzo y Gómez, 2012), inferiores (Bingol y Ergun, 2011; Polidori *et al*., 2015) y similares (Sarriés y Beriain, 2006) a los indicados en la tabla 3. Según a* y C*, la variación entre ambos tiempos de maduración (M0 *vs.* M7) fue de 28,34% y 29,76%, respectivamente. Esta variación podría explicar el aumento y la intensidad del color rojo en la carne de potro como consecuencia del proceso de maduración. Este hecho ya ha sido explicado por Sarriés y Beriain (2006) siendo atribuido a la baja tasa de respiración mitocondrial como resultado del almacenamiento refrigerado. La reducción en el consumo de oxígeno hace que el espesor de la capa de oximioglobina aumente (que sea más profundo), y que el color rojo de la carne mejore; pero, en contrapartida, este color rojo se caracteriza por su baja estabilidad. Los valores de índice de rojo (a*) en la carne de potro descritos por otros autores (Lanza *et al*., 2009; Bingol y Ergun, 2011; Lorenzo y Gómez, 2012) fueron similares a los obtenidos en el presente estudio para el músculo LD madurado 7 días (M7). Sin embargo, los resultados obtenidos por Polidori *et al*. (2015) fueron similares a los del músculo no madurado (M0). Por el contrario, estos resultados fueron muy inferiores a los descritos por King *et al*. (2012) (a*: 32-15) o Kurvea *et al*. (2016) (a*: 28,5) en carne de vacuno.

Los valores de olor y color característico aparecen en la tabla 3. La maduración no mostró ningún efecto sobre el color ($P > 0,05$), pero sí sobre el olor ($P \leq 0,001$). Parece ser que la maduración fue importante en la evaluación de este segundo parámetro. Los panelistas describieron que la carne de potro procedente del músculo no madurado (M0) era "carne sin olor". Sin embargo, no describieron lo mismo en la carne madurada 7 días quedando reflejado en una valoración más elevada. En este caso, el olor continuaba siendo a carne fresca, pero lo

percibían de forma más intensa. Aunque no excedió el límite (75 mm), sí se produjo una visible degradación del olor característico. Esto puede estar relacionado con la oxidación lipídica sufrida durante la maduración, ya que mostraron una correlación positiva ($p \leq 0,001$; $r = 0,83$). A pesar de la falta de estudios sobre el proceso de maduración en la carne de potro y su influencia sobre la evaluación sensorial de la misma, Lorenzo y Gómez (2012) mostraron resultados similares en carne de potro conservada entre 7 y 10 días sin sufrir ningún proceso de maduración previo y describieron la carne como "moderadamente aceptable". A pesar de que la maduración sí mostró un efecto sobre el color medido de forma instrumental ($P \leq 0,001$) (tabla 3), el panel entrenado no apreció ninguna diferencia visual entre ambos tipos de carne para el "color característico" entre la carne procedente de M0 y M7, y describiendo su color como "ligeramente marrón y poco brillante".

Efecto del Tiempo de conservación (TC) en filetes

Los resultados obtenidos para los parámetros de calidad de la carne durante los días 0, 3, 6 y 9 de TC se muestran en la tabla 3. La conservación en filetes (TC) mostró un efecto significativo en la oxidación lipídica ($P \leq 0,001$). Esto implicó que los niveles de rancidez aumentaron con la presencia de oxígeno y el tiempo de exposición. Estos resultados coinciden con los descritos por otros autores (Gómez y Lorenzo, 2012; Zakrys *et al*., 2008). Si se extrapola a carne de potro, el valor máximo de oxidación no superó el umbral de rancidez descrito por Campo *et al*. (2006) en carne de vacuno (2,28 mg MDA/ kg carne fresca). Así mismo, Gómez y Lorenzo (2012) observaron que después de 10 días, la carne de potro no alcanzaba el límite mencionado.

El efecto de TC fue significativo ($P \leq 0,001$) sobre el contenido de metamioglobina. Este parámetro aumentó de forma considerable entre el día 0 y 3 de TC (88,02%). Esta variación podría ser la causa del desarrollo de tonos marrones o verdosos en la carne y, por tanto, de la disminución de la estabilidad del color (Sarriés y Beriain, 2006). Además, se describieron correlaciones negativas de MMb% con a* ($P \leq 0,01$; $r = - 0,72$) y positivas de MMb% con el color característico ($P \leq 0,01$; $r = 0,59$). Esto coincide con Zakrys *et al*. (2008) quienes asociaron la presencia de oxígeno con un aumento de oxidación de la oximioglobina y degradación del color.

De acuerdo con las coordenadas de color, el TC mostró un efecto significativo en la luminosidad ($P \leq 0,01$), índice de rojo ($P \leq 0,001$) y cromaticidad ($P \leq 0,05$), pero no en el índice de amarillo y tonalidad ($P \geq 0,05$) (tabla 3). La luminosidad (L*) se mantuvo constante durante los 6 primeros días (35,22) y en ese momento, este valor disminuyó (31,53) y la carne se volvió más oscura. Sin embargo, Zakrys *et al*. (2008) observaron el efecto contrario en muestras de vacuno debido al oxígeno libre. Es probable que este oscurecimiento se produjese debido al alto contenido de hierro hemínico que provoca una baja reflectancia de la luz (Seydim *et al*., 2006). Con respecto al índice de rojo (a*), su valor disminuyó día a día (12,27 el día 0; 9,09 el día 3; 8,14 el día 6; 8,44 el día 9). Otros autores (Bingol y Ergun, 2011; Gómez y Lorenzo, 2012; Leygonie *et al*., 2011; Lorenzo y Gómez, 2012) describieron valores similares con muestras de carne en diferentes condiciones de envasado. También se puede apreciar una relación inversa entre los valores de TBA y la estabilidad del color (valores a*) ($P \leq 0,01$; $r = 0,62$). Esta relación también se corroboró en otros estudios con el empleo de envases enriquecidos en oxígeno (Gómez y Lorenzo, 2012; Kim *et al*., 2010; Lorenzo y Gómez, 2012). Estos resul-

tados indican que el oxígeno libre provocó la disminución de la estabilidad del color y el aumento de la oxidación lipídica (Zakrys *et al*., 2008). Es más, Spanier (1992) afirmó que la cantidad de hierro, el cual actúa como catalizador (proveniente de la hemoglobina, la mioglobina y el citocromo) y la cantidad de hierro hemínico y no hemínico está relacionada con el proceso de oxidación lipídica. Esto podría explicarse por el alto contenido de mioglobina (5,84 mg / g de músculo) y hierro hemínico (1,97 mg / 100 g de carne) de la carne de potro, en este estudio.

Además, se encontraron diferencias con un nivel de significación de $P < 0,1$ entre M0 y M7 durante el TC. La evolución de ambos tipos de carne mostró una tendencia diferente en cuanto a la luminosidad (Figura B.1.) y el índice de rojo (Figura B.2.). Por un lado, los valores de L* en la carne procedente de M0, se mantuvieron constantes hasta el día 6 (36,28) y luego disminuyeron a 30,08 el día 9, tornando más oscuras. Por otra parte, el TC no afectó a la evolución de L* en la carne procedente de M7, manteniendo un valor constante de 33,92. Las muestras de músculo no madurado (M0) sufrieron una variación de 26,62% entre los días 3 y 6 (10,48 *vs.* 7,69). Por el contrario, la variación en las muestras del músculo madurado (M7) fue más evidente (45%) y ocurrió antes, entre los días 0 y 3 (10,48 *vs.* 7,69). Esto significa que el color de la carne madurada durante 7 días tuvo una estabilidad más baja que el color de las muestras que no se maduraron (M0). Esta disminución en el índice de rojo podría estar influenciada por la oxidación de ácidos grasos poliinsaturados propios de la carne de potro, y por el elevado incremento de metamioglobina sufrido durante los 3 primeros días. El índice de amarillo (b*) está relacionado con el color de con la grasa (Franco *et al*., 2011b), pero no contribuye significativamente a la apariencia de la carne (Leygonie *et al*., 2011). Los valores obtenidos fueron inferiores a los descritos en otros estudios (Sarriés y Beriain, 2006; Lanza *et al*., 2009; Gómez y Lorenzo, 2012; Lorenzo y Gómez, 2012) y ligeramente superiores a los citados por Franco *et al*. (2011a) en carne de potro. Los valores de cromaticidad (C*) fueron similares a los descritos en carne de potro por Franco *et al*. (2011b) y más bajos que los indicados por Lanza *et al*. (2009). Por otro lado, sólo los valores de tonalidad (h*) obtenidos en el día 0 de TC fueron similares a los observados en otros estudios (Franco *et al*., 2011b; Lanza *et al*., 2009), ya que tras este día (3, 6 y 9 días de TC) los valores fueron moderadamente superiores.

El tiempo de conservación (TC) presentó un efecto importante sobre el olor y el color característico de la carne de potro ($P \leq 0,001$) (tabla 3). Dicho efecto destacó claramente en la valoración del olor entre 3 y 6 días de TC (55 *vs.* 77 mm). El tercer día de conservación mantenía un olor intenso pero característico. El día 6, se superó el límite establecido (75 mm) percibiendo ligeras sensaciones a picor y acidez. Este punto marca la diferencia entre carne con olor característico y carne con olor que ha dejado de ser característico y se ha degradado. Los ácidos grasos poliinsaturados están muy presentes en la carne de potro y son muy susceptibles a la oxidación (Badiani *et al*., 1997; Sarriés y Beriain, 2006; Tateo *et al*., 2008), siendo este factor una de las causas principales de la degradación del olor y la consiguiente aparición de olores a moho y putrefacción apoyada por la actividad microbiana (Gómez y Lorenzo, 2012). De acuerdo al color característico, el límite fue superado tras 3 días de TC (77,5 mm); momento en el que comenzaron a aparecer las primeras tonalidades verdosas. Por lo tanto, la degradación del color fue muy precoz. Lorenzo *et al*. (2012) y Gómez y Lorenzo (2012) describieron un tiempo de conservación

para carne de potro más largo ya que mantuvieron su color característico hasta 7 días y fue el atributo determinante. Cabe destacar las correlaciones encontradas entre la valoración de color y el MMb%, y a* ($P \leq 0,001$; $r = 0,58$, $r = -0,66$, respectivamente) y L* ($P \leq 0,01$; $r = -0,42$). Esto se tradujo en la degradación del color característico como resultado de una alta oxidación y disminución del índice de rojo y de la luminosidad. En el presente estudio, el atributo sensorial que marcó el máximo tiempo de conservación de la carne de potro fue el color, ya que su degradación fue previa a la del olor e inferior a 3 días.

Conclusiones

En lo que a carne de potro se refiere, un proceso de maduración del músculo LD fuera de la canal puede no ser necesario, ya que a diferencia de otras especies como el vacuno los valores de terneza 24 horas *post mortem* son adecuados. Los valores de los parámetros de oxidación y color limitan la estabilidad de la carne de potro a menos de 3 días y está directamente afectada por el tiempo de maduración. Aunque son necesarios más estudios acerca de la maduración y conservación de la carne de potro, se podría conseguir un tiempo de conservación más largo de carne de potro fileteada, siempre que el oxígeno sea controlado mediante el empleo de técnicas de envasado al vacío o atmósfera controlada/modificada.

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MANUSCRIPT III

Colour and texture evolution of foal meat during the storage time as quality attributes, from animals reared under sustainable conditions.

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Prepared for submision

Colour and texture evolution of foal meat during the storage time as quality attributes, from animals reared under sustainable conditions.

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Short title: Age and diet effect on foal meat quality evolution

Abstract

This research was conducted to study the effect of slaughter age (13 vs. 26-months) and finishing diet (standard vs. linseed) on the colour, texture and oxidation stability of foal meat during storage. Forty-six crossbred foals (Galician Mountain x Burguete genotype) were used. Between both studied factors, the slaughter age was the main factor to difference the animals. The meat from the older foals was darker (L*: 28.90 vs. 32.49; $P < 0.001$), redder (a*: 20.35 vs. 17.59; $P < 0.001$) and tougher (WBSF: 53.27 vs. 45.88 N; $P < 0.05$) than the meat from the younger foals. Optimal tenderness was reached on day 8 by most of the meat samples in the younger group whereas the older group required 12 days. Regarding the oxidative stability, the lowest lipid oxidation values were described in the meat from adult foals. The protein oxidation did not vary significantly ($p > 0.1$) within each studied group throughout the storage time. In conclusion, the crossbreeding Galician Mountain x Burguete allows to obtain heavy carcasses and meat with good quality properties.

Keywords: Foal, slaughter age, linseed, meat quality, storage time

Implications

Foal meat could have an important role in the meat market and be competitive to others kinds of red meat as beef or lamb because of its intrinsic quality properties. Foals production is based on an extensive system which is helpful to preserve the ecosystems and sustainable with the environment. If foal meat was well-defined, well distinguished and there was a good traceability chain, we could provide the market with a natural, healthy and sustainable product.

1. Introduction

Recent studies establish that foal meat quality could be influenced by the livestock production system (Lorenzo *et al.*, 2014), breed and crossbreed (Franco *et al.*, 2013), slaughter age (Domínguez *et al.*, 2015) or finishing diet (Franco and Lorenzo, 2014). Regarding slaughter age, Sarriés and Beriain (2006) described meat from 24-month old Burguete foals to be brighter, redder, and tougher than meat from 16-month old animals. According to finishing diet, 3 kg of commercial supplementation instead of 1.5 kg improved the colour and increased intramuscular fat content fourfold in 18-month old Galician Mountain foals (Franco *et al*., 2013). Linseed-enriched feeds have been studied in beef (Corazzin *et al*., 2012) or lamb meat (Realini *et al*., 2017) due to the improvement in the fatty acid profile, and consequently in meat quality. However, their influence has not been described in foal meat.

It is known that colour stability is essential in determining consumer preferences (Quevedo *et al*., 2013) meanwhile, tenderness is a very important aspect of eating quality and variations affect the decision to repurchase (Bindon and Jones, 2001). The high myoglobin (Ruiz *et al.*, 2018) and PUFA content (Sarriés *et al*., 2006) make foal meat be susceptible to oxidation processes. Thus, colour and texture are important attributes to be studied in foal meat.

Animals from Galician Mountain local breed are sold with 6-8 months of age and dressing percentage lower than 50% (Franco *et al*., 2011). Burguete foals (improved breed) are slaughtered at 16 months of age showing dressing percentage upper than 63% (Sarriés and Beriain, 2005). This study aims to assess the slaughter age and finishing diet effect on colour, texture and lipid and protein oxidation in meat from Galician Mountain x Burguete crossbred foals during the storage period after slaughter.

2. Material and methods

2.1. Animal management

Forty-six Galician Mountain x Burguete crossbred foals were used. In the North of Spain, horsemeat production is based on extensive systems. The foals were kept with their mothers and allowed to suck freely on pasture from the birth to the weaning at the age of 6-7 months (1st period). Next, foals were randomly divided in two groups to be slaughtered at two different ages (**SA**): 22 foals were slaughtered at 13 months (403 ± 30 days) (**13M**) and 24 foals were slaughtered at 26 months of age (784 ± 37 days) (**26M**). Both groups of animals were fed on the same pasture following a rotational grazing (2nd period). This period lasted 3 months (± 15 days) and 16 months (± 15 days) for 13M and 26M, respectively. Finally, foals were supplemented for 104 days (± 10 days) with a finishing diet (**FD**) based on concentrate (3rd period). The vegetation during the three periods aforementioned was composed by seeded (*Lolium perenne* and *Trifolium repens*) and natural fields (*Agrostis* spp., *Lotus corniculatus*, *Holcus lanatus*, *Bromus mollis*, *Pseudoarrenatherum longifolium*, etc.). During the last period (3rd period), 11 foals from 13M group and 12 from 26M group were randomly chosen and supplemented with standard concentrate (**SC**). Then again, the others 11 foals from 13M and 12 from 26M group were randomly chosen and supplemented with linseed-rich concentrate (5%) (**LC**), both concentrates were isoenergetic and isoproteic. Thus, 4 experimental groups were obtained: 13M-SC, 13M-LC, 26M-SC, 26M-LC. Foals were supplemented with 2 kg of the mentioned concentrates to carry out an optimal animals' fattening and to improve the meat lipid profile (linseed supplementation). These supplementations were increased from 0.3 kg to 2 kg per foal/day for the first 10 days (adaptation term). The concentrates composition is shown in Chapter 4, and the animals' production parameters have been already described

(Ruiz *et al*., 2017). The intakes were estimated according to the methodology and equations proposed by NRC (1989):

$$DE\ (Mcal/\ kgDM) = 4.22 - (0.11 \times BP) + (0.0332 \times ADF) + (0.00112 \times ADF^2)$$

$$Intake\ \overline{DE}\ (Mcal/\ foal\text{-}day) = (1.4+0.03 \times \overline{LW}) + (4.81 + 1.17 \times \overline{A} - 0.023 \times \overline{A}^2) \times \overline{ADG}$$

$$Intake\ \overline{DM}\ (kg/\ foal\text{-}day) = [Intake\ \overline{DE}\ (Mcal/\ foal\text{-}day)]/\ [DE\ (Mcal/\ kgDM)]$$

$$Intake\ \overline{DM}\ (\%LW) = [Intake\ \overline{DM}\ (kg/\ foal\text{-}day)/\ \overline{LW}\ (kg)] \times 100$$

where DE = Digestible energy; BP = Brute protein, ADF = Acid detergent fibre; DM = Dry matter; LW = Live weight, A = Age, ADG = Average daily gain. $\overline{DE}$,$\overline{LW}$,$\overline{A}$,$\overline{ADG}$,$\overline{DM}$, average values for 13M and 26M foals.

The estimated $\overline{DM}$ intakes during the 2nd period were 2.18 and 2.37 %LW for 13M and 26M foals, respectively, whereas the estimated $\overline{DE}$ intake was 13.16 and 16.51 Mcal/ foal-day for 13M and 26M foals, respectively. For the finishing period (3rd period), the estimated $\overline{DM}$ intakes (pasture plus concentrate) were 2.80 and 2.59 %LW for 13M and 26M foals, respectively, whereas the estimated $\overline{DE}$ intake were 20.67 and 26.09 and Mcal/ foal-day for 13M and 26M foals, respectively. All estimated intakes fell within the range 1.8-3.5 %LW, reported by Dulphy *et al*. (1997).

Foals were transported 50 km to the abattoir the day before slaughter in compliance with current European regulations (Council Regulation 1/2005EC, 2005), and were stunned with a captive bolt, slaughtered and dressed according to the specifications outlined in the European legislation (Council Directive 93/119/EC, 1993).

2.2. Physic-chemical analyses

Forty-six *Longissimus thoracis et lumborum* (**LTL**) muscles were removed from the left half-carcasses at 24 h *post-mortem*. Chemical composition was determined at 0 days as proposed Domínguez *et al.* (2017) and total and soluble collagen content were determined from the hydroxyproline content (Bonnet and Kopp, 1986). The LTL muscle was sliced into 20 mm (±0.2) thick steaks and (FIRMAQ, V-900, Lorca, Spain) and four steaks per LTL were sent for the storage time study (0, 4, 8 and 12 days). All were vacuum-packed (99%) and kept in refrigerated chambers at 2±1ºC during the corresponding days. Once these elapsed, samples were frozen during 3 months (± 10 days). After that, samples were thawed during the 24 hours prior to colour and texture determination under refrigerated conditions (2±1ºC).

Meat colour was analysed at days 0, 4, 8 and 12. Lightness (L*), redness (a*), yellowness (b*), chromaticity (C*) and hue (h*) (CIE, 1978) were assessed. Samples were allowed to bloom for one hour. Five readings were made after 1 hour of blooming of each steak, varying the angle of observation for each reading, and the average value was used for data analysis. A Minolta CM-2002 spectrophotometer with a D65 illuminant and a standard 10mm aperture. Reflectance values were obtained from the visible spectrum (360-740nm) and used to classify the samples by colour and calculate the proportion of each pigment form. Reflex attenuance is calculated as A = log (1/R), where "R" is reflectance in the interval [0,1]. Relative myoglobin (**DMb**) (%), metmyoglobin (**MMb**) (%) and oxymyoglobin (**OMb**) (%) contents were obtained from the reflex attenuance at the isobestic points 572, 525, 473nm and 700nm according to (AMSA, 2012). In addition, visual colour were described following the methodology developed by Ruiz et al. (2016) which, through the visual colour evaluation of a trained panel, established the relationship between the colour values (L*, a*, b*) and the visual colour evaluation (Table 1), and developed 5 colour patterns linked to 5 states of degradation (Figure 1).

Table 1 *Colour degradation scores and scale position (0-150mm) defined by a trained panel and its relation to the colour values in Burguete foal meat*.

Degradation scores (mm)	Visual colour (mm)	L*	a*	b*
1 (0)	0	37,02	18,03	11,25
2 (0 < x ≤ 37)	34	37,84	16,20	13,02
3 (37 < x ≤ 75)	75	37,10	15,94	12,92
4 (75 < x ≤ 112)	111	32,87	14,99	12,63
5 (112 < x ≤ 150)	138	32,77	13,35	12,31

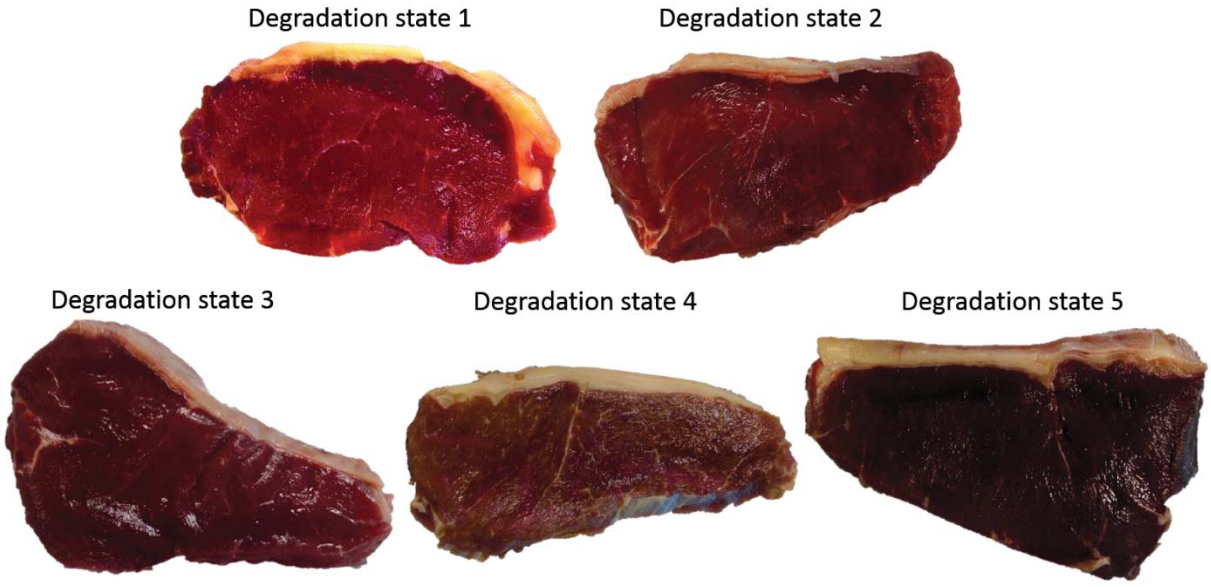

Figure 1 *Visual patterns of foal meat colour corresponding to 5 colour degradation states according to the instrumental colour values (Ruiz et al., 2016).*

Texture were determined at days 0, 4, 8, 12 according to Sarriés and Beriain (2006). The variables considered in raw meat were compression at 20% (**C20**), 80% (**C80**) and 100% (**C100**) (N/cm^2). Vacuum-packaged meat was cooked in a water bath at 75ºC to an internal temperature of 70ºC for 45 minutes. Maximum shear force (N) was assessed using a Warner-

Bratzler shear force device. For both, raw and cooked meat analyses, 8 replicates per sample of 1 cm^2 (square cross section), with muscle fibres parallel to the longitudinal axis of the sample were analysed. A TA-XT2i Stable Micro Systems texturometer was used. Lipid and protein oxidation were determined at days 0, 4, 8 and 12 days. The thiobarbituric acid value (**TBA**) was calculated by distillation (Vyncke, 1975) and the carbonyls content was determined as indicated Vuorela *et al.* (2005).

2.3. Statistical analyses

Statistical analysis was conducted by SPSS (SPSS 23.0, Chicago, IL, USA). Analysis of variance (ANOVA) was performed using the General Linear Model (GLM). The model used to study the effect of slaughter age and finishing diet on meat quality was:

$$Y_{ij} = \mu + SA_i + FD_j + SA_i \times FD_j + \varepsilon_{ij}$$

where: Y_{ijk} is the observed parameter; μ is the minimum mean square error over the population; SA_i is the slaughter age effect (i = 1 denotes 26 months of age; i = 2 denotes 13 months of age); FDj is the finishing diet effect (j = 1 denotes standard concentrate; j = 2 denotes linseed-enriched concentrate); SAxFD is the interaction effect of the i^{th} slaughter age and the j^{th} finishing diet and ε_{ij} is the residual random error associated with the observation. This model was also employed to select the wavelengths used in the discriminant analyses.

The model used to study the effect of finishing diet and storage time on meat quality from 13M and 26M foals was:

$$Y_{ij} = \mu + FD_i + ST_j + FD_i \times ST_j + \varepsilon_{ij}$$

where Y_{ij} is the observed parameter; μ is the minimum mean square error over the population; FD_i is the finishing diet effect (i = 1 denotes standard concentrate; i = 2 denotes linseed-

enriched concentrate); ST_j is the storage time effect (i = 1 denotes 0 days; i = 2 denotes 4 days; i = 3 denotes 8 days; i = 4 denotes 12 days); FDxST is the interaction effect of the i^{th} finishing diet and the j^{th} storage time and ε_{ij} is the residual random error associated with the observation. Significant differences ($P < 0.05$) between storage times were tested using Tukey's test. Correlations between variables ($P < 0.05$) were determined by Pearson's linear correlation coefficient.

Canonical discriminant analysis were performed to classify the samples. The first discriminant procedure examined visible spectroscopy wavelengths. The selected wavelengths were taken from the first analysis of variance mentioned and listed in order of their weight in the discriminant function (based on p-values). Once listed, those wavelengths that were representative for all the significant ranges were selected. The second discriminant analysis was performed on the texture parameters using stepwise model analysis using a significance level of 0.05 as the variable entry criterion. The leave-one-out cross-validation method was used.

3. Results

3.1. Slaughter age and finishing diet effect on foal meat characterization

Table 2 shows chemical composition, colour values, relative pigment percentage and texture parameters according to SA and FD effects. Few interactions (SAxFD) were found ($P < 0.05$) on ash content, b* and h*, C20 and firmness values. Slaughter age was found to have an effect on most of the parameters, whereas FD basically affected colour and cooked-meat texture parameters. In terms of chemical composition, the 26M samples showed lower moisture and more than twice intramuscular fat (IMF) percentages ($P < 0.001$) than the 13M samples. Higher

values of total collagen were found in the 26M than in 13M samples ($P < 0.01$). The soluble fraction (%), however, was higher in the 13M than in the 26M samples ($P < 0.05$).

Table 2 *Effect of slaughter age (SA) (13 vs. 26 months) and finishing diet (FD) (standard vs. linseed) on physical-chemical characteristics of meat from Galician Mountain x Burguete crossbred foals (mean and SEM).*

	Slaughter age		Finishing diet			*Significance*		
	13 months	26 months	Standard	Linseed	SEM	SA	FD	SA x FD
Chemical composition								
pH	5.59	5.62	5.64	5.57	0.02	ns	**	ns
Moisture (%)	74.19	72.33	73.52	73.00	0.32	***	ns	ns
Protein (%)	22.54	22.96	22.62	22.87	0.27	ns	ns	ns
Ash (%)	1.28	1.28	1.32	1.24	0.03	ns	**	***
IMF[1] (%)	0.56	1.72	1.10	1.18	0.18	***	ns	ns
Total collagen (g/100g meat) (TC)	0.36	0.44	0.40	0.40	0.02	**	ns	ns
Soluble collagen (%TC)	4.11	2.39	3.40	3.11	0.81	*	ns	ns
Colour parameters								
L*	32.49	28.90	31.82	29.57	0.78	***	**	ns
a*	17.59	20.35	17.89	20.05	0.65	***	**	ns
b*	10.01	11.49	9.92	11.58	0.79	ns	*	**
C*	20.29	23.46	20.52	23.23	0.91	***	**	ns
h*	29.56	28.79	28.94	29.41	1.25	ns	ns	*
%DMb[2]	25.11	22.36	23.21	24.26	2.72	ns	ns	ns
%MMb[3]	16.93	21.97	18.92	19.99	1.90	**	ns	ns
%OMb[4]	57.95	55.67	57.86	55.76	2.74	ns	ns	ns
Texture parameters								
C20[5]	4.84	5.37	5.30	4.91	0.05	ns	ns	*
C80[6]	29.63	28.84	31.34	27.13	0.25	ns	ns	ns
C100[7]	79.99	78.55	79.65	78.89	0.45	ns	ns	ns
Shear force (N)	45.88	53.27	52.71	46.44	2.99	*	*	ns
Firmness (N/cm^2)	8.79	10.87	10.56	9.09	0.76	**	ns	*

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. ns = non-significant ($P > 0.05$).
[1]IMF: Intramuscular fat; [2]DMb, [3]MMb, [4]OMb: Deoxymyoglobin, metmyoglobin, oxymyoglobin; [5]C20, [6]C80, [7]C100: Compression at 20%, 80% and 100% (N/cm^2).

The colour results showed that the 26M samples showed lower L* and higher a* and C* values than the 13M samples ($P < 0.001$). With regard to the FD effect, the SC samples seemed to

have higher L* and lower a*, b* values than the LC samples. With respect to relative pigment percentages, the only difference in concentration was observed in %MMb and this can be attributed to SA (16.93 for 13M vs. 21.97 for 26M) ($P < 0.05$). Regarding texture, C20, C80, and C100 in raw meat did not change significantly with SA although C80 did show a trend in that direction ($P > 0.10$). Once cooked, however, the 26M samples presented higher shear force (N) and firmness (N/cm^2) values than the13M samples ($P < 0.05$). As for FD effects, the meat from the SC group showed higher in shear force (N) values than that from the LC group.

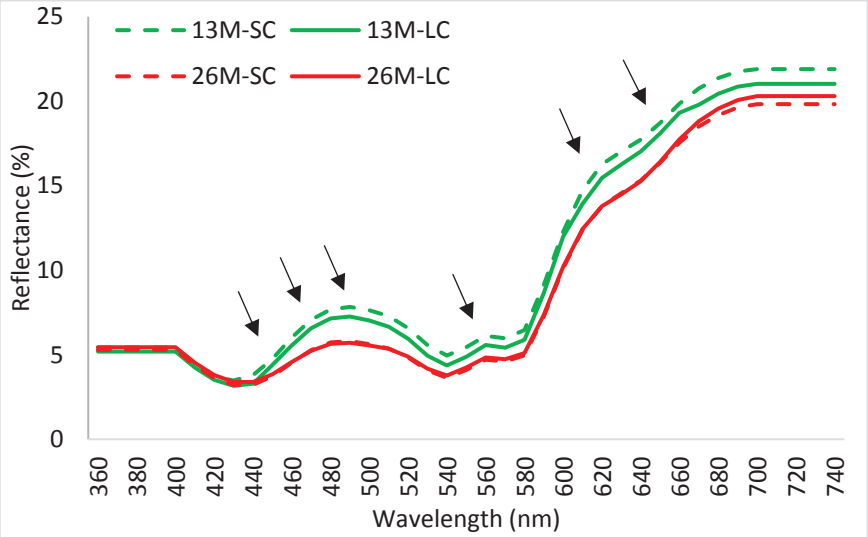

Figure 2 *Mean reflectance in the visible spectrum of Longissimus thoracis et lumborum (24 hours post-mortem) from foals slaughtered at 13 versus 26 months of age (13M, 26M) and finished with standard versus linseed-enriched concentrates (SC, LC).*

Discriminant analysis helped to classify samples by SA and FD using the visible reflectance spectra. The average reflectance (%) spectra obtained for each group of samples are shown in Figure 2. Six wavelengths, 650, 620, 580, 500, 480, 460 nm ($P < 0.001$), were retained for the

stepwise discriminant analysis. The clearest distinction between slaughter-age groups occurred at reflectance points 500 and 460 nm.

Foals slaughtered at 13 and 26 months were classified with 64% and 83% accuracy, respectively. A second discriminant analysis were developed with texture parameters (total and soluble collagen content, C20, C80 and shear force) to classify the samples by SA and FD. Shear force and total collagen were the best parameters classifying by age. In this case, 13M and 26M foals were classified with 82% and 68% accuracy, respectively. Summarising, while the samples were able to be classified by both spectra and texture with an average about 74.0% accuracy regarding SA, no discriminant were possible by FD. Considering these results, meat colour and texture during storage were studied separately in the 13M and the 26M samples.

3.2. Colour, texture and oxidation stability of foal meat during storage

Table 3 summarizes the effect of finishing diet (FD) and storage time (ST) on colour and relative pigment values in 13M and 26M foal-meat samples. High significant interactions (FDxST) were found for colour values in the 13M samples ($P < 0.01$). Both FD and ST had a strong effect on colour values ($P < 0.01$) but not on relative pigment contents ($P > 0.05$). Unlike the 13M samples, the 26M samples showed no significant interactions (FDxST). In the 26M samples, FD was shown to have a significant effect on colour ($P < 0.001$) and %MMb ($P < 0.01$), whereas ST had only a slight influence on redness (a*) ($P < 0.05$).

Colour values in the 13M samples were different on each day of ST (days 0, 4, 8, and 12) ($P < 0.01$) due to FD. The meat from the SC group showed higher L* and lower a* values on day 0, whereas L* value was lower and a* values was higher on day 12 than the meat from the LC

group. With respect to ST effects, the meat from the SC group showed a decrease in L* and an increase in a* and C* ($P < 0.001$), from day 0 to day 12, thus becoming darker and redder. Nevertheless, LC group showed an earlier decrease of a* value ($P < 0.001$), from 0 to 4 days. Regarding 26M foals, the meat from the LC group showed higher values of a*, b*, C* and h* than those from the SC group throughout ST. A slight FD effect on relative pigment content was found in the 13M samples. Those from the SC group showed higher ($P < 0.05$) values of %OMb than those from the LC group on days 0 and 4 (61.67 vs. 54.22: day 0; 61.42 vs. 52.07: day 4, for SC and LC respectively). With respect to ST effects, no significant differences were found; but it is worth highlighting a notable decrease in %OMb from day 4 to day 8 (61.42% vs. 55.49%, respectively) in the SC group, which contrasts with the stability shown by the LC group (51.81%). The 26M samples showed no difference in %OMb ($P < 0.05$), but %MMb was higher in the LC than in the SC group throughout ST ($P < 0.01$).

Table 3 *Effect of finishing diet (FD) (standard vs. linseed) and storage time (ST) (0, 4, 8, 12 days) on colour values and relative pigment content of foal meat from Galician Mountain x Burguete crossbred foals slaughtered at 13 vs. 26 months of age (mean and SEM).*

	Standard concentrate				Linseed concentrate					*Significance*		
	0 days	4 days	8 days	12 days	0 days	4 days	8 days	12 days	SEM	FD	ST	FD x ST
13-month old foals												
L*	33.76^{b}	33.64^{b}	30.54^{a}	29.73^{a}	31.21^{y}	32.17^{y}	35.15^{x}	34.20^{x}	0.61	**	***	***
a*	16.84^{a}	17.76^{a}	20.72^{b}	20.47^{b}	18.33^{y}	16.32^{x}	15.55^{x}	16.03^{x}	0.51	***	***	***
b*	10.32^{a}	11.76^{a}	15.05^{b}	16.72^{b}	9.70^{x}	11.03^{y}	12.77^{z}	12.41^{z}	0.56	***	***	**
C*	19.79^{a}	21.67^{a}	25.62^{b}	26.43^{b}	20.79	19.73	20.17	20.29	0.39	***	***	***
h*	31.38^{a}	33.52^{a}	35.99^{ab}	32.25^{b}	27.73^{x}	33.99^{y}	39.39^{z}	37.72^{z}	0.80	***	***	***
%Mb[1]	21.90	24.97	26.61	24.88	28.32	28.96	27.64	24.22	2.27	ns	ns	ns
%MMb[2]	16.41	15.60	18.22	25.40	17.46	18.96	20.81	24.65	2.09	ns	ns	ns
%OMb[3]	61.67	61.42	55.49	49.72	54.22	52.07	51.55	51.13	2.99	*	ns	ns
26-month old foals												
L*	29.87	28.75	30.58	29.72	27.92	28.03	28.40	27.83	0.89	ns	ns	ns
a*	18.93^{a}	20.96^{b}	19.58^{ab}	20.39^{ab}	21.76	22.50	22.33	23.00	0.87	***	*	ns
b*	9.52	10.63	10.12	10.51	13.46	13.56	13.62	14.16	0.87	***	ns	ns
C*	21.25	23.53	22.05	22.95	25.66	26.32	26.21	27.06	1.17	***	ns	ns
h*	26.49	26.65	27.36	27.22	31.09	30.59	30.91	31.11	0.95	***	ns	ns
%Mb	24.52	25.30	26.12	25.57	20.19	23.90	24.46	26.02	2.18	ns	ns	ns
%MMb	21.42	21.70	21.46	22.92	22.52	25.43	25.80	27.40	1.51	*	ns	ns
%OMb	54.05	53.00	52.41	52.51	57.29	50.67	49.74	46.58	2.68	ns	ns	ns

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. ns = non-significant ($P > 0.05$).

^{abc}: within row and standard finishing diet statistical differences for each predictor variable.

^{xyz}: within row and linseed finishing diet statistical differences for each predictor variable.

[1]DMb, [2]MMb, [3]OMb: Deoxymyoglobin, metmyoglobin, oxymyoglobin.

Table 4 summarizes the effect of FD and ST on texture parameters in 13M and 26M samples. Highly significant interactions ($P < 0.01$) were found (FDxST) for C20 and firmness in the 13M samples. Both FD and ST had an effect on texture values ($P < 0.01$). Contrary to the younger group (13M), no significant interactions (FDxST) were found between the main effects in the 26M samples.

All texture parameters in the 13M samples were only affected by FD ($P < 0.01$) on day 0. With respect to the ST effect, the most noticeable change in the texture variables in both the SC and LC samples appeared between day 0 and day 4, when C20, C80 and shear force decreased ($P < 0.001$). In the 26M samples, the FD effect was again observed only at day 0 of ST ($P < 0.05$), when the shear force and firmness values were higher in the SC samples than in the LC samples ($P < 0.05$). Due to the ST effect, C20 and C80 decreased ($P < 0.01$) from day 0 to day 12; as did the shear force and firmness values ($P < 0.001$).

Table 4 *Effect of finishing diet (FD) (standard vs. linseed) and storage time (ST) (0, 4, 8, 12 days) on texture parameters of foal meat from Galician Mountain x Burguete crossbred foals slaughtered at 13 vs. 26 months of age (mean and SEM)..*

	Standard concentrate				Linseed concentrate					*Significance*		
	0 days	4 days	8 days	12 days	0 days	4 days	8 days	12 days	SEM	FD	ST	FD x ST
13-month old foals												
C20[1] (N/cm^2)	5.65^{b}	2.93^{a}	2.30^{a}	2.24^{a}	4.03^{y}	2.42^{x}	2.33^{x}	2.32^{x}	0.23	**	***	***
C80[2] (N/cm^2)	33.73^{b}	27.23^{ab}	21.45^{a}	26.11^{ab}	25.52^{y}	19.71^{x}	22.13^{xy}	20.17^{xy}	1.19	***	***	**
C100[3] (N/cm^2)	75.97	70.38	73.54	72.75	81.13	74.99	75.46	73.50	0.34	ns	ns	ns
Shear force (N)	53.96^{c}	35.62^{b}	28.07^{ab}	27.92^{a}	52.58^{z}	44.70^{z}	33.03^{y}	29.22^{x}	2.72	**	***	ns
Firmness (N/cm^2)	10.65^{b}	8.65^{ab}	7.36^{a}	7.61^{a}	11.09^{z}	11.41^{z}	7.53^{x}	8.51^{y}	0.70	***	***	**
26-month old foals												
C20 (N/cm^2)	4.95^{b}	2.84^{a}	2.46^{a}	2.12^{a}	5.79^{y}	3.13^{x}	2.52^{x}	2.47^{x}	0.33	ns	***	ns
C80 (N/cm^2)	28.94^{b}	23.47^{ab}	21.28^{a}	23.64^{ab}	28.73	25.19	24.74	22.04	1.20	ns	*	ns
C100 (N/cm^2)	83.33	79.54	75.88	77.85	76.65	68.28	73.06	70.16	0.44	**	ns	ns
Shear force (N)	51.46^{b}	38.51^{a}	32.30^{a}	26.60^{a}	40.31^{y}	37.85^{y}	28.51^{x}	29.29^{x}	2.37	ns	***	ns
Firmness (N/cm^2)	10.48^{b}	8.91^{ab}	6.88^{a}	7.01^{a}	7.10^{y}	9.26^{y}	5.11^{x}	6.83^{xy}	0.56	*	***	ns

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. ns = non-significant ($P > 0.05$).

^{abc}: within row and standard finishing diet statistical differences for each predictor variable.

^{xyz}: within row and linseed finishing diet statistical differences for each predictor variable.

[1]C20, [2]C80, [3]C100: Compression at 20%, 80% and 100%.

The sample distributions by degree of tenderness, based on the ranges suggested by Shackelford *et al*. (1991) ranges, are shown in Figure 3. Of the 13M sample, 45% were rated "tough" and 36 % "intermediate", whereas most (83%) of the 26M samples were rated "tough" at day 0 of ST. At day 4, less than 50% both SA samples were rated "tender". At day 8, 77% of the 13M samples and just over 50% of the 26M samples were rated "very tender". Finally, at day 12, 86% of the 13M samples and 71% of the 26M samples were rated "very tender".

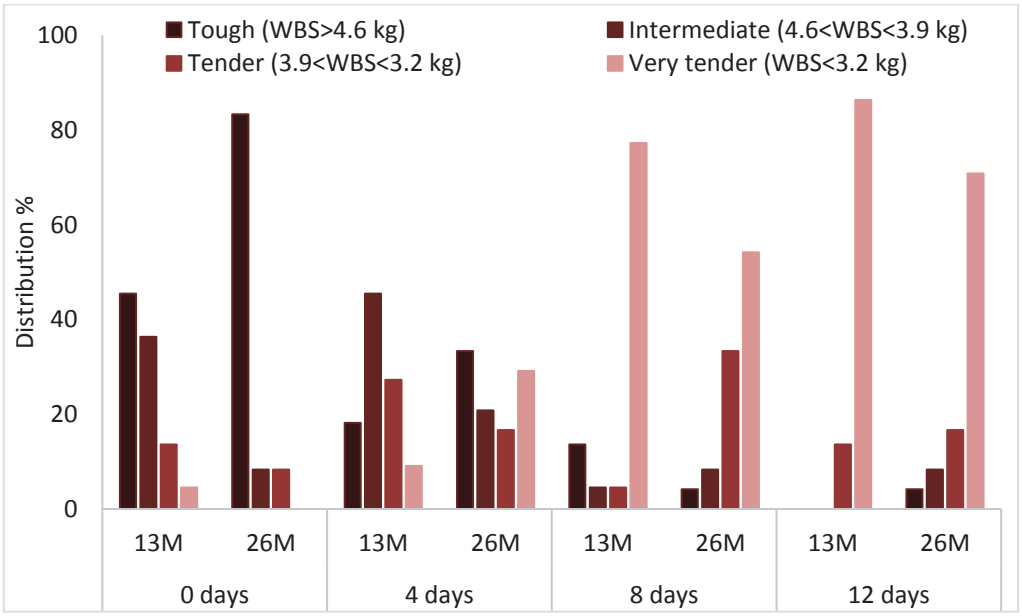

Figure 3 *Percentage distribution (%) of meat at days 0, 4, 8 and 12 of storage time from foals slaughtered at 13 versus 26 months of age (13M, 26M) according to WBSF classification.*

Lipid and protein oxidation are represented in Figure 4 and 5, respectively. Regarding lipid oxidation, 13M samples showed high interaction (FDxST) ($P < 0.01$). Values of TBAR'S were lower ($P < 0.01$) in meat from the SC than in that from the LC group

throughout ST (0.40 vs. 0.43 at day 0; 0.40 vs. 0.47 at day 4; 0.42 vs. 0.51 at day 8; 0.54 vs. 0.64 at day 12 for SC and LC, respectively) (mg MDA/kg meat). Both SC and LC samples were affected by ST ($P < 0.001$). No interaction (FDxST) or significant differences due to FD were shown for 26M samples, whereas ST induced an increase in oxidation ($P < 0.001$) from 0.36 at day 0 to 0.49 at day 12 (mg MDA/ kg meat). According to protein oxidation, carbonyls content were only affected by FD ($P < 0.01$) and not by SL. The meat from 13M foals showed lower values in samples from SC group (3.11 vs. 4.23 for SC and LC, respectively) (nmol/ mg protein) , whereas the meat from 26 M foals, showed higher values in the same samples' group (4.22 vs. 3.43 for SC and LC, respectively). Negative correlation were found in 13M samples between protein oxidation and C80 (r=-0.343; $P<0.01$) and in 26M samples between protein oxidation and shear force (r=-0.22; $P<0.05$).

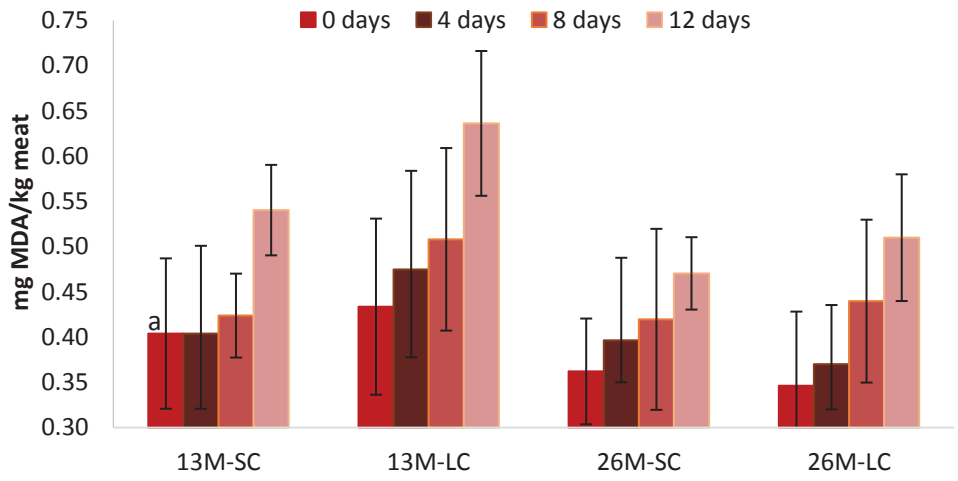

Figure 4 *Lipid oxidation during storage time (0, 4, 8, 12 days) of meat from foals slaughtered at 13 versus 26 months of age (13, 26) and finished with standard (SC) versus linseed finishing concentrate (LC), where "a,b,c,d" and "w,x,y,z" indicate significant differences within SC and LC, respectively.*

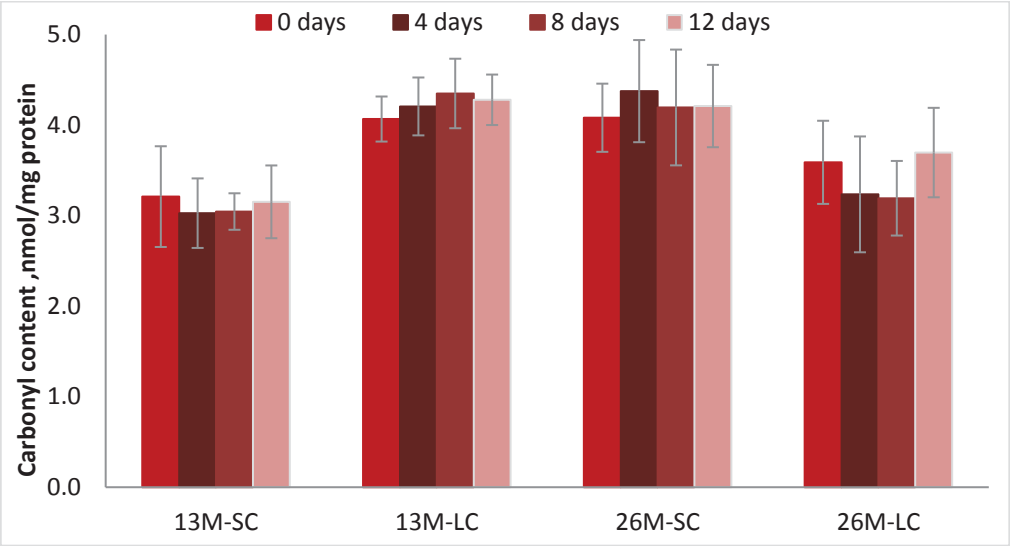

Figure 5 *Protein oxidation during storage time (0, 4, 8, 12 days) of meat from foals slaughtered at 13 versus 26 months of age (13, 26) and finished with standard (SC) versus linseed finishing concentrate (LC).*

4. Discussion

4.1. Slaughter age and finishing diet effect on foal meat characterization

Chemical composition have been recently discussed by Domínguez *et al.* (2017). Briefly, the chemical results were similar to those reported for 15–24 month horse meat samples (Sarriés and Beriain, 2005). However, the same authors reported higher fat values (2.39%). The low IMF value described in the 13M samples might be due to lack of maturity because of the shorter rearing period. For collagen, Sarriés and Beriain (2005) reported soluble collagen values twice as high as those found in this study (10.23) (%). Regarding colour, the 26M samples were darker and redder than the 13M samples. Sarriés and Beriain (2006) also observed an increase in a* value with age, and could be due to the high production of heme pigments and/or physical activity, typical in extensively reared livestock. Both the darkening and the reddening might be related to the longer pasture period, higher grass intake and greater physical activity of the 26M

foals. With regard to the FD effect, the LC samples appeared darker and redder than the SC samples. However, these values deviate from those found in beef by Corazzin *et al.* (2012), or in lamb by Realini *et al.* (2017), who described no effect of linseed supplementation on colour parameters. With respect to relative pigment percentages, Renerre and Mazuel (1985) claim that the 50% of potential beef consumers would reject a product with a proportion of MMb of 20% or over. In this work, the 13M samples did not reach the %MMb threshold whereas the 26M samples are around the 20%. These results bring to light that futures consumers' studies with foal meat are necessary to know the relation between pigments content and the meat acceptability.

Regarding texture, myofibrils and collagen structure in the older foals did not result in higher resistance to compression, as C20 and C80 values did not changed significantly with age. These results depart from those of Sarriés and Beriain (2006), who observed higher compression values due to more resilient muscle structure in 24M than in 16M foal meat. These results coincide with those of Sarriés *et al.* (2006), who reported on the influence of slaughter age on texture parameters in cooked meat. As for FD effects, the meat from the SC group showed higher in shear force (N) values than that from the LC group, in contrast to findings made by Realini *et al.* (2017) who observed no texture change due to linseed enrichment.

For the visible spectra and texture classification, the degree of classification accuracy in these analyses was lower than obtained for lamb (83.3%) (Juárez *et al.*, 2008). Nevertheless, the analyses did show that the 13M samples are very different in colour and texture from the 26M samples. Twenty-four hours *port-mortem*, the 26M samples showed higher intramuscular fat and total collagen content, and were darker, redder and tougher than the 13M samples. This is very important, since colour and texture are

the main attributes driving the purchase decision (Quevedo *et al.*, 2013) and the repurchase decision (Bindon and Jones, 2001), respectively. These findings could therefore be helpful in defining and classifying foal meat by slaughter age.

4.2. Colour, texture and oxidation stability of foal meat during storage

For 13M samples of foal meat, the high significant interaction (FDxST) found for colour values ($P<0.01$) means that the effect of ST in meat from 13M foals varies with the type of finishing regime. Both FD and ST had a strong effect on colour values but not on relative pigment contents. Vacuum packaging may have affected these results, despite colour having been measured after 1 hour of blooming.

The high lightness and redness variations in 13M samples of foal meat due to both FD and ST effects do not agree with other authors (Gómez and Lorenzo, 2012) who described a continuous increase in redness in meat from 15M foals from 0 days ($a^*=15.53$) to 14 days of ST ($a^*=20.24$). Regarding 26M foals, redness values from both FD groups were all higher than those reported by Gómez y Lorenzo (2012) (17.03) in meat from Galician Mountain foals. The results obtained showed a low FD and ST effect and an unclear trend in the relative pigment contents among samples as well for both 13M and 26M samples of foal meat. Storage in vacuum packaging reduces susceptibility to oxidation (dos Santos *et al.*, 2015) and could account for the lack of variation in pigment content. Despite the values of metmyoglobin around 20% of the 26M samples throughout ST, recent studies in beef claim that meat with a^* values equal to or above 14.5, would be acceptable to 95% of consumers (Holman *et al.*, 2017). Thus, based on the colour values found in this study, meat from both 13M and 26M foals would be

acceptable for a storage period of more than 12 days. In addition, these results show that colour changes are not entirely due to myoglobin forms. This is in line with findings by Hernández *et al.* (2016) who state that colour and pigment parameters are not equivalent but complementary. These results bring to light that futures consumers' studies with foal meat are necessary to know the relation between pigments content and the meat acceptability.

Regarding the relationship between the colour values with the visual colour, the values of lightness (L*) of meat from 13M foals, would correspond to states 3 and 4 (characteristic/non-characteristic colour) (37.10 > L* > 32.87); whereas L* values of meat from 26M foals, would belong to state 5 (non-characteristic colour) (32.77 > L*) ((Ruiz et al., 2016) throughout the entire storage time. This fact could be related to the oxygen absence under vacuum conditions. Nevertheless, redness (a*) is considered the best predictor parameter of foal meat colour (Ruiz *et al*., 2015), and the values were, during the whole storage time, over than 17.0. In this case, would belong to states 1 (a* > 18.03) or 2 (18.03 > a* > 16.20), being the colour defined as characteristic (Figure 1). According to yellowness (b*), it is very difficult to describe the colour degradation states of foal meat as b* behaviour is complex and the variation obtained by Ruiz *et al*. (2016) did not showed clear trends. Thus, following the foal meat description designed by Ruiz *et al*. (2016) and already employed by Ruiz *et al*. (2018), colour kept "Characteristic" throughout the storage time and described as "intense red, with low lightness and with a slight brownish trend".

Regarding texture, the interactions found (FDxST) for C20 and firmness in the 13M samples means that the effect of ST on texture appears to vary with the finishing diet, whereas these mentioned effects had an independent behaviour in the 26M samples.

The decrease in compression in both the 13M and 26M raw meat samples between day 0 and day 4 of ST signalled high calpain and cathepsin activity and myofibril deterioration (Lepetit and Culioli, 1994). However, from day 4 to day 12, both the 13M and 26M samples showed a low tenderization. Although this process evolved at a similar rate in the 13M and 26M samples, the former were quicker to achieve higher tenderness, perhaps due to the differences in total and soluble collagen content. According to tenderness classification, meat from both 13M and 26M foals should be stored for a minimum of: 4 days to improve tenderness, 8 days in order to allow more than 50% of the samples to classify as "very tender" and 12 days to get more that 70% of the samples classified in this same category. A minimum storage period of 4 days for foal meat was described by Sarriés and Beriain (2006) describing it as tough and unsuitable for consumption.

The results for lipid oxidation show that the linseed supplementation induce a high lipid oxidation (more obvious in the younger group). Despite the effect of ST showed a similar trend in both 13M and 26M samples, meat from 13M foals seemed to be lower stable than meat from 26M foals. This fact could be related to the longer grazing period of the older animals and the natural antioxidants from pasture (Sarriés and Beriain, 2005). Although there is a lack of information about the effect of linseed supplementation in foal meat, it is known that linseed fatty acids in beef, increase susceptibility to oxidation and loss of colour stability (Faustman *et al.*, 2010). The rancidity threshold (2 mg MDA/kg fresh meat) reported by Campo *et al.* (2006) in beef was not exceeded in either the 13M or the 26M samples. Lipid oxidation, however, was higher than reported by Gómez and Lorenzo (2012) in foal meat (from 0.12 in day 0, to 0.18 at day 12).

Far from TBAR'S results, protein oxidation did not change noticeably. The carbonyls content trend was opposite according to the FD; in the youngest group the oxidation increased with the linseed supplementation, in contrast to what happened in the oldest one. All values are in line with those from Gómez and Lorenzo (2012) (2.09-4.06 nmol carbonyl/mg protein) in the same conditions. The low variability throughout the SL could be due to the vacuum packaging, which help to reduce any oxidation process (dos Santos *et al.*, 2015). In general, in non-oxidized muscle tissue, the carbonyl content is 1 nmol/mg protein whereas in oxidized tissue is in the range 2 to ~14 nmol/mg protein (Lund *et al*., 2007). In the present work the carbonyl mean value was approximately 3.75 nmol/mg protein, indicating that vacuum package could not avoid protein the oxidation process in the foal meat during storage.

5. Conclusions

From the commercial perspective, it is necessary to offer consumers meat in which the essential colour and texture meat properties are well-defined. Slaughter ages of 13 and 26 months produce meat with different quality characteristics and both maintain acceptable colour and lipid stability during 12 days of storage. Eight days and 12 days, respectively, are required for the majority of the 13-month and 26-month samples to reach an acceptable degree of tenderness (a "very tender" rating). The most obvious change due to linseed supplementation is an increase in lipid oxidation, most noticeably in the meat from the younger foals. On the contrary, protein oxidation behaviour is not so clear and furthered studies must be deeper studied.

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MANUSCRIPT IV

Effect of slaughter age and finishing diet on sensory evaluation and consumers' preference of foal meat

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Effect of slaughter age and finishing diet on sensory evaluation and consumers' preference of foal meat

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Running title: Sensory evaluation of foal meat

Abstract

This study focused on the sensory evaluation and consumer preferences of foal meat according to the age of slaughter of the animals (13 and 26 months) and the finishing diet (standard and flax concentrate). It was conducted by a trained panel of 10 members and a hedonic panel of 474 consumers. Consumers in Pamplona and Ourense were assigned different scenarios depending on the amount of product information disclosed. The trained panel described the meat of 13 and 26 month old foals in a similar way. On the contrary, consumers considered the meat of the younger group to be juicier and more tender, with juiciness and tenderness being the most important keys to its overall acceptability. In addition, the information had a positive effect only on the assessment of Pamplona's consumers. Therefore, the city and the level of information are essential factors in introducing foal to the market. The lack of knowledge of colt meat and its limited presence in the meat markets makes it necessary to develop more sensory studies, to obtain patterns of description and to know more deeply the tastes of consumers.

Keywords: Foal meat, slaughter age, linseed, consumers, information level

1. Introduction

The interest in foal meat has increased notably due to the healthy characteristics declared by different authors (Lorenzo et al., 2014). These nutritional properties are in line with recent health recommendations regarding the consumption of fatty acids and lipids, and make colt meat a potential alternative in different national markets (Domínguez et al., 2017). In addition, horsemeat production is based on an extensive production system that favours the maintenance of the environment and the ecosystems that support sustainable agriculture (Belaunzaran et al., 2015).

There are many studies that deal with extrinsic attributes as indicators of meat quality (Lusk and Fox, 2001). Nevertheless, slaughter conditions or type of production system are two of the most important factors for consumer decisions in different regions of Spain (Olaizola et al., 2005). Production factors such as breed/crossbreed (Franco et al., 2013), slaughter age and finishing diet (Sarriés and Beriain, 2006), and *post-mortem* factors such as storage time (Lorenzo and Gómez, 2012), have been studied, but not from the consumer's point of view.

Morales et al. (2013) confirmed that there are predominant intrinsic attributes for consumers to assess the quality of beef, namely tenderness, juiciness and flavour, which are the most important acceptance factors after colour. In foal meat, there are some studies that describe this type of meat using a trained panel (Franco et al., 2013; Sarriés and Beriain, 2005). Nevertheless, there is a lack of research on foal meat and consumer preferences or sensory acceptance. These issues are fundamental to understanding the potential market for foal and to be able to offer the market a well-defined product. In addition, more recent studies have attempted to explore hedonic scores by considering different levels of product information (Beriain et al., 2016; Realini et al., 2014)

Thus, the objective of this work was to evaluate the sensory quality of foal meat from animals slaughtered at 13 and 26 months of age and supplemented with standard or linseed enriched concentrate (5%) using a trained and a consumer panel in two different cities (Pamplona and Ourense).

2. Materials and methods

2.1. Animal management and sampling

Forty-six Galician Mountain x Burguete crossbred foals were used. The foals were kept with their mothers and allowed to suck freely on pasture from the birth to the weaning at the age of 6-7 months. Next, foals were randomly divided in two groups to be slaughtered at two different ages (SA): 22 foals were slaughtered at 13 months (403 ± 30 days) (13M) and 24 foals were slaughtered at 26 months of age (784 ± 37 days) (26M). Both groups of animals were fed on the same pasture following a rotational grazing. This period lasted 3 months (± 15 days) and 16 months (± 15 days) for 13M and 26M, respectively. Finally, foals were supplemented for 104 days (± 10 days) with a finishing diet (FD) based on concentrate. During the last period (3rd period), 11 foals from 13M group and 12 from 26M group were randomly chosen and supplemented with standard concentrate (SC). Then again, the others 11 foals from 13M and 12 from 26M group were randomly chosen and supplemented with linseed-rich concentrate (5%) (LC). Foals were supplemented with 2 kg of the mentioned concentrates to carry out an optimal animals' fattening. These supplementations were increased from 0.3 kg to 2 kg per foal/day for the first 10 days (adaptation term). The intakes were estimated according to NRC (1989). The pasture estimated (DM) intakes were 2.18 and 2.37 %LW for 13M and 26M foals, respectively, whereas the estimated (DE) intake was 13.16 and 16.51 Mcal/ foal-day for 13M and 26M foals, respectively. For the finishing period, the estimated (DM) intakes (pasture plus

concentrate) were 2.80 and 2.59 %LW for 13M and 26M foals, respectively, whereas the estimated (DE) intake were 20.67 and 26.09 and Mcal/ foal-day for 13M and 26M foals, respectively.

Foals were transported 50 km to the abattoir the day before slaughter in compliance with current European regulations (Council Regulation 1/2005EC, 2005), and were stunned with a captive bolt, slaughtered and dressed according to the specifications outlined in the European legislation (Council Directive 93/119/EC, 1993).

2.2. Physic-chemical analysis

The physic-chemical composition (pH, moisture, protein, intramuscular fat) has already been published joined to the cooking losses (Domínguez et al., 2017). The intramuscular fat content was quantified according to ISO 937:1978 (ISO, 1978) by gravimetric difference (Pateiro et al., 2013) and shear force (WBSF) was determined according to the methodology followed by Sarriés and Beriain (2006) using a Warner-Bratzler (N) shear force device. These data are described in Table 1.

Table 1. *Chemical composition of foal meat from animals slaughtered at 13 and 26 months of age and supplemented with standard and linseed feed (mean ± standard deviation).*

	Slaughter age		Finishing diet	
	13 months	26 months	Standard	Linseed
pH	5.59 ± 0.21	5.62 ± 0.07	5.64 ± 0.18	5.57 ± 0.11
Moisture (%)	74.19 ± 0.68	72.33 ± 1.37	73.52 ± 1.56	73.00 ± 1.27
Protein (%)	22.54 ± 0.89	22.96 ± 0.91	22.62 ± 0.99	22.87 ± 0.84
Intramuscular fat (%)	0.56 ± 0.33	1.72 ± 0.73	1.10 ± 0.63	1.18 ± 0.99
Cooking loss (%)	21.7 ± 2.86	22.7 ± 2.16	23.0 ± 2.86	21.4 ± 1.96
Shear force (N)	45.88 ± 5.73	53.27 ± 5.71	52.71 ± 4.99	46.44 ± 6.73

2.3.Trained Panel

Prior to the sensory evaluation, samples were thawed at 4ºC for 24 hours. Steaks were cooked in a convection oven at 180ºC to an internal temperature of 70ºC, which was measured using a handheld probe thermometer (HI-985011, Hanna Instruments, Spain). The cooked foal steaks were cut into pieces (10×10×25 mm) and placed on white plastic trays covered with aluminium foil, and immediately served. The taste panel evaluation was conducted by ten panellists trained and selected according to ISO 8586:2012. The panellists completed 120 hours of training (training sessions were run once a week and lasted about 90-100 minutes), and all of them had a minimum of 2000 hours of sensory testing experience. Panel members were situated in a private red-lighted cabinet during sessions (ISO 8589:2007). The red light was used in order to mask appearance characteristics of products because in the present study they are not variables to evaluate in the product, as appearance or colour.

The study focuses on the variables related to odour, flavour and texture. It was used the xlstat-sensory software which allows to search the optimal design. The experimental design was a randomized block completed with 10 panellist, six sessions and 4 products. In a complete block, all levels of the interest factors are present once within each block, so all products are seen once by each judge. Each sample was evaluated six time by each panellist. To avoid first sample effects the experimental design, that is the order of sample presentation, was rotated and balanced to ensure that all samples were treated in the same way and thus, the test was fair. Carry over effects were measured by Xlstat software. The design was balanced to minimize first-order and carry-over effects (Macfie et al., 1989).

Water to clean the palates and remove residual flavours was given to them at the beginning of the performance and in between samples, which were individually labelled with three-digit aleatory numbers and were randomly served one at a time. A 10 cm unstructured scale was

used, were 0 was “absence/the lowest intensity of the attribute” (left side) and 10 was “the highest intensity of the attribute” (right side). Then, each one was measured using a 10 cm ruler to score it from 0 to 10 points. Pondered variables: characteristic odour intensity (0=low intensity; 10=very intense), and characteristic flavour intensity (0=low intensity; 10=very intense), fat odour intensity (0=low intensity; 10=very intense), fat flavour intensity (0=low intensity; 10= very intense), tenderness (0=very tough; 10=very tender), juiciness (0=very dry; 10=very juicy), fibrousness (0=slightly fibrous; 10=very fibrous) and greasiness (0=slightly greasy; 10=very greasy.

2.4.Consumer panel

2.4.1. Screening of hedonic panel and design of information

The study was carried out with 474 individuals from two different Spanish cities: Pamplona, Northern Spain (n = 247) and Ourense, North-West Spain (n = 227). Thus, two samples per LTL muscle were employed; one in each city. Taking into account the methodology followed by Beriain et al. (2016), the distribution of means in the random, age-stratified sample of 474 consumers resembled that of the population of the regions selected according to gender, age, education and household income level (Spanish National Institute of Statistics, 2015). The non-experienced consumers were contacted by telephone and offered a small monetary incentive in exchange for their participation in the survey as it was carried out by the aforementioned authors.

The balanced distributed sessions were of two types, differing in the experimental marketing scenario and amount of information disclosed (Beriain et al., 2016): Scenario 1 = blind (B) (just saying that meat samples were foal meat); Scenario 2 = full information (F) including details on: (1) geographical breed origin (Galician Mountain x Burguete crossbred, autochthonous

breeds from Galicia and Navarra, respectively); (2) the location of rearing and slaughtering: Galicia. Thus, the impact of product information absence or presence on the consumer ratings was researched. Consumers tested the product after being informed according to their assigned information scenario (B or F). Due to the lack of foal meat sensory references, some questions were based on beef researches related to the consumer process evaluation and the main intrinsic and extrinsic sensory attributes (Beriain et al., 2016)

2.4.2. Taste sample preparation

The methodology used for taste panels was described by Beriain et al. (2014). The frozen steaks were thawed at 1ºC for approximately 24 hours and cooked on a grill (Magefesa, Spain) according to AMSA (2015) guidelines until they reached 70ºC of internal temperature. Nine tasting sessions, each involving 25–30 consumers, were carried out in each city. For each session, three steaks of 2.5-cm-thick per treatment (from different carcasses) were cooked in each session (12 steaks [4 treatments x 3 steaks/treatment]). Whole steaks were cut into 1.5 x 2 x 1.5 cm cubes and kept in heat-retaining containers until the moment of serving. A total of four samples of meat from LTL were randomly and individually presented at each consumer. Participants were asked to evaluate the samples in the order they were served, which was previously designed to avoid the order of presentation effect and first order and carry over effects (Beriain et al., 2014; Macfie et al., 1989)

The testing panel was asked to the aroma, flavour, tenderness, juiciness, sweetness and their overall acceptance of the samples. A 9-point hedonic scale was used, being 1 = “Dislike extremely”, 2 = “Dislike very much”, 3 = “Dislike moderately”, 4 = “Dislike slightly”, 5 = “Neither like nor dislike”, 6 = “Like slightly”, 7 = “Like moderately”, 8 = “Like very much” and 9 = “Like

extremely". Before tasting each sample, consumers were asked to eat some unsalted toasted bread and then rinse their mouths out with water.

2.5.Statistical analysis

The data collected from the taste panels were analysed by the method used previously by Beriain *et al*.(Beriain et al., 2016) That is, the same model was applied for all sensory attributes. An analysis of variance (ANOVA) using the General Lineal Model (GLM) procedure was performed for all variables considered in the study (SPSS 23.0, Chicago, IL, USA).

Firstly, for the trained panel evaluation, the effect of slaughter age and finishing diet were included in the first model. The model used was:

$$Y_{ij} = \mu + SA_i + FD_j + SA_i \times FD_j + \varepsilon_{ijk}$$

where, Y_{ij} is the observation of dependent variables, μ is the overall mean, SA_i is the effect of slaughter age (i = 1 denotes 13 months of age; i = 2 denotes 26 months of age), FD_j is the effect of finishing diet (j = 1 denotes standard concentrate; j = 2 denotes linseed-enriched concentrate), $SA_i \times FD_j$ is the effect of the interaction between the i^{th} slaughter age and the j^{th} finishing diet and ε_{ijk} is the residual random error associated with the observation.

Secondly, for the consumers' evaluation, the effect of slaughter age, finishing diet and information scenario were included in the model. The model used was:

$$Y_{ijk} = \mu + SA_i + FD_j + S_k + SA_i \times FD_j + SA_i \times S_k + FD_j \times S_k + \varepsilon_{ijkl}$$

where, Y_{ijk} is the observation of dependent variables, μ is the overall mean, SA_i is the effect of slaughter age (i = 1 denotes 13 months of age; i = 2 denotes 26 months of age), FD_j is the effect of finishing diet (j = 1 denotes standard concentrate; j = 2 denotes linseed-enriched concentrate), S_k is the effect of information scenario (k = 1 denotes blind information; k = 2 denotes full information), $SA_i \times FD_j$ is the effect of the interaction between the i^{th} slaughter

age and the j^{th} finishing diet, $SA_i \times S_k$ is the effect of the interaction between the i^{th} slaughter age and the k^{th} scenario, $FD_j \times S_k$ is the effect of the interaction between the j^{th} finishing diet and the k^{th} scenario and ε_{ijkl} is the residual random error associated with the observation.

In addition, Pearson's correlation test between the mean values of the meat quality attributes of the foal meat were determined. Principal component analysis (PCA) was applied to study the relationship between the chemical composition and sensory variables of both, the trained panel and consumers' evaluation. Varimax rotation was applied to the factors to facilitate interpretation and maximize the explained variance.

3. Results and discussion

3.1. Sensory evaluation of the trained panel

Table 2 summarizes the effect of slaughter age and finishing diet on the sensory attributes assessed by the trained panel. Both SA and FD showed a minor influence on them. Meat from 13M and 26M groups showed similar intensity values for the attributes of flavour and tenderness, reaching average scores of 5.84 and 4.61, respectively. They also showed similar intensity values for the variables of fat odour, taste and greasiness, which reached low scores (2.91, 2.78 and 2.44 on average, respectively). Significant interactions were found between the main categories (SA x FD) for odour ($P < 0.05$) and fibrousness ($P < 0.01$) attributes. On the other hand, samples from the 13M group had the highest odour scores due to linseed supplementation (5.81 vs. 5.26, for the LC and SC groups, respectively), while samples from the 26M groups showed the opposite behaviour (6.02 vs. 6.24, for the LC and SC groups, respectively).

Table 2. *Trained panellists' scores for sensory attributes. Effect of slaughter age (SA) (13 vs. 26 months) and finishing diet (FD) (standard vs. linseed-rich concentrate) (mean and SEM).*

	Slaughter age		Finishing diet			*P-value*		
	13 months	26 months	Standard	Linseed	SEM	SA	FD	SA x FD
Characteristic odour	5.54±0.47	6.13±0.23	5.76±0.53	5.92±0.37	0.121	0.001	0.348	0.027
Characteristic flavour	5.85±0.32	5.83±0.44	5.81±0.37	5.87±0.39	0.122	0.915	0.727	0.300
Fat odour	3.03±0.55	2.79±0.37	2.89±0.51	2.93±0.45	0.122	0.159	0.789	0.249
Fat flavour	2.69±0.42	2.87±0.55	2.74±0.52	2.81±0.46	0.120	0.320	0.687	0.725
Tenderness	4.56±0.71	4.45±0.63	4.31±0.94	4.70±0.65	0.153	0.600	0.077	0.907
Juiciness	3.74±0.59	4.11±0.66	3.67±0.63	4.19±0.65	0.131	0.041	0.004	0.190
Fibrousness	5.01±0.74	4.98±0.65	5.11±0.40	4.88±0.51	0.141	0.869	0.259	0.007
Greasiness	2.47±0.44	2.40±0.46	2.43±0.54	2.44±0.34	0.112	0.645	0.957	0.054

With regard to fibrousness, its score decreased due to linseed supplementation in samples from 13M group (5.40 vs. 4.62, for SC and LC groups, respectively), whereas it increased in samples from 26M groups (4.82 vs. 5.14, for SC and LC groups, respectively). According to the slaughter age, the odour ($P < 0.001$) and juiciness ($P < 0.05$) attribute scores of the 13M samples were lower than those obtained for the older animals. These results disagree with the data reported by Sarriés and Beriain (2005), who described that 16M foal meat was juicier than that of 26M foals, while the odour was similar in both age groups. The higher scores for the odour and juiciness found in the 26M group samples compared to those obtained in the 13M group may be due to the higher intramuscular fat content of the older animals (1.72% vs. 0.56%, for 26M and 13M groups, respectively) (Domínguez et al., 2018). Franco et al. (2011) suggested that animals slaughtered at an early growth period have a low level of maturity and do not develop adipose tissue because they transfer energy into the muscle, while older animals transfer energy into adipose tissue. In addition, significant positive correlations were found between intramuscular fat content and odour and juiciness attributes ($r=0.18$, $r=0.39$; $P < 0.05$). Regarding the finishing diet, only the juiciness showed significant differences ($p < 0.01$), presenting higher values in LC than in the SC group. This fact could be related to lower

values in the cooking losses of samples of foals supplemented with linseed (23.0 vs. 21.4, for the SC and LC group, respectively) (Table 1), as also indicated Domínguez et al., (2018).

The negative correlation found between WBSF values and tenderness should be highlighted ($r=-0.30$; $P < 0.05$), meaning that higher WBSF values lead to lower tenderness scores. This result is in accordance with those reported by Franco et al. (2011) in foals slaughtered at 9 and 12 months of age. Nevertheless, although the WBSF values were different between the 13M and 26M samples (Table 1), the trained panel was not able to appreciate any difference according to tenderness.

3.2. Consumer panel information and sensory evaluation

Table 3 describes the sociodemographic consumer profiles in the two cities and the information scenarios according to gender, age, education and incomes. An equalized percentage of men and women was reached in both cities, more than 65% of them between 20 and 50 years old. The majority of the tasters (higher than 70%) had a university degree and around 40% showed incomes from 1.500 to 3.000€.

Table 3. *Market experiment design. General description of the consumer sample by information scenario in two Spanish cities (mean percentages).*

	Gender		Age (years)				Education			Income			
	Male	Female	20 - 34	35 - 50	51 - 65	65 <	High	Secondary	Elementary	Low	Modest	Medium	High
Pamplona													
Blind (n=125)	56.67	43.33	24.59	39.34	22.13	13.93	70.25	23.97	5.76	2.65	16.81	44.25	36.28
Full (n=122)	43.97	56.03	49.14	31.03	17.24	2.59	76.07	17.09	6.84	3.66	7.32	45.76	35.69
Ourense													
Blind (n=118)	50.43	49.57	47.46	33.05	15.25	4.24	75.42	16.10	8.47	5.54	27.69	46.16	20.31
Full (n=109)	43.93	56.07	55.05	28.44	13.76	2.75	71.96	19.63	8.41	5.05	38.39	45.46	14.14

In general, meat consumption was relatively high, with over 70% of consumers eating chicken, pork and beef meat once per week or more. Nevertheless, over 55% and 85% of consumers in Pamplona and Ourense, respectively, never consume foal meat. Because of this high difference, the sensory study in Pamplona was analysed separately from Ourense.

The three fixed effects studied were: age at slaughter (13M vs. 26M), completion diet (SC vs. LC) and the information scenario (B vs. F) (Table 4) on the attributes assessed by the panelists. No significant interactions were found between the main factors ($p > 0.05$). This fact has recently been reported by Ruiz et al. (2017) between the slaughter age and the finishing diet. Overall, in both cities, 13Msamples were better evaluated than 26M samples, showing significant differences ($p < 0.05$) in terms of tenderness and juiciness attributes. The higher juiciness described in the younger group may be related to the higher moisture and lower cooking loss values found in the 13M group compared to the other group (Table 1). No significant differences were found due to the effect of the finishing diet in either Pamplona or Ourense. This may be due to the low percentage of linseed included in the diet (5%) and the short fattening period (Domínguez et al., 2018), which did not result in a noticeable sensory difference between the groups. Along the same lines, other studies on foal (Domínguez et al., 2018), beef (Realini et al., 2017) or lamb meat (Urrutia et al., 2015) hardly found the effect of linseed supplementation on meat quality.

Table 4. *Consumers' scores for aroma, flavour, tenderness, juiciness, sweetness and acceptance of foal meat in two Spanish cities. Effect of slaughter age (SA) (13 vs. 26 months), finishing diet (FD) (standard vs. linseed-rich concentrate) and information scenario (S) (blind vs. full) (mean and SEM).*

	Slaughter age			Finishing diet			Scenario			*P-value*		
	13 months	26 months	SEM	Standard	Linseed	SEM	Blind	Full	SEM	SA	FD	S
Pamplona												
Aroma	5.51±0.42	5.66±0.32	0.09	5.59±0.35	5.57±0.38	0.10	5.40±0.39	5.82±0.36	0.095	0.285	0.877	0.002
Flavour	5.57±0.38	5.51±0.66	0.10	5.65±0.33	5.44±0.51	0.10	5.38±0.26	5.76±0.69	0.101	0.675	0.154	0.009
Tenderness	5.76±0.55	5.03±0.87	0.14	5.44±0.54	5.35±0.65	0.15	5.14±0.61	5.73±0.52	0.150	0.001	0.692	0.007
Juiciness	5.61±0.50	5.14±0.64	0.13	5.45±0.49	5.31±0.80	0.14	5.13±0.51	5.71±0.80	0.133	0.018	0.477	0.003
Sweetness	5.76±0.60	5.70±0.74	0.13	5.77±0.59	5.69±0.76	0.14	5.64±0.60	5.85±0.74	0.137	0.056	0.221	0.298
Acceptance	5.65±0.29	5.36±0.60	0.10	5.60±0.32	5.41±0.51	0.10	5.30±0.32	5.78±0.63	0.107	0.753	0.685	0.001
Ourense												
Aroma	5.51±0.34	5.28±0.48	0.08	5.50±0.35	5.29±0.49	0.08	5.48±0.35	5.30±0.36	0.083	0.047	0.064	0.135
Flavour	5.45±0.52	5.25±0.72	0.12	5.45±0.52	5.26±0.72	0.12	5.24±0.59	5.48±0.61	0.124	0.254	0.282	0.167
Tenderness	5.80±0.54	4.98±0.74	0.16	5.26±0.48	5.52±0.71	0.17	5.42±0.73	5.35±0.72	0.189	0.001	0.302	0.803
Juiciness	5.53±0.36	4.77±0.64	0.15	5.11±0.38	5.18±0.89	0.16	5.12±0.74	5.17±0.75	0.170	0.001	0.796	0.840
Sweetness	4.94±1.79	4.96±0.69	0.25	5.01±0.89	4.89±1.69	0.25	4.79±0.86	5.14±1.67	0.221	0.025	0.785	0.406
Acceptance	5.59±0.47	5.18±0.59	0.12	5.41±0.60	5.36±0.74	0.13	5.31±0.69	5.47±0.73	0.132	0.952	0.748	0.328

The given information described a high and positive effect on almost all attributes (except for sweetness) and general acceptance (P < 0.01) in Pamplona, but not in Ourense. These results show the importance of the level of information between different cities or regions(Kallas et al., 2014; Realini et al., 2013) and a possible relationship with the different frequency of consumption in both cities. In this sense, Resano and Sanjuán (2017) point out that differences between regions must be taken into account when developing marketing strategies for the meat market. In addition, Lusk and Fox (2001) highlighted the importance of defining the relevance of different types of information (origin, diet, age, safety, animal welfare, etc.) to identify the main aspects that impact on consumer behaviour when developing marketing or branding strategies.

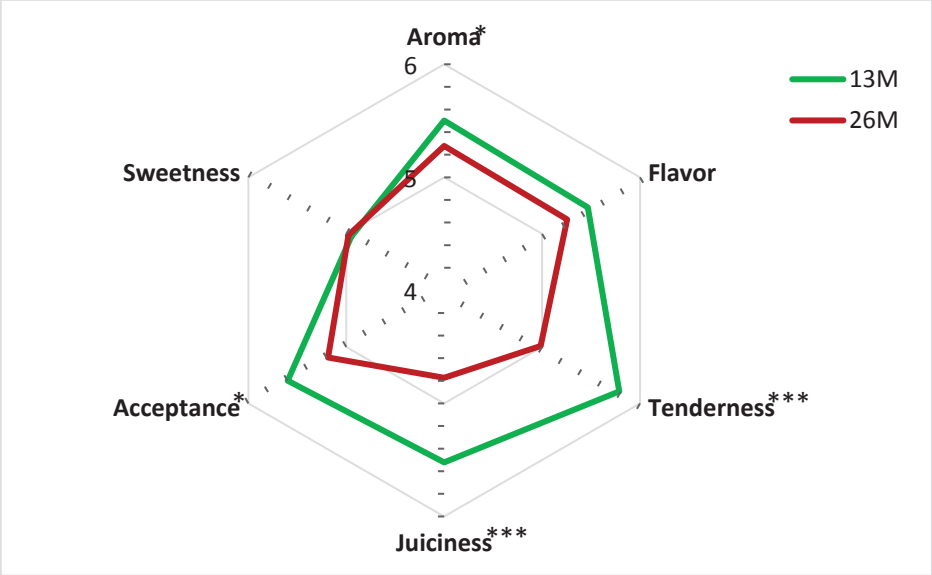

Figure 1. *Consumers' scores for aroma, flavour, tenderness, juiciness, sweetness and acceptance of foal meat in Pamplona. Effect of the slaughter age (13 vs. 26-month old foals) (13M vs. 26M) under the blind (a) and full (b) scenario. Scale range: 4-6.*

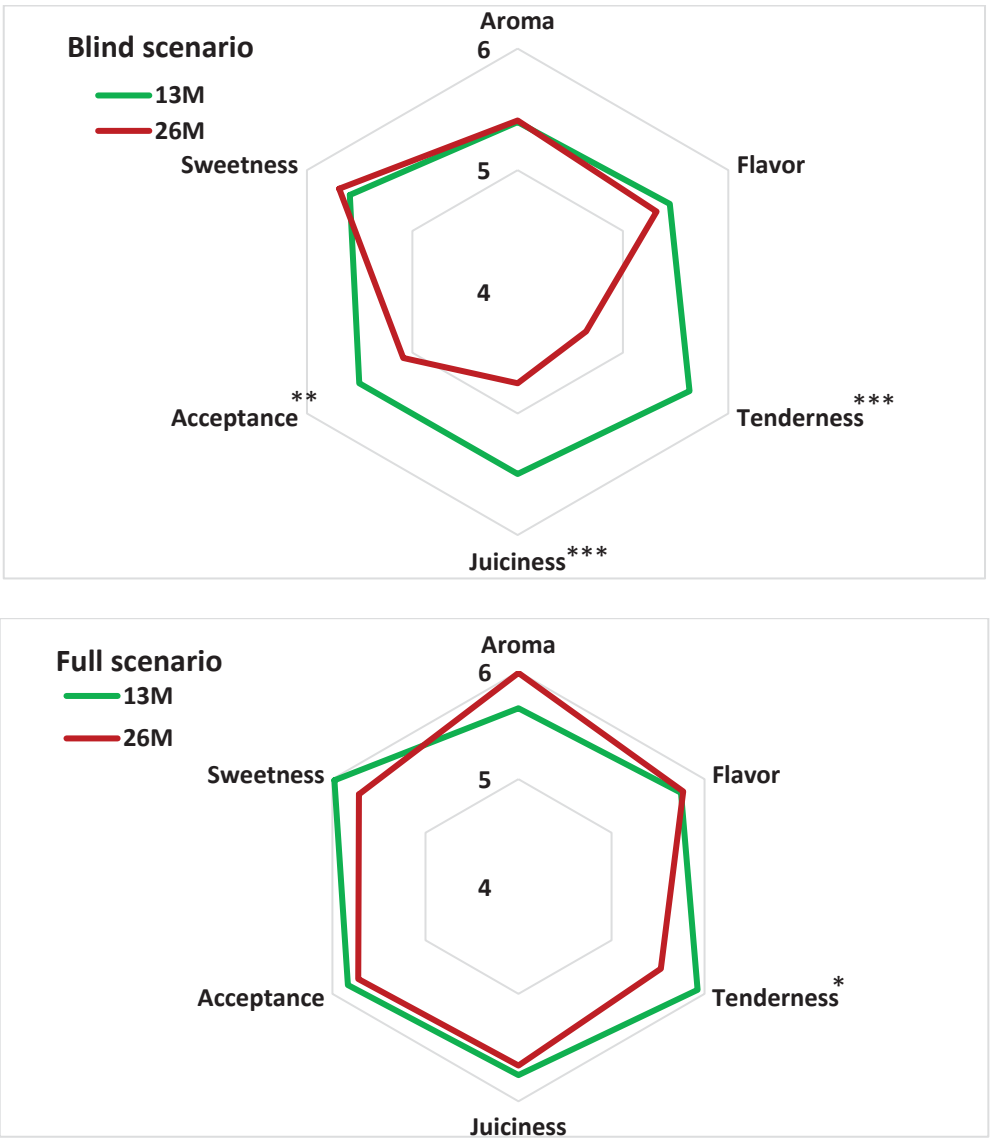

Figure 2. *Consumers' scores for aroma, flavour, tenderness, juiciness, sweetness and acceptance of foal meat in Ourense. Effect of the slaughter age (13 vs. 26-month old foals) (13M vs. 26M). Scale range: 4-6.*

Figures 1 and 2 represent the consumers' evaluation in Pamplona and in Ourense. This outcome agrees with data previously reported by beef meat (Beriain et al., 2014, 2016). It should be pointed out that all the values assigned for every attribute and in both cities were in the range of 5-6 ("Neither like nor dislike" - "Like slightly"). So, in spite of the wider knowledge of foal meat perceived in Pamplona, similar assessments were obtained in both

cities. Taking into account the lack of consumption habit of foal meat, noticeably marked in Ourense, these average assessment values could be a positive result from a future marketing perspective.

Table 5 displays the Pearson's (r) coefficients of correlation between the mean scores of sensory attributes. It reveals that there were positive correlations in both information scenarios. Thus, a higher score of one attribute had a positive impact on the scores of the other ones (Beriain et al., 2016). The attributes with higher effect on the overall acceptability of foal meat were juiciness ($0.91 < r < 0.73$), tenderness ($0.97 < r < 0.84$), and flavour ($0.97 < r < 0.65$) ($P<0.01$), for samples from 13M and 26M groups, respectively with or without complementary information. The importance of these attributes was previously described by Beriain et al. (2016) in beef meat. The highest correlations between overall acceptability and juiciness, tenderness and flavour scores were obtained in samples from 26M group. These high correlations could be useful to explain the lower scores obtained for samples from 26M foals with respect to the youngest group, as consumers assessed samples from 26M group as lower tenderness ($P < 0.001$) and juiciness ($P < 0.01$). In addition, it should be mentioned that negative correlations were found between WBSF and tenderness ($r=-0.78$; $P < 0.01$), juiciness ($r=-0.66$; $P < 0.05$) and overall acceptability ($r=-0.73$; $P < 0.05$). Nevertheless, only the full information scenario described lower sensory scores with higher WBSF values.

The tenderness and juiciness of 26M samples in the full information scenario showed a positive correlation with the intramuscular fat content ($r=0.68$, $r=0.67$; $P < 0.05$, for tenderness and juiciness, respectively). Consumers were able to slightly perceive higher intramuscular fat content in 26M samples compared to 13M samples, even when the percentage (1.72 vs. 0.56, for 26M and 13M, respectively) was lower than 3%, which is the threshold stated by Savell and Cross (1988) to observe palatability differences. Thus, in spite of the correlations

obtained, consumers did not give higher assessments to 26M than to 13M groups regarding tenderness or juiciness attributes. The results found in this study showed that tenderness and juiciness attributes were determining for consumers to accept foal meat, as happens in beef (Beriain et al., 2016; Sánchez et al., 2012).

Table 5. *Correlation mean scores for hedonic attribute given by the panel of consumers, of the two types of meat (from 13 and 26-month old foals) in the two information scenarios (blind and full) and in the two Spanish cities (Pamplona and Ourense).*

Pamplona	**Blind Scenario (n=125)**							
	Slaughter at 13 months				**Slaughter at 26 months**			
	Flavour	**Tenderness**	**Juiciness**	**Acceptance**	**Flavour**	**Tenderness**	**Juiciness**	**Acceptance**
Aroma	0.344	0,557*	0.460	0,552*	0,591*	0,506*	0,717**	0,611*
Flavour		0.361	0.173	0,650**		0,775**	0,641**	0,848**
Tenderness			0,856**	0,836**			0,793**	0,910**
Juiciness				0,808**				0,826**
	Full Scenario (n=122)							
	Slaughter at 13 months				**Slaughter at 26 months**			
	Flavour	**Tenderness**	**Juiciness**	**Acceptance**	**Flavour**	**Tenderness**	**Juiciness**	**Acceptance**
Aroma	0,651*	-0.420	-0.105	0.163	0,655*	0.514	0.437	0,659*
Flavour		-0.015	0.203	0,679*		0,900**	0,816**	0,945**
Tenderness			0,850**	0,688*			0,871**	0,969**
Juiciness				0,800**				0,894**
Orense	**Blind Scenario (n=118)**							
	Slaughter at 13 months				**Slaughter at 26 months**			
	Flavour	**Tenderness**	**Juiciness**	**Acceptance**	**Flavour**	**Tenderness**	**Juiciness**	**Acceptance**
Aroma	0,628*	0.390	0.511	0,769**	0,840**	0,749**	0,771**	0,781**
Flavour		0.311	0,591*	0,881**		0,966**	0,888**	0,966**
Tenderness			0,865**	0,632*			0,908**	0,958**
Juiciness				0,805**				0,903**
	Full Scenario (n=109)							
	Slaughter at 13 months				**Slaughter at 26 months**			
	Flavour	**Tenderness**	**Juiciness**	**Acceptance**	**Flavour**	**Tenderness**	**Juiciness**	**Acceptance**
Aroma	0.335	-0.398	-0.445	0.098	0,684*	0.445	0.325	0,614*
Flavour		0.260	0.405	0,878**		0,733**	0.564	0,916**
Tenderness			0,895**	0,604*			0,944**	0,919**
Juiciness				0,730**				0,820**

The relationship between the trained and consumer panel and the physic-chemical characteristics is shown in Figure 3. It revealed not only the low relationship between the trained panel and consumers' attributes, but also the low relationship between the physic-chemical properties and the sensory attributes. The principal component 1, related to consumers' evaluation, explained 35% of the variability, whereas the principal component 2, related to trained panel evaluation, explained 21% of the variability. Finally, the principal component 3 (15% of the variability) was related to physic-chemical parameters (WBSF, cooking loss (CL), moisture and intramuscular fat content). In general, the WBSF seemed to be positively related to fibrousness and negatively to the juiciness and tenderness evaluated by both, the trained panel and consumers. On the other hand, intramuscular fat content seemed to be slightly related to the greasiness and the fat flavour evaluated by the trained panel, but it was not related to the consumers' evaluation. Therefore, the trained panel was able to perceive the greasiness and the fat flavour and relate it positively to juiciness and tenderness, whereas consumers were not able to perceive the higher intramuscular fat content of the older foals (26M). This fact could help to explain the higher scores given by the trained panel to samples from 26M group in contrast to scores given by consumers.

Figure 3 confirms the results showed in Tables 2 and 4 according to sensory attributes. While, the trained panel (Table 2) described little variation between the intrinsic attributes of meat from 13 and 26-months old foals, consumers (Table 4) clearly differentiated meat from 13-months old foals, giving an important role to tenderness and juiciness attributes. In contrast to the results of Beriain et al. (2014) in beef, this study showed barely any interesting correlation between the attributes defined by the panel of experts and the untrained consumers. Thus, beef meat is not fully comparable

to foal meat. In addition, beef together with lamb and pork meat, has been widely studied and is very important in the meat sector (Belaunzaran et al., 2015). Therefore, further studies are necessary to obtain knowledge about the foal meat characteristics and consumers liking and perception.

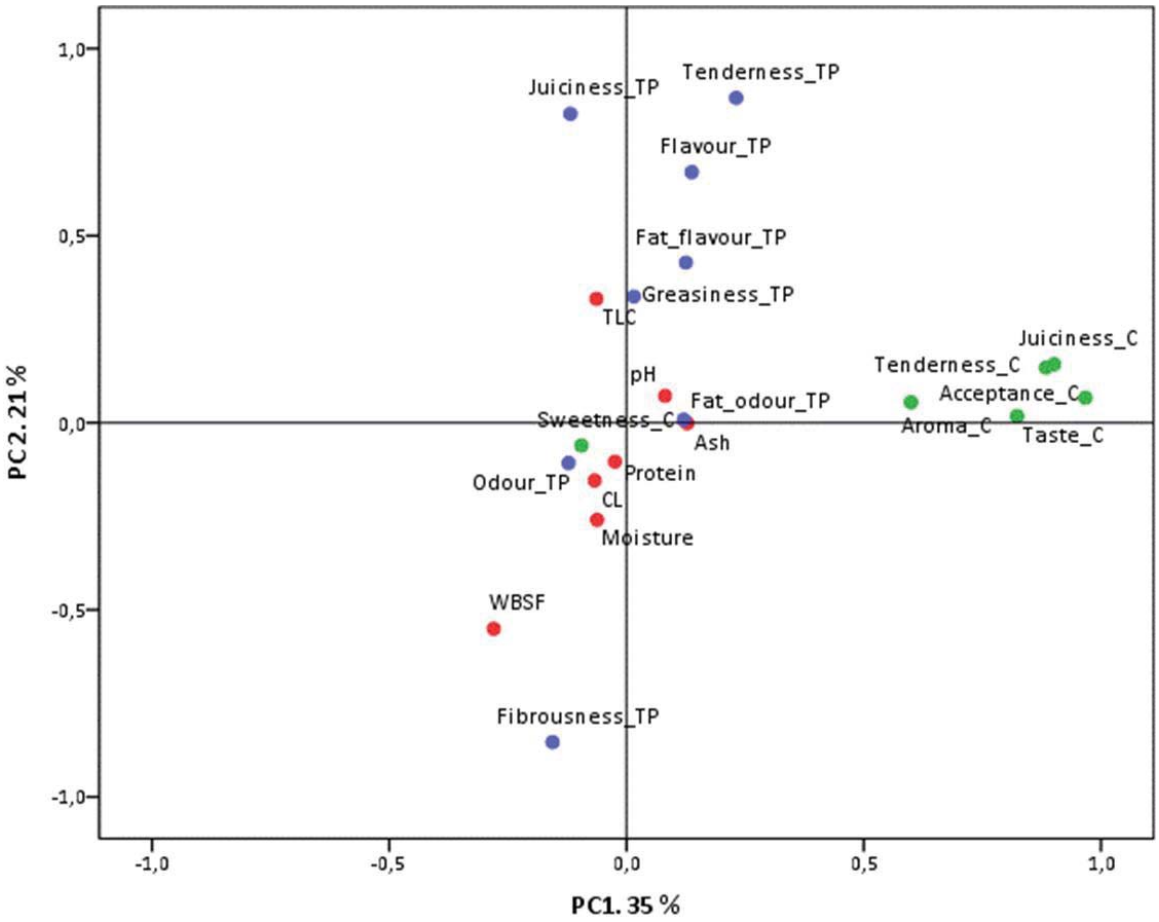

Figure 3. *Biplot representation of principal components PC1 and PC2 studied on foal meat samples for the chemical parameters and the sensory attributes evaluated by the trained panel (TP) and consumers (C). (TLC: total lipid content; CL: cooking loss; WBSF: Warner Bratzler shear force).*

4. Conclusions

It can be concluded that slaughter age seemed to be slightly important factor for the trained panel, whereas consumers noticeably preferred meat from younger foals due to

their higher juiciness and tenderness, which were the most important attributes for overall acceptability of foal meat. According to finishing diet, the linseed percentage in the finishing concentrate should be increased (higher that 5%) or the fattening period should be longer in order to perceive differences between the two groups studied. Finally, the lack of knowledge about foal meat and its low presence in meat markets make it necessary to develop further sensory studies to obtain better description patterns to get to understand consumers' likings.

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MANUSCRIPT V

Application of ATR-FT/MIR spectroscopy to evaluate the chemical composition and quality parameters of foal meat

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Application of ATR-FT/MIR spectroscopy to evaluate the chemical composition and quality parameters of foal meat

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Abstract

The aim of this work was to study the evaluation of chemical composition and quality of foal meat and the capability of FT/MIR spectroscopy to classify samples according to different slaughter ages and finishing diets. Important characteristics as moisture and total lipids content were well predicted with R_v^2 values of 82 and 66 %, respectively. Regarding fatty acids, the best models were obtained for arachidonic, vaccenic, DPA and DHA with R_v^2 values over 65 %. Quality parameters, as instrumental colour and texture and sensory attributes did not reach high prediction coefficients, being the colour coordinates from the image analysis the best predicted. With the spectra data of the region 2198-1118 cm-1, samples were accurately classified according to slaughter age (78 %) and finishing diet (72 %). This preliminary research shows the potential of MIR spectroscopy to develop further studies.

Keywords: foal meat quality, FT/MIR spectroscopy, evaluation, chemical composition, wavelength selection.

1. Introduction

Nowadays, high assurance of quality and safety during food production is being extremely demanded. Thus, strict controls both throughout the production and during the marketing process are required (Karoui et al., 2010). This demand and the agricultural industries supervision make highly desirable, food analysis methods which are speed, ease to use, with non-preparation or minimum sample preparation, with non-destruction of samples, low cost and environmentally sustainable (de Oliveira et al., 2014; Lozano et al., 2017). In this way, the NIR (Neat Infrared Spectroscopy) and ATR-FT/MIR spectroscopy (Attenuated total Reflectance-Fourier Transform Mid-Infrared Spectroscopy) are practical options, as they fulfil the requirements aforementioned. MIR spectroscopy applications in food analysis are diverse although its current use is limited. Some studied foods are honey (Tewari and Irudayaraj, 2004), tomato (Ścibisz et al., 2011), fish (Hernández-Martínez, 2013) or meat (Lozano et al., 2017; Shi and Yu, 2017). Specially for meat, several topics have been studied: (1) determination of chemical composition, (2) detection of microbiological spoilage, (3) authentication of products or (4) detection of meat adulterations (Karoui et al., 2010). Nevertheless, there is a lack of knowledge regarding meat quality determination according to not only chemical composition, but also texture, colour parameters, or even sensory attributes. Ripoll, Albertí, Panea, Olleta, & Sañudo (2008), did obtained successful results predicting some chemical parameters (moisture, fat, water holding capacity), the shear force and sensory tenderness by NIR spectroscopy. Worse results were obtained for compression or juiciness and overall appraisal estimation.

At present, studies of foal meat has noticeable arisen because of its healthy properties and sustainable production, which make it an alternative for consumers, whose demand

of healthy and safe products is continuously increasing (Lorenzo et al., 2014). Nevertheless, this kind of meat must be more exhaustively defined according to different factors as slaughter age or feeding which play and important role in meat quality (Franco and Lorenzo, 2014; Sarriés and Beriain, 2006). In this way, rapid, non-destructive and sustainable techniques as FT/MIR spectroscopy would be helpful to distinguish foal meat regarding the mentioned factors.

Thus, the objective of the present work was to investigate the potential of the MIR spectroscopy technique for the evaluation of foal meat quality of 13 and 26-month old foals supplemented with standard and linseed concentrate. One specific objective was to determine the wavelength ranges which contribute to the meat differentiation by the finishing diets or animals' slaughter age. The second specific objective was to determine if the studied meats could be differentiated and if it would be possible to separate them into homogeneous groups due to slaughter age or finishing diet.

2. Material and Methods

2.1. Animal management, sampling

Forty foals obtained by crossing Galician Mountain mares with a Burguete stallion were used. The animals were reared under extensive conditions. A fuller description of the animal management has been published in (Ruiz et al., 2017). Twenty-two foals were slaughtered at 13 months (13M) and another 24 at 26 months of age (26M). Prior to slaughter, all the foals were supplemented for a fattening period of about 104 days. Two study groups were formed by randomly assigning 11 foals from the 13M group and 12 foals from the 26M group to be supplemented on pasture with a standard concentrate (SC) (2 kg per foal and day) and pasture; and 11 foals from the 13M group and 12 foals

from the 26M group to be supplemented on pasture with a linseed-rich concentrate (5%) (LC) (2 kg per foal and day) and pasture.

The foals were slaughtered and dressed according to the specifications outlined in the European legislation (Council Directive 93/119/EC, 1993).

Immediately after slaughter, hot carcass weight was determined and once chilled for 24 hours at 4±1ºC, cold carcass weight (kg) and dressing percentage (%) were determined (Ruiz et al., 2017). At this point, the left half-carcasses were transported in refrigeration to the research centre pilot plant at 4±1ºC, where *Longissimus thoracis et lumborum* (LTL) muscles were removed from the left half-carcasses. In order to carry out the analyses, the LTL section ranged between the seventh and the twelfth rib was employed, following the same cutting order for all the analyses. They were then sliced into five 20 mm (±0.2) thick steaks (FIRMAQ, V-900, Lorca, Spain), vacuum-packaged and frozen at -18ºC (± 2) until analyses. Forty six LTL samples aged 24 hours were analysed.

2.2. Physic-chemical analysis

The moisture, protein, ash, total lipids content, pH and water holding capacity were performed following the procedure of Domínguez et al. (2017). Total and soluble collagen content were determined from the hydroxyproline content (Bonnet and Kopp, 1986). The spectral reflectance of the samples, provided by a Minolta CM2002 spectrophotometer with a D65 illuminant and a 10 standard observer, was used to calculate the proportion of each pigment form. Relative myoglobin (DMb) (%), metmyoglobin (MMb) (%) and oxymyoglobin (OMb) (%) contents were obtained from the reflex attenuance at the isobestic points 572, 525, 473nm and 700nm (AMSA, 2012). The reflex attenuance is defined as A = log (1/R), with “R” the reflectance in the [0,1]

(Karamucki et al., 2013). In addition, fatty acid profile was determined as it is shown in Domínguez et al. (2017). But, for this study, the most remarkable fatty acids from the nutritional point of view were selected. These are: stearic (C18:0), oleic (C18:1n9c), linoleic (C18:2n6c), linolenic (C18:3n3), arachidonic (C20:4n6) and vaccenic (CLA precursor) (C18:1n11t) acids. Finally, eicosapentaenoic (EPA, C20:5n3), docosapentaenoic (DPA, C22:5n3), docosahexaenoic (DHA, C22:6n3) and *n*-6 total PUFA content were studied as well.

To assess meat colour by Image Analyses, Grease (Gr), Red (R), Green (G), Blue (B) values were assessed. All values go from 0 to 225. The value "0" means: 100 % of the light is reflected, and value "225" means: 100 % of the light is absorbed. Samples were examined under the microscope and both sides photographed. The microscope images obtained were digitized by the employment of an image analysis software (ImageJ 1.47). The average red, green and blue values were obtained. The CIEL*a*b* system was also employed. Lightness (L*), redness (a*), yellowness (b*), chromaticity (C*) and hue (h*) (CIE, 1978) were assessed following the methodology employed by Sarriés & Beriain (2006).

2.3. Sensory analysis

Tenderness, juiciness and overall appraisal were also determined by a consumer panel integrated by 247 panellist from Pamplona. The methodology used for taste panels was described by Beriain, Sánchez, & Carr (2014). Participants were asked to evaluate the samples in the order they were served, which was designed to avoid the order of presentation effect and first order and carry over effects (Macfie et al., 1989). Panellists were asked to rate their liking of the tenderness, juiciness and their overall appraisal of

the samples. A 9-point hedonic scale was used, being 1 = “Dislike extremely”, 2 = “Dislike very much”, 3 = “Dislike moderately”, 4 = “Dislike slightly”, 5 = “Neither like nor dislike”, 6 = “Like slightly”, 7 = “Like moderately”, 8 = “Like very much” and 9 = “Like extremely”.

2.4. Mid-infrared spectra acquisition

All the samples were defrosted 24 hours at 4 ±1 ºC, previous to the analysis with the ATR-FT/MIR spectroscopy. The measuring instrument used in this research was a FTIR Vertex 80v spectrometer (Bruker, Germany). This equipment allows to work under vacuum conditions in the optical system which reduces the possible interferences produced by the water steam or the carbon dioxide in the measurements. This device is located in a clean room certified according to ISO 14644-1:2015. There, the concentration of particles present in the air and the temperature (constantly maintained at 22 ºC) were controlled. The measurements were made with an accessory A225 / QPlatinum-ATR (Bruker, Germany) made of a diamond crystal.

The followed process was always the same. Firstly, a reference spectrum was taken with the ATR device empty. This reference was taken before each measurement as it was observed that if more than one measure was made with the same reference, the spectra obtained were not clean, and presented high noise signal. Thus, after obtaining each reference, the spectrum of each of the samples was measured. It was necessary to place the meat sample on the crystal, ensuring that all crystal surface was completely covered and that there was a perfect contact between the sample and the crystal. For this, meat samples were lightly pressed with the plunger present in the ATR accessory. In total, 6 repetitions were performed per sample analysed. For each sample, 32 scans in the 4000–400 cm^{-1} spectral range were recorded with a resolution of 4 cm^{-1}.

2.5. Statistics analysis

Statistical analysis was conducted by means of SPSS (SPSS 23.0, Chicago, IL, USA) to obtain the descriptive statistics. The mean plus standard deviation and the coefficient of variation (%) were obtained. Pearson's (r) coefficients of correlation between the mean scores for the various meat quality characteristics of the foal meat were determined. In addition, correlations between the most relevant wavelengths found throughout the spectra were studied. A positive correlation coefficient close to 1 indicates that when band intensity increases, the signal of the other band increases as well. This means that the same link or structural unit is in different bands with different modes of vibration. A negative correlation coefficient close to -1 reflects that an increase in a signal coincides with the decrease of the other compared signal. In no case, correlation must be understood as synonymous with causality.

With the most representative wavelength range obtained by the models, principal component analysis (PCA) was applied to reduce the number of variables. PCA determines linear combinations of the original variables to summarize the data with minimal loss of information. Varimax rotation was applied to the factors to facilitate interpretation and maximize the explained variance. Once the PC variables were obtained, canonical discriminant analysis was carried out to classify the samples. A stepwise model analysis using a significance level of 0.05 as the variable entry criterion was applied for the discriminant procedure. The leave-one-out cross-validation method was used to validate the model. Canonical discriminant analysis method was developed to classify the animals into the different slaughter ages and finishing diet. Discriminant analysis was developed including the medium spectroscopy wavelengths, and was conducted using stepwise model analysis, which performed the best-subset selection of

the quantitative predictor by a procedure of entrance-remove of variables in the model. The significant level of a variable to enter in the model was 0.05.

2.6. Chemometric analysis

The chemical bonds associated with each peak of the FTIR spectra were determined by analysing the physic-chemical composition and sensory properties of the samples, by correlations among the bands with larger intensity, and by comparing the wavenumbers with the literature (Lozano et al., 2017).

Then, the analysis was particularized to the physical and chemical and sensory properties of the samples and prediction models were built, using the results obtained by the described methods as reference values. Spectral data pre-treatments such as standard normal variate (SNV), multiplicative scatter correction (MSC) and first or second order derivatives were applied to the spectra with the purpose of reducing noise and scattering effects. The models were built using a specific program of chemometrics, OPUS Quant 2 (Bruker, Ettlingen, Germany). The calibration models were developed using the Partial Least Square (PLS) regression method and validated by cross-validation. The main advantage of cross-validation is that a small number of samples are required because the same set of samples is used to calibrate and validate the method. Before starting the calibration, one sample is excluded from the entity of samples and the remaining samples are used to calibrate the system. Once the model is built, it is tested using the excluded sample. Then, the cycle is repeated separating a different sample until all samples have been used for validation once. The optimum number of factors in the PLS calibration models was indicated by the lowest number that gave the minimum value of the root mean square error (RMSE) in cross validation, in order to avoid

overfitting of the models. In each variable analysed, the range of wavenumbers with more information in their absorbance and the best pre-treatment method were selected using the OPUS Quant 2 program. During the calibration and prediction stages, the outliers were detected and kept out of the prediction models. These models enable a quantitative estimation of the physic-chemical composition, colour parameters, and sensory attributes of the different samples depending on the absorbance intensity.

3. Results and Discussion

3.1. Chemical composition of the samples

A full description of foal meat in terms of composition and quality and according to the slaughter age and finishing diet, is shown in Table 1.

In terms of chemical composition, the most noteworthy point is the lower moisture and more than twice total lipids content presented by the 26M samples in contrast to the 13M samples. Fatty acids are, generally different depending on slaughter age, but not on finishing diet. According to GrRGB values, all of them were higher in 26M and LC than in 13M samples and SC samples, respectively. Regarding CIELab colour space, the 26M samples showed lower L* and higher a* and C* values than the 13M samples. With regard to the FD effect, the SC samples seemed to have higher L* and lower a*, b* values than the LC samples. Regarding WBSF, the 26M samples presented higher shear force (N). As for FD effects, the meat from the SC group showed higher values of shear force (N) than that from the LC group. Meat from 13M foals showed higher tenderness, juiciness and overall appraisal values than 26M samples.

Table 1. *Physic-chemical and sensory description of Longissimus thoracis et lumborum muscle from Galician Mountain x Burguete crossbred foals slaughtered at 13 and 26 months of age (13M, 26M) and supplemented with standard and linseed concentrate (SC, LC) (n=46).*[a]

	Slaughter Age				Finishing Diet			
	13M	**CV%**	**26M**	**CV%**	**SC**	**CV%**	**LC**	**CV%**
Chemical composition								
Moisture (%)	74.2 ± 0.15	0.2	72.3 ± 0.28	0.4	73.5 ± 0.27	0.4	72.9 ± 0.33	0.5
Protein (%)	22.5 ± 0.19	0.8	22.9 ± 0.19	0.8	22.6 ± 0.18	0.8	22.9 ± 0.21	0.9
Ash (%)	1.28 ± 0.02	0.0	1.27 ± 0.03	2.4	1.32 ± 0.03	2.3	1.23 ± 0.03	2.4
Total lipids content (%)	0.57 ± 0.08	14.0	1.73 ± 0.16	9.3	1.20 ± 0.22	18.3	1.16 ± 0.14	12.1
Total collagen (g/100g meat)	0.36 ± 0.04	11.1	0.44 ± 0.05	11.4	0.40 ± 0.01	2.5	0.40 ± 0.11	27.5
Soluble collagen (%TC)	4.11 ± 0.58	14.1	2.39 ± 0.63	26.4	3.40 ± 0.87	25.6	3.11 ± 0.87	28.0
pH	5.59 ± 0.02	0.4	5.62 ± 0.02	0.4	5.64 ± 0.02	0.4	5.57 ± 0.02	0.4
Water Holding Capacity	21.7 ± 0.61	2.8	22.7 ± 0.44	1.9	22.9 ± 0.41	1.8	21.5 ± 0.60	2.8
DMb (%)	25.1 ± 5.11	20.4	22.4 ± 13.44	60.0	23.2 ± 8.64	37.2	24.3 ± 7.84	32.3
MMb (%)	16.9 ± 9.63	57	22.0 ± 5.91	26.9	18.9 ± 3.23	17.1	20.0 ± 5.62	28.1
OMb (%)	58.0 ± 8.14	14.0	55.7 ± 11.21	20.1	57.9 ± 7.84	13.5	55.8 ± 9.69	17.4
***Fatty acids* (g/100g lipids)**								
Stearic acid	7.21 ± 0.20	2.8	5.10 ± 0.19	3.7	6.12 ± 0.30	4.9	6.09 ± 0.29	4.8
Oleic acid	23.0 ± 1.03	4.5	30.8 ± 0.88	2.9	25.5 ± 1.39	5.5	28.6 ± 1.03	3.6
Linoleic acid	18.4 ± 0.85	4.6	11.9 ± 0.74	6.2	15.9 ± 1.16	7.3	14.1 ± 0.88	6.2
Linolenic acid	11.1 ± 0.37	3.3	11.9 ± 0.59	5.0	11.1 ± 0.53	4.8	11.8 ± 0.48	4.1
Arachidonic acid	1.73 ± 0.12	6.9	0.99 ± 0.08	8.1	1.48 ± 0.14	9.5	1.20 ± 0.11	9.2
Vaccenic acid	0.05 ± 0.01	20.0	0.03 ± 0.00	0.00	0.04 ± 0.01	25.0	0.04 ± 0.00	0.0
EPA	0.97 ± 0.08	8.3	0.37 ± 0.03	8.1	0.73 ± 0.09	12.3	0.58 ± 0.07	12.1
DPA	1.81 ± 0.10	5.5	0.92 ± 0.07	7.6	1.48 ± 0.14	9.5	1.21 ± 0.12	9.9
DHA	0.48 ± 0.03	6.3	0.21 ± 0.02	9.5	0.38 ± 0.04	10.5	0.29 ± 0.03	10.3
n-3 PUFA	14.9 ± 0.35	2.4	13.8 ± 0.60	4.3	14.2 ± 0.58	4.1	14.4 ± 0.45	3.1
n-6 PUFA	21.1 ± 1.00	4.7	13.6 ± 0.84	6.2	18.3 ± 1.35	7.4	16.1 ± 1.03	6.4
Quality parameters								
Gr	117.7 ± 8.09	6.9	135.1 ± 7.34	5.4	124.4 ± 13.75	11.1	128.4 ± 5.53	4.3
R	141.6 ± 6.93	5.0	161.9 ± 9.63	6.0	150.6 ± 17.89	11.9	152.9 ± 7.42	4.9
G	85.9 ± 6.73	7.8	98.7 ± 5.54	5.6	90.3 ± 9.71	10.8	94.3 ± 4.15	4.4
B	99.0 ± 8.88	8.8	112.9 ± 7.39	6.5	103.2 ± 11.81	11.4	108.6 ± 5.43	5.0
L*	32.49 ± 2.31	7.1	28.90 ± 3.25	11.3	31.82 ± 2.99	9.4	29.57 ± 3.36	11.4
a*	17.59 ± 1.32	7.5	20.35 ± 3.15	15.5	17.89 ± 1.85	10.3	20.05 ± 3.17	15.8
b*	10.01 ± 1.67	16.7	11.49 ± 3.81	33.2	9.92 ± 1.83	18.5	11.58 ± 3.74	32.3
C*	20.29 ± 1.55	7.6	23.46 ± 3.51	15.0	20.52 ± 2.03	9.9	23.23 ± 4.53	19.5
h*	29.56 ± 4.25	14.4	28.79 ± 4.98	17.3	28.94 ± 4.72	16.3	29.41 ± 4.58	15.6
WBSF (N)	45.88 ± 5.73	12.5	53.27 ± 5.71	10.7	52.71 ± 4.99	9.5	46.44 ± 6.73	14.5
Tenderness [b]	5.76 ± 0.64	11.1	5.03 ± 0.88	17.5	5.44 ± 0.60	11.0	5.35 ± 0.55	10.3
Juiciness [b]	5.61 ± 0.63	11.2	5.14 ± 0.85	16.5	5.45 ± 0.63	11.6	5.31 ± 0.74	13.9
Overall appraisal [b]	5.65 ± 0.45	8.0	5.36 ± 0.61	11.4	5.60 ± 0.53	9.5	5.41 ± 0.69	12.8

Values shown as mean plus standard deviation and coefficient of variation (CV).

[a] Abbreviation: TC: Total collagen; DPA: Docosapentaenoic acid, EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, PUFA: Polyunsaturated fatty acids; Gr: Grease; R: Red; G: Green: B: Blue; %DMb/ %MMb/ %OMb: Percentages of deoxymyoglobin, metmyoglobin, oxymyoglobin.
[b] Sensory scale: 1-9 (Dislike extremely-Like extremely)

The differences due to finishing diet were less relevant. The most remarkable correlations were found between the overall appraisal with tenderness and juiciness (r=0.89, r=0.87, respectively; P > 0.01). Moisture was positively correlated to tenderness (r=0.38; P > 0.01) and overall appraisal (r=0.31; P > 0.05), whereas total lipids content was negatively correlated to tenderness (r=-0.40; P > 0.01) and juiciness (r=-0.35; P > 0.05). All colour variables from the image analyses (GrRGB) and the CIELab system were negatively correlated to overall appraisal (P > 0.05).

3.2. Spectral data

Dealing with the spectra in the medium infrared, Lozano et al. (2017) recently described the two most important spectra ranges as far as foal meat is concerned: 3200-2500 cm^{-1} and 2300-980 cm^{-1}. These two sections are shown in Fig. 1 and 2 for meat samples from 13M and 26M foals supplemented with SC and LC concentrate samples (13M-SC, 13M-LC, 26M-SC, 26M-LC). Both ranges have been slightly widened not to lose possible wavelength which could show some differences between each group.

Fig. 1 represents the section from 3000 cm^{-1} to 2700 cm^{-1}. This region is the one of hydrogen's stretching related to -C-H stretching vibration, involving double bounds =C-H and aliphatic CH_3 and CH_2 (Jović et al., 2013; Lucarini et al., 2017). It is shown that 26M-LC samples described higher absorbance peaks than 13M-SC/LC and 26M-SC between 2990 and 2800 cm^{-1}. This means that asymmetric and symmetric stretching vibrations of -C-H of aliphatic CH_3 at 2959 and 2873 cm^{-1} were taking place more intense than in

the other groups. Moreover, the absorbance difference at 2925 and 2851 cm^{-1} (peaks related to asymmetric and symmetric stretching vibrations of -C-H of aliphatic CH_2) between 26M-LC and the others 3 groups, was even higher, and thus more intense. Domínguez et al. (2017) showed that 26M foals' samples and those from animals supplemented with linseed concentrate presented higher amount of monounsaturated fatty acids, and reached higher total lipids content (Table 1). This higher amount of aliphatic CH_3 and CH_2 compounds define larger fatty acids chains, indirectly represented with a higher absorbance.

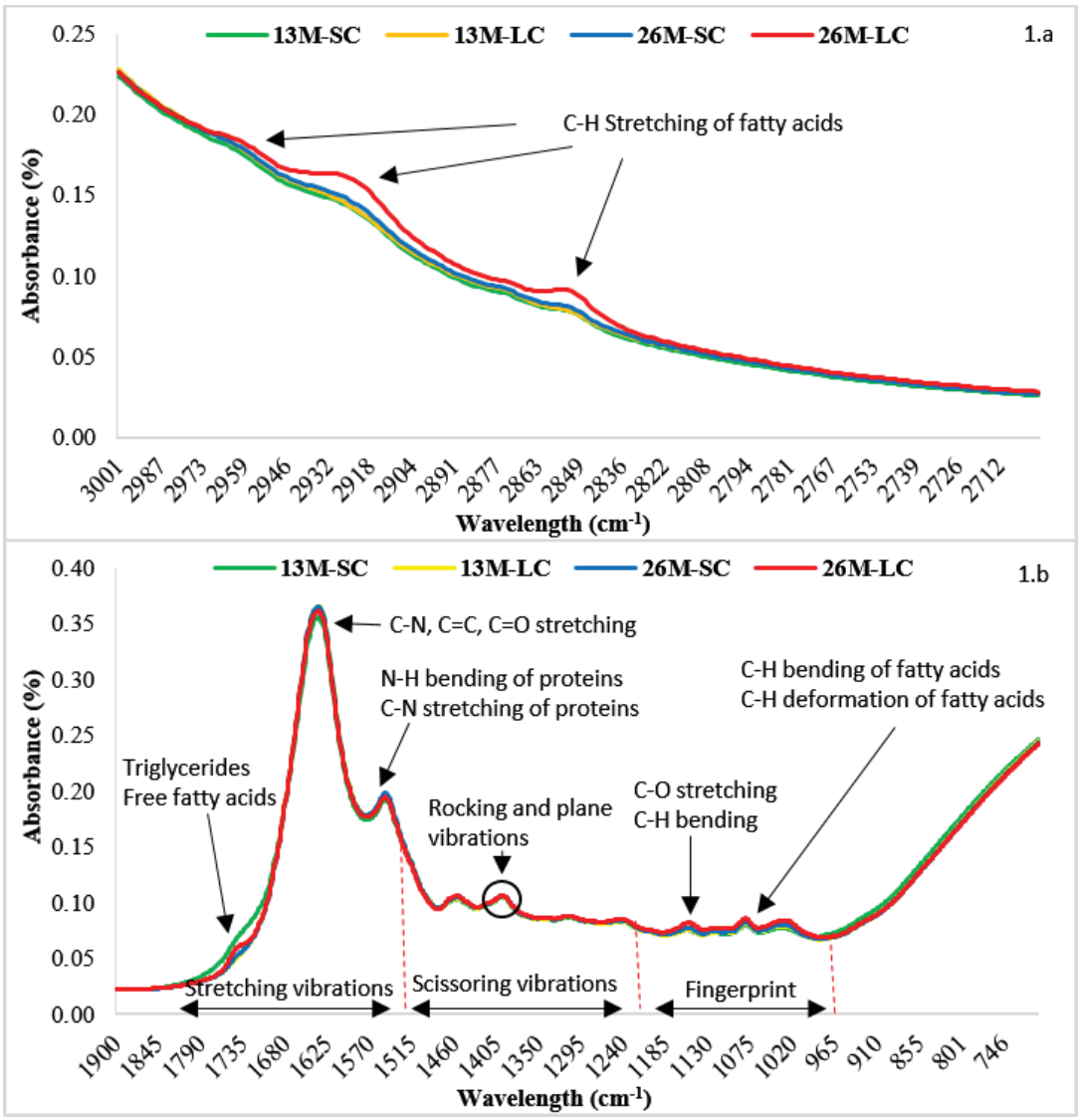

Figure 1. *Range selected from 3000-2500 cm^{-1} (a) and from 1900-980 cm^{-1} (b) according to the literature data (Lozano et al., 2017). Samples from 13 and 26-month old foals (13M, 26M) and supplemented with standard and linseed concentrate (SC, SL).*

Fig. 2 and 3 represents the region from 1900 cm^{-1} to 750 cm^{-1}. This region involves double bond's stretching (1750-1650cm^{-1}), other deformations and bendings (1500-1200cm^{-1}) and the called "fingerprint range" (1200-950 cm^{-1}) (Jović et al., 2013; Lozano et al., 2017; Lucarini et al., 2017). The 13M-SC samples, which presented higher percentage of saturated and polyunsaturated fatty acids (data shown in Domínguez et

al., 2017) and higher lipid oxidation than the rest of groups (0,40 vs. 0.36 mg MDA/kg fresh meat) (data nor shown), showed a higher absorbance from 1800 to 1700 cm^{-1}. Bands at 1742 cm^{-1} are related to triglycerides and free fatty acids. Vlachos et al. (2006) explained that an oxidation of fatty acids is likely to happen proximately to 1700cm^{-1}, and that the accurate peak position and intensity would depend on the fatty acid composition. Thus, it might be possible that the higher absorbance obtained for 13M-SC samples, was linked to the high amount of polyunsaturated fatty acids and a possible lipid oxidation.

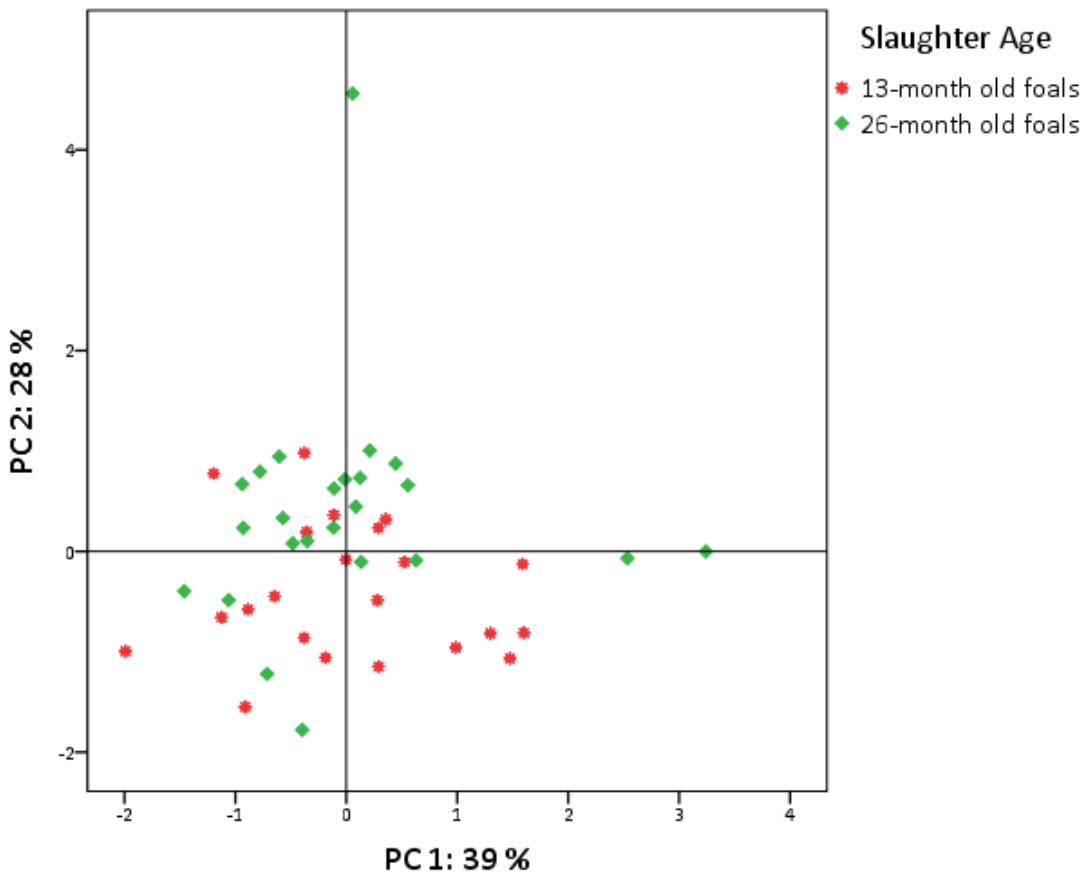

Figure 2. *Distribution of foal samples according to slaughter age (SA) obtained from the PCA carried out with the third selected wavelength range (2198-1118 cm^{-1}), where 13M and 26M are 13 and 26-months old foals and SC and LC are standard and linseed concentrate.*

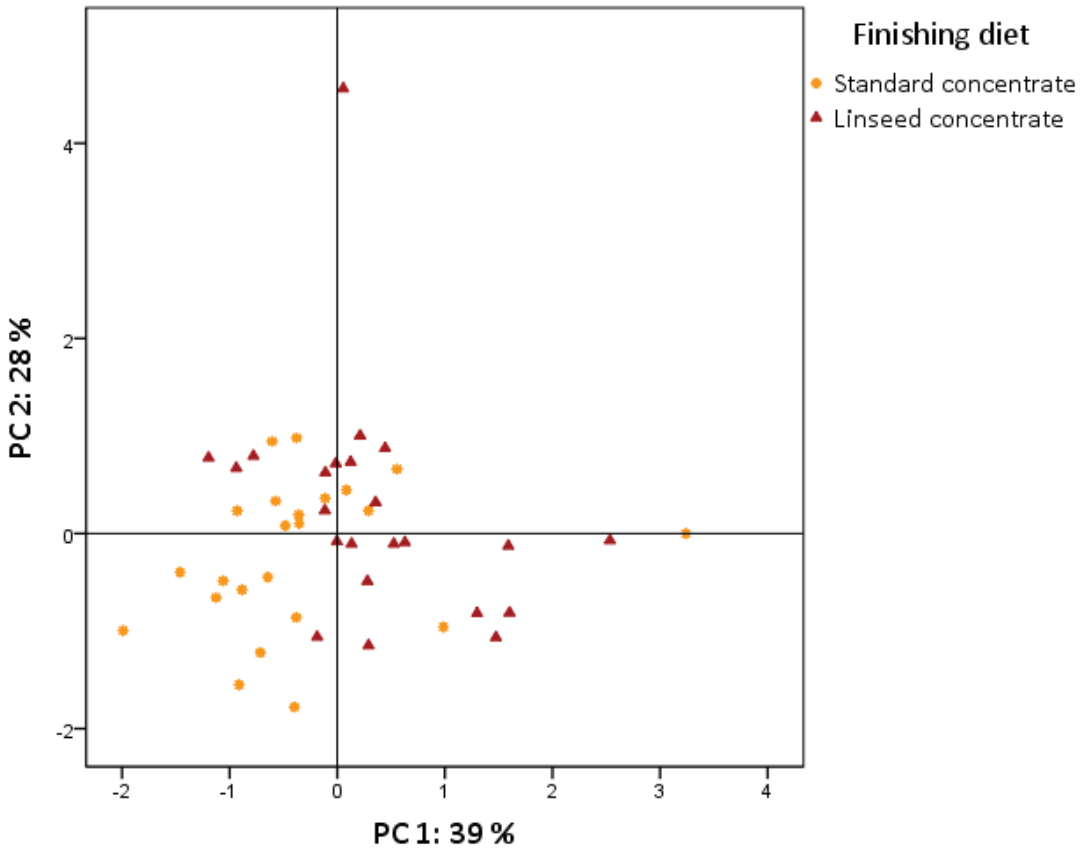

Figure 3. *Distribution of foal samples according to finishing diet (FD) obtained from the PCA carried out with the third selected wavelength range (2198-1118 cm^{-1}), where 13M and 26M are 13 and 26-months old foals and SC and LC are standard and linseed concentrate.*

Bonds of N-H, C=C, C-N and the combination of N-H with C-H (Amides II) are typical of protein amino acids and appear at bands 1657 and 1542 cm^{-1} (Lozano et al., 2017). Figure 2 does not show any difference in the absorbance although it seems to be slightly higher in 26M-SC foals samples. Along the fingerprint range it can be seen a smooth absorbance increase in the 26M-LC foal samples, but not determinant. This region is related to stretching vibrations of the C-O bond stretching vibration and the C-H bond bending vibration (Hernández-Martínez, 2013); and the peak at 1117 cm^{-1} is assigned to bending and twisting vibration of the fatty acids (Rohman et al., 2011). Correlations help

to better understand the bands' changes due to different vibrations. The absorbance ~ 2959 cm^{-1} was positively correlated to the peak ~2925 cm^{-1} (0.78), ~2837 cm^{-1} (0.94), ~2851 cm^{-1} (0.74), ~1658 cm^{-1} (0.81), ~1542 cm^{-1} (0.67) and ~1117 cm^{-1} (0.73). According to several authors, these wavenumbers are related to typical vibration modes of the lipids fatty acids and proteins (C-H bonds). A weak and negative correlation between 2959 and 1742 cm^{-1} was found (-0.38**). This means that the stretching vibration of carbonyl bond of esters and free fatty acids (Hernández-Martínez, 2013; Lozano et al., 2017) related to this peak is different from the vibration way at 2959 cm^{-1}. Another example of different vibration is better understood by the positive correlation found between the peak at ~1117 cm^{-1} and ~2925 (0.88**), ~2873 (0.88**) and ~2851 cm^{-1} (0.90**). All these peaks are related to vibration of the fatty acids. But ~1117 cm^{-1} is assigned to bending and twisting vibration whereas the other ones are related to stretching vibrations.

3.2.1. Principal Component and Discriminant Analyses previous to chemometric analysis

According to spectra data results and the literature cited, the two aforementioned spectra ranges were used: 3200-2500 cm^{-1} and 2300-980 cm^{-1}. Results showed that 5 PC variables explained the 95 % of the total variability. One preliminary discriminant analysis was developed (Table 2).

Table 2. *Classification matrix, from FT/MIR spectrum of 13 and 26-month old Galician Mountain x Burguete crossbreed foals (13M, 26M) and of those supplemented with standard and linseed concentrate (SC, LC).*

		Classify into		Total
		13M	**26M**	
13-month old	**%**	**72.7**	27.3	100.0
26-month old		25.0	**75.0**	100.0
		SC	**LC**	
Standard Concentrate	**%**	**43.5**	56.5	100.0
Linseed Concentrate		47.8	**52.2**	100.0

While the samples from the two slaughter-age groups (13M and 26M) were classified with about 74.0% accuracy, a poor classification was obtained according to the finishing diet, with just a 47.8 % accuracy. Better results were reported by Xing, Ngadi, Gunenc, Prasher, & Gariepy (2007) classifying intact pork meat (85 % accuracy) and Juárez, Alcalde, Horcada, & Molina (2008) classifying six lamb breeds (83 % accuracy) by visible spectroscopy. The results obtained let infer that the variables obtained from the PCA, which involved the most relevant spectra data, are mainly related to different characteristics due to the slaughter age than due to finishing diet. Moreover, according to the relationship appreciated in the spectra between different wavelengths and the fatty acids, it could be highlighted that the total lipids contents would be one of the most evident causes why 13M and 26M foals are different.

3.3. Calibration and validation models to predict the chemical composition and quality parameters of foal meat

In order to look into the intrinsic properties of foal meat, regression models were built based on the two spectrum ranges (3200-2500 cm^{-1} and 2300-980 cm^{-1}) to estimate the

chemical composition and quality parameters of the samples analysed from the spectral information. Table 3 shows mathematical treatments and wavelengths for each calibration and validation equation according to chemical composition, fatty acids profile and quality parameters, respectively. The lack of MIR spectroscopy studies dealing with meat composition and meat quality makes necessary the comparison with studies of NIR spectroscopy in large extent, although the spectra range is not the same.

Table 3. *Results of the PLS regression models for the FT/MIR data matrix of meat composition and quality from 13 and 26-month old Galician Mountain x Burguete crossbreed foals (13M, 26M) supplemented with standard and linseed concentrate.*

			Calibration			Validation				
	n	*p*	R_c^2 (%)	RMSEC	RPD	R_v^2 (%)	RMSECV	RPD	Treatment	Selected regions (cm^{-1})
Chemical composition										
Moisture	41	9	93.67	0.34	3.97	81.57	0.53	2.33	Max. and min. normalization	3278-2918; 1839-1478; 1119-759
Protein	42	1	30.36	0.75	1.20	22.71	0.78	1.14	Max. and min. normalization	3998- 3637; 1839-1478; 1119- 399
Ash	42	5	97.92	0.02	6.36	40.55	0.09	1.30	2nd derivate	3278- 2918; 2198- 1838
Total lipids content	38	5	26.30	0.85	1.16	65.99	0.46	1.72	2nd derivate	2559-1838; 1479- 1118
Total collagen	40	8	98.12	0.01	7.39	70.65	0.04	1.85	1st derivate + MSC	3638-3277; 1479- 1118
Soluble collagen	45	1	9.78	2.67	1.05	0.615	2.72	1.00	MSC	2198-1838
pH	40	1	31.19	0.07	1.21	22.39	0.07	1.14	Removal of constant slope	1479- 1118
WHC	37	1	29.88	1.56	1.19	20.03	1.60	1.13	Removal of constant slope	759- 399
DMb	46	10	97.88	6.88	1.55	25.78	8.02	1.16	2nd derivate	3278-2918; 1839-1118
MMb	45	3	35.31	5.21	1.24	21.63	5.48	1.13	1st derivate + MSC	1839-1478
OMb	46	3	29.23	8.27	1.19	16.25	8.58	1.09	MSC	1839-1118; 759-399
Fatty acids										
Stearic acid	42	10	97.73	0.23	6.63	61.77	0.79	1.62	1st derivate + SNV	3638-3277; 2559-2198; 1839-1118
Oleic acid	43	10	96.70	0.28	5.51	60.07	0.83	1.59	1st derivate + SNV	3638-3277; 2559-2198; 1839-1118
Linoleic acid	38	8	88.13	1.83	2.90	55.49	3.15	1.50	1st derivate + SNV	3638-3277; 1839-1118
Linolenic acid	43	7	98.47	0.32	8.07	38.36	1.82	1.27	2nd derivate	3278-2918; 2559-2198; 1119-759
Arachidonic acid	40	9	97.03	0.12	5.80	77.67	0.29	2.12	MSC	2919- 2558; 1119-759
Vaccenic acid	43	5	93.70	0.01	3.98	67.11	0.01	1.74	1st derivate + SNV	2559-2199
DPA	43	10	97.03	0.12	5.80	76.39	0.29	2.06	1st derivate + SNV	3638-3277; 2919- 2558; 1839-1118
EPA	42	10	87.47	0.13	2.83	54.64	0.22	1.50	None	3104-2501; 2130- 893
DHA	43	3	97.03	0.12	5.80	76.39	0.03	2.06	1st derivate + SNV	3638-3277; 2919-2558; 1839-1118
n-3 PUFA	39	4	90.73	0.58	3.29	41.19	1.37	1.30	2nd derivate	3278-2918; 2559-2198
n-6 PUFA	40	8	94.87	1.46	4.42	73.89	2.90	1.96	MSC	2919-2558; 1119-759

			Calibration			Validation				
	n	*p*	R_c^2 (%)	RMSEC	RPD	R_v^2 (%)	RMSECV	RPD	Treatment	Selected regions (cm^{-1})
Quality parameters										
Grease	43	7	90.96	4.47	3.33	65.68	7.85	1.17	1st derivate + Vector normalization	3998-3637; 2198-1118
Red	42	7	98.06	2.53	7.18	58.47	10.5	1.55	1st derivate + MSC	3998-3637; 1119-759
Green	43	7	91.65	3.24	3.46	70.32	5.55	1.84	1st derivate + MSC	3998-3637; 2198-1118
Blue	39	8	96.03	2.77	5.02	73.63	6.26	1.95	1st derivate + MSC	3998-3637; 2198-1118
L*	46	1	6.80	2.59	1.04	5.28	2.69	1.00	Max. and min. normalization	3090-2497; 2300-980
a*	46	1	25.70	1.76	1.16	15.85	1.83	1.09	Max. and min. normalization	3200-2500; 2300-980
b*	46	1	31.01	2.16	1.20	21.55	2.25	1.13	Max. and min. normalization	3200-2500; 2300-980
C*	46	1	36.74	1.78	1.26	22.60	1.93	1.14	Max. and min. normalization	3200-2500; 2300-980
h*	46	1	11.71	4.06	1.06	4.49	4.13	1.02	Max. and min. normalization	3200-2500; 2300-980
WBSF	40	3	44,62	10,3	1,34	25,7	11,6	1,60	1st derivate + SNV	1119-759
Tenderness	46	1	36.17	0.49	1.24	27.41	0.50	1.17	Max. and min. normalization	3200-2500
Juiciness	46	1	18.91	0.56	1.12	8.04	0.58	1.04	Max. and min. normalization	3200-2500
Overall appraisal	46	1	29.49	0.39	1.19	23.89	0.39	1.05	1st derivate	3200-2500

n: number of samples used in calibration; p: number of terms included in the equation; R^2_c: coefficient of determination for calibration; RMSEC: root mean square error of calibration (%);R^2_v: coefficient of determination for cross-validation (%); RMSECV, root mean square error of cross-validation (%); RPD, ratio of prediction to deviation; MSC, multiplicative scattering correction; SNV, standard normal variate

In Table 3, it is shown that moisture, total lipids content and total collagen are the only chemical parameters with coefficient of determination for validation higher that 60 % whereas the rest of variables did not reach 25 %, except for ash content (40.55 %). It should be explained that the ratio of prediction to deviation (RPD) is noticeably poor with regard to validation results, except for moisture (2.33). High quality moisture calibrations have been reported in literature, with coefficients of determination higher than 0.70 in NIR (Ripoll et al., 2008). This means that the regression models have a low accuracy to predict different chemical composition parameters of foal meat. The calibration model for protein had a low R_v^2 (22.71 %). Difficulties in predicting meat protein content have been previously reported in beef (Oliván et al., 2001; Ripoll et al., 2008) with NIR spectroscopy. One of the causes of the low accuracy of protein prediction in meat could be related to the analytical differences between the Kjeldahl determination (which measures nitrogen) and the MIR/NIR techniques (which measures protein bonds) (Lanza, 1983). Nevertheless, better results have been described in beef predicting fat and protein content (Lozano et al., 2017) by MIR spectroscopy, whereas similar results of RPD were described by Ripoll et al. (2008) for chemical composition with NIR spectroscopy. One hypothesis might be the higher homogeneity of beef, which could favour better regression results. For both moisture and protein content, the maximum and minimum normalization treatment were used, being 1839-1478 and 1119-759 cm^{-1} the wavelength ranges employed. Total lipids content reached a high coefficient of calibration (76.30 %) but the validation process made it decrease to 66 %. Higher coefficient have been reported in beef meat (Ripoll et al., 2008) (76 %) or lamb meat Cozzolino et al. (2000) (73 %) with NIR spectroscopy. This could be due to the fact that measurements were taken on the intact sample instead of on a homogenized

sample. Total collagen content showed also good coefficients of validation (70.65 %), far from those reported by other authors with NIR spectroscopy in beef meat (Weeranantanaphan et al., 2011). For both total lipids and total collagen content, it must be mentioned that RPD values (1.72 and 1.85, respectively) were close to 2, threshold which defines an acceptable regression model. In addition, in both cases the wavelength selected was 1479-1118 cm^{-1}. With regard to pigment content (%), among oxymyoglobin (OMb), deoxymyoglobin (DMb) and metmyoglobin (MMb) only the reduced myoglobin state (DMb) showed a high coefficient of determination for calibration (over 95 %) although it was noticeably reduced for validation models (R_v^2 < 26 %). The three of them seemed to be related to, at least, the wavelength range 1839-1478 cm^{-1}.

According to the fatty acids, the coefficients values for calibrations were all over 88 %. Nevertheless, after the validation process, linoleic, linolenic, EPA and omega-3 polyunsaturated fatty acids did not reach 60%. The best prediction models, with coefficients of validation over 75 %, were obtained for arachidonic, DPA, DHA (with RPD values over 2) and omega-6 polyunsaturated fatty acids (with a RPD value close to 2). The most repeated selected wavelength ranges were 3838-3277, 2919-2558 and 1839-1118 cm^{-1}. The best treatments were first derivate plus standard normal variate (SNV) and multiplicative scatter correction (MSC). These models are in concordance with the spectra results and with the literature mentioned according to fatty acids. Generally, the healthy properties of the aforementioned fatty acids are well known for humans. Therefore, these results are of great practical importance and they make in evidence the usefulness of FT/MIR spectroscopy technique and the need of gaining deeper knowledge on this research field. It must be mentioned as well, that the vaccenic acid (precursor of conjugated linoleic acid, CLA), reached coefficients of validation of 67.11

% and a RPD value of 1.74. The potential properties of CLA for human health (Dilzer and Park, 2012) supports the need of developing further studies to be able to detect it, qualitative and quantitatively.

According to colour variables (Table 3), GrRGB variables reached high values for the coefficient of determination for calibration (over 90 %) and validation (over 60 %, with the exception of red), whereas the most typical colour values (L*, a*, b*, C*, h*) reached coefficient of determination for calibration and validation below 90 and 22 %, respectively, being h* the worst predicted variable (4.49 %). The selected wavelength ranges for GrRGB colour variables were 3998-3637 and 2198-1118 cm^{-1} and the first derivate + MSC was used as prediction treatment for most of the cases. On the other hand, no enclosed wavelength range were described for L*, a*, b*, C* and h*. The lack of references dealing with the estimation of colour parameters with both NIR and MIR spectroscopy makes impossible a deeper discussion of the results. For WBSF, the prediction results were poor (R^2_v = 25.70 %). WBSF was positively correlated with cooking losses (Domínguez et al., 2018), and meat moisture was also highly and negatively related to intramuscular fat content (r = -0.89; P < 0.01). Thus, as Ripoll et al. (2008) stated, these strong relationships between moisture, fat and cooking losses with WBSF could explain the WBSF behaviour better than its own calibration model. Geesink et al. (2003) did not obtain useful models for WBSF, owing to limited muscle variance (CV: 21%). This fact could be another reason for the weak prediction result in our research (ranged 10-14%). With regard to sensory attributes, coefficients of determination for calibration were poor, being the highest 36.17 % for tenderness. The three sensory variables were negatively correlated to WBSF (tenderness; r = -0.82, P<0.01; juiciness; r = -0.89, P<0.01; overall appraisal; r = -0.66, P<0.05) and the low

accuracy of WBSF prediction could be reflected in the sensory attributes. As it was reported by Ripoll et al. (2008) and Andrés et al. (2007) for sensory traits in beef and lamb respectively, tenderness, juiciness and overall appraisal are hard to estimate as they are subjective judgements and their correlations to other instrumental determinations are weak.

3.3.1. Principal Component and Discriminant Analyses after chemometric analysis

After the results obtained from the chemometric analysis, three different wavelengths ranges were selected according to the prediction accuracy for chemical composition, the studied fatty acids and the quality parameters. These are: 3278-2918 cm^{-1} for the chemical composition variables group, 2919-2558 cm^{-1} for the fatty acids group and 2198-1118 cm^{-1} for the quality parameters group. Taking these ranges into account, canonical discriminant analysis were again carried out in order to improve the first classification obtained before developing the calibration and validation models (Table 4).

Table 4. *Classification matrix from FT/MIR spectrum of 13 and 26-month old Galician Mountain x Burguete crossbreed foals (13M, 26M) and supplemented with standard and linseed concentrate (SC, LC). Discriminant analyses results after selecting the most suitable wavelength range from the prediction models according to chemical composition (a), fatty acids (b) and quality parameters (c).*

		Classify into		Total
(a) Range 3278-2918 cm^{-1}				
		13M	**26M**	
13-month old	%	**63.6**	36.4	100.0
26-month old		29.2	**70.8**	100.0
		SC	**LC**	
Standard Concentrate	%	**56.5**	43.5	100.0
Linseed Concentrate		34.8	**65.2**	100.0
(b) Range 2919-2558 cm^{-1}				
		13M	**26M**	
13-month old	%	**86.4**	13.6	100.0
26-month old		37.5	**62.5**	100.0
		SC	**LC**	
Standard Concentrate	%	--	--	100.0
Linseed Concentrate		--	--	100.0
(c) Range 2198-1118 cm^{-1}				
		13M	**26M**	
13-month old	%	**77.3**	22.7	100.0
26-month old		20.8	**79.2**	100.0
		SC	**LC**	
Standard Concentrate	%	**78.3**	21.7	100.0
Linseed Concentrate		34.8	**65.2**	100.0

--: Any possible samples' clssification according to finishing diet with that selected range.

It could be stated that the best wavelength range in order to classify samples according to slaughter age and finishing diet is the one which better predicts the quality parameters: 2198-1118 cm^{-1}. Regarding slaughter age, samples were classified with an accuracy around 78.3 % whereas regarding finishing diet, samples were classified with

accuracy around 71.8 %. Figure 2 shows the distribution obtained for the samples after selecting the range aforementioned (2198-1118 cm^{-1}). When comparing the first classification to the aforementioned new one, it could be highlighted that the classification percentages of the samples were improved for both slaughter age and finishing diet: according to slaughter age, with an increase of 5.1 and 4.2 % of well-classified for 13M and 26M samples, respectively. Regarding finishing diet, this classification improvement was made evident by an increase of 34.8 and 13.0 % of well-classified for SC and LC samples, respectively.

4. Conclusion

The spectra showed some remarkable differences mainly due to the fat and the fatty acid composition. Prediction models were not reliable for most of the studied variables, but they were accurate for moisture, collagen and lipids, most of the fatty acids and for the GrRGB colour coordinates. Classifications by discriminant analysis showed good results after the use of regression treatments delimiting the range of the spectrum to 2198-1118 cm^{-1} and coinciding with the wavelengths in which a large number of protein and fatty acid bonds are recognized. From the commercial point of view, given the results, the use of MIR could help the accurate sale of "old or young" meat. Less precision was achieved according to the animals feeding. Considering the potential of this technique, more studies are needed in order to improve the estimation of meat characteristics. Nevertheless, the estimation of the sensorial properties is very complex and would require a deeper study of the spectrum. Since the present study intends to be a preliminary study and an approach to know the possibilities of this technique application , for future researches it would be necessary to use a larger sample number

and study other physical characteristics such as protein denaturation or sarcomere length which could help to understand changes in the bonds.

Author contributions

M. Ruiz performed the statistical analysis of the data and drafted the manuscript. K. Insausti joined to M.J. Beriain and M.V. Sarriés designed the experimental study. M.J. Beriain and M. Ruiz were in charge of samples preparation and instrumental measurements. J.M. Lorenzo dealt with the chemical composition analyses. M.J. Beriain developed the chemometrics analysis. M. Ruiz, K. Insausti, M.J. Beriain and M.V. Sarriés participated in the interpretation of data and performed the critical revision of the manuscript.

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GENERAL DISCUSSION

This PhD thesis has focused on the study of foal meat throughout the whole production chain from the animals' slaughter to the consumption of their meat. Foal meat is different from other types of meat production for several reasons among which, sustainability can be highlighted. Nevertheless, there is a lack of uniformity and standardization in the carcass and horsemeat production. From a commercial point of view, it is therefore necessary to develop research studies to help characterise livestock production and define the quality of the carcass and meat obtained in order to (1) provide the consumer with a quality product with an accurate description and (2) standardise and integrate the value chain of the equine meat sector. The global objective of the present PhD thesis was to assess the effect of slaughter age (13 vs. 26 months) and finishing diet (standard vs. 5% linseed enriched concentrate) on carcass and meat quality from GMxB foals, followed by a consumers' preferences study.

Therefore, according to the first objective of the present PhD thesis, the slaughter age was the production factor that more influenced the carcass quality. The carcasses from animals later slaughtered showed better conformation degree of fatness. From the commercial point of view, it is very interesting as the prime cuts obtained show higher percentages, above all, of those from the hindquarter, where the most valuables ones are located. By contrast, the meat:bone ratio did not improve with age, what means that the aptitude for meat production was not favoured by an increase in the slaughter age in spite of being twice as old. The first responsible was the amount of subcutaneous fat covering the carcasses which was almost 3 times higher in the older group. Comparing to the original breeds, the dressing percentages (52.7-54.7%) of this work were slightly higher with respect to those of Galician Mountain (around 51%) but not respect to Burguete breed (over than 63 %). On the other hand, the ratio

meat:bone of the present work was very similar to those obtained in GM foals from slaughtered at the age of 15 months ("*2.4. Foal carcass quality*" section). Thus, these results induces the necessity to develop further studied in order to find the optimal slaughter age. According to carcass grading, the tissue composition explained the 36 and 33% of the total variability for degree of fatness and conformation scores, what means the great relevance for industries to establish grading systems. Far from the slaughter age effect, the inclusion of linseed concentrate (5%) in the finishing diet was not enough to show remarkable differences on the carcass quality. Thus, an increase of the linseed percentage or an extension of the finishing period would be necessary in futures studies.

The second objective involved the characterization of foal meat and the determination of its quality evolution under different experimental conditions of ageing and storage time; first with Burguete foals and secondly with the Galician Mountain x Burguete crossbred (GMxB) foals.

Burguete is a commercial and heavy breed autochthonous from Navarra, which shows a high rusticity. Their conformation and dressing percentages make it be very suitable for meat production. It is worth highlighting that this meat showed a high amount of myoglobin and total iron. Regarding the ageing time, 7 days of ageing favoured the reddening and the tenderization (Sarriés and Beriain, 2006; Shackelford et al., 1991); although it should be remarked that non-aged meat was, nearly, "very tender" (3.37 Kg). The ageing process also induced a slight lipid oxidation and thus, a smooth meat odour intensification. Nevertheless, none of them reached the threshold considered for meat degradation (2 mg MDA/Kg fresh meat, for lipid oxidation; 75 mm, for sensory attributes). By contrast, sensory colour kept similar for both aged and non-aged meat. These results bright to light 2 interpretations, (1) an ageing process inside the loin could preserve the fresh meat properties during 7 days, or (2) an ageing process could not be necessary as meat did not need to get tender, which is the main purpose of an ageing process.

On the other hand, once the loin was sliced and steaks were placed in trays overwrapped with PVC permeable to O_2, the oxidation reactions rapidly increased during the storage time reducing the sensory colour stability, and consequently, reducing the storage time of foal meat below than 3 days. This happened for both non-aged and aged meat, and could be related to the high polyunsaturated fatty acids and myoglobin content, which are very susceptible to oxidation reaction (Zakrys et al., 2008). So that, the vacuum packaging could be more appropriated for this kind of meat (Gómez and Lorenzo, 2012; Lorenzo and Gómez, 2012). The work that was conducted with Burguete foals show that it was not necessary to age foal meat because after 24 hours it was tender enough to make it commercially attractive. Nevertheless, the aging in the whole piece for 7 days did not reduce its storage time when it was sliced and packaged for sale remaining its acceptable quality until 3 days with an overwrap packaging. The results obtained in this work, would let change the ageing and storage conditions of the meat from the GMxB foals.

Regarding the meat quality of GMxB foals, the slaughter age showed an important effect over all meat variables set, whereas the finishing diet had a minor impact. According to chemical composition, most of the parameters fell within the range shown in "*2.5.1.1. Chemical composition*" section. The most remarkable difference was due to the fat content, which was 3 times higher in the older group. Sarriés and Beriain (2005) also observed higher fat content in animals slaughter at a later age. Nevertheless, the fat values reported for GMxB foals (< 1.72 %), are far from those described in previous works of Burguete breed (3-5.2 %) (Ruiz et al., 2018; Sarriés and Beriain, 2005). These differences between breeds could be understandable since Burguete foals are, from many years, animals highly adapted to rustic areas that have developed a high capacity of energy reserves, whereas Galician Mountain is a local and small-sized breed less developed. Regarding fatty acids, PUFA and MUFA (g/100 g total fatty acids) were the most abundant in meat from the young and adult group, respectively (Domínguez et al., 2018). Therefore, concentration of PUFA decreased as slaughter age increased (Sarriés et al., 2006). On

the other hand, the linseed concentration could not be high enough to find differences in fatty acid profile. It should be also highlight the contribution of LA (linoleic acid) and ALA (α-linoleic acid) to the nutritional recommendation for humans' health as it has been suggested in "Chapter 2". The fatty acid LA was the predominant n-6 contributing around 87.5 % of total n-6 in IM tissue. A ration of 200 g of meat from foals slaughtered at the age of 13 and 26 months, would supply 204 and 412 mg of LA mg, respectively. Thus, the absolute contribution of such foal meat to the recommended consumption of LA is small (17 and 12 g/d for men and women, respectively: Institute of Medicine Food and Nutrition, 2002). In terms of n-3 fatty acids, the fatty acid ALA was the predominant n-3 contributing around 80% of total n-3 in IM tissue. In this case, a ration of 200 g of meat from foals slaughtered at the age of 13 and 26 months, would supply 127 and 395 mg of ALA, respectively. The absolute contribution of such foal meat to the recommended consumption of ALA is very important as this amount of ALA could cover 25% of the recommended nutritional needs (1.6 and 1.1 g/d for men and women, respectively: Institute of Medicine Food and Nutrition, 2002). According to amino acids content, both the increase in slaughter age and the inclusion of linseed in the diet had very low effect (Domínguez et al., 2018), as reported previous authors (Domínguez et al., 2015; Lorenzo et al., 2014b; Polidori et al., 2015).

Regarding colour parameters, a later slaughter age and the inclusion of linseed concentrate in the finishing diet resulted in darker and redder meat (Sarriés and Beriain, 2006). Both the darkening and the reddening might be related to the longer pasture period, higher grass intake and greater physical activity of the adult foals. According to previous works shown in "*2.5.1.3. Colour*" section, lightness values of meat from GMxB foals were similar to those reported for Burguete foals whereas redness values were close to those reported for Galician Mountain foals. With respect to texture, the earlier slaughter age and the linseed concentrate favoured the tenderness of cooked meat. It could be due to the higher soluble collagen content of meat samples from the younger foals. Similar results were found by Ruiz et al. (2018) in meat

from 16-month old Burguete, whereas other studies did not found any differences (Franco et al., 2011a, 2011c; Sarriés and Beriain, 2006). Tenderness values are close to those of Burguete and far from those of Galician Mountain whose meat is twice tender (*"2.5.1.4. Texture"* section). In this case, the collagen and fibres characteristics of GMxB foals might be similar to those of Burguete breed. Overall, an increase in the slaughter age seemed to show relevant differences in meat quality, whereas the inclusion of linseed (5%) in the finishing diet seemed to have a minor impact. This fact can be shown in figure 8 that means that the obtained meat could be distinguished depending on if it comes from young or adult animals.

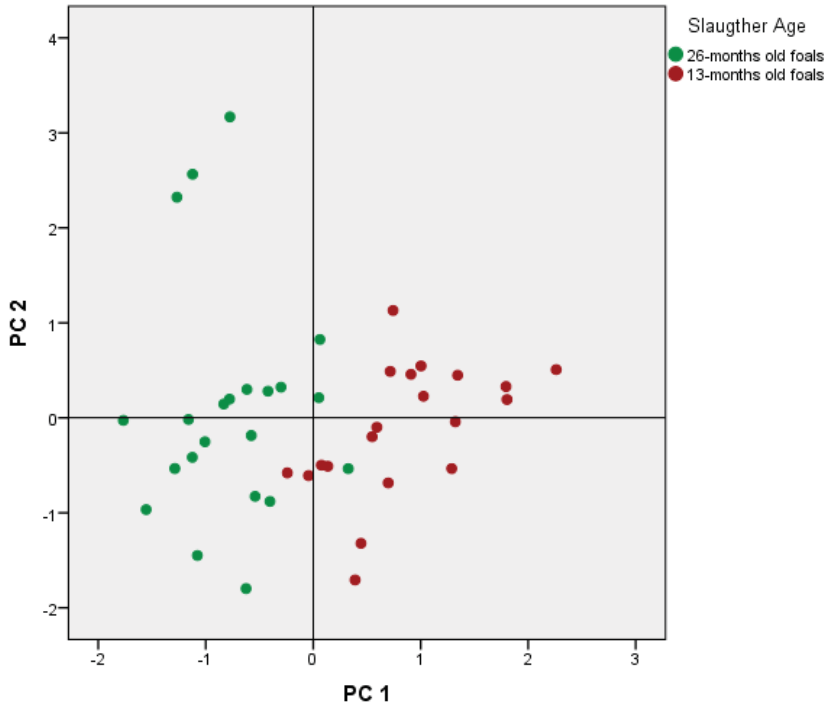

Figure 8. Plot of slaughter age on the bidimensional space formed by components 1 and 2 obtained by the principal component analysis of physical–chemical variables.

Once the meat was characterised 24 hours *post-mortem*, its quality evolution was studied throughout the storage time. From the commercial point of view, texture and colour are the two main quality attributes at the purchase and repurchase moment. During the storage time, in general, meat from the younger group was lighter and less red than the older group. Regarding finishing diet, redness was similar in meat from animals supplemented with standard

concentrate (17.0 - 21.0), whereas the inclusion of linseed concentrate made the meat from adults, redder (21.7 - 23.0) than that of the younger foals (15.5 - 18.5). All these results are noticeable higher than those obtained in 16-month old Burguete foals during the storage time (a*: 12 - 8) (Ruiz et al., 2018). According to Ruiz et al. (2016), the meat, vacuum packed, from the GMxB foals kept the "characteristic colour" and was defined as "*Intense or not intense red colour, with or without brightness and even smoothly brownish*" during the entire storage time (12 days). Nevertheless, the meat overwrapped packed from Burguete foals turned into "non-characteristic" after 3 days. This fact could be related to the different metmyoglobin percentages, and implies with no doubt that, the employment of vacuum instead of overwrap packaging favoured the colour preservation. From the texture point of view, meat quality improved during the first 4 days. Nevertheless, the majority of meat samples from young foals (80%) needed 8 days whereas the majority from adult foals needed 12 days to reach the best degree of tenderness ("very tender") (Sarriés and Beriain, 2006; Shackelford et al., 1991).

The colour and texture degradation occurs, above all, due to oxidation processes as lipid and protein oxidation. During the storage time, lipid oxidation increased due to the slaughter age and finishing diet. It was higher in meat from the younger group, perhaps due to their high amount of PUFA content (Domínguez et al., 2018). Nevertheless, this oxidation increase did not reach the rancidity threshold (2 mg MDA/ kg fresh meat) proposed by Campo et al. (2006). In meat from Burguete foals, this threshold was almost exceeded after 9 days under overwrap conditions (Ruiz et al., 2018). It was the vacuum packaging which reduced the meat oxidation (dos Santos et al., 2015). Regarding protein oxidation, the carbonyl mean value of the whole collection of GMxB foal samples, was approximately 3.75 nmol/mg protein, felling within the range of oxidized tissue 2 to 14 nmol/mg protein (Lund et al., 2007). This means that vacuum packaging could not avoid the oxidation of proteins during storage but reduce considerably its impact. Once the meat was cooked, the trained panel barely showed different descriptors values by slaughter age and finishing diet. The meat from the younger group showed lower

characteristic odour and was less juicy, and it could be related to the lower fat content of the younger foals. Regarding finishing diet, the meat was juicier in samples from animal supplemented with linseed concentrate. In this case, it could be related to the lower cooking loss values (%) of the linseed group (Domínguez et al., 2018).

All these results let see that, both kinds of meat showed good but different quality properties that should be offered as different products in the market. The development of "Quality Labels" would help the equine sector to strengthen the value chain of foal meat, offering meat perfectly characterised.

Nevertheless, no consumers' studies have been carried out with foal meat in order to know the consumers perception. Thus, following with the third objective, consumer panellists were assigned to one of two different experimental scenarios based on the amount of product information disclosed (1: blind test, 2: information of the origin, rearing and slaughter of the breeds). In general, finishing diet did not influenced the foal meat evaluation and slaughter age just affected the juiciness and tenderness evaluation. Consumers considered meat from the younger group juicier and more tender than meat from the older foals. Thus, juiciness and tenderness turned into the most important clues for foal meat acceptance. The influence of information level was different depending on the city. Tasters from Pamplona better evaluated the meat when information about the product was given. By contrast, the degree of information was not important for consumers in Ourense as they did not change their evaluation scores. This could be due to the lack of knowledge about foal meat, their perception, or the consumption data: 55% and 85% from Pamplona and Ourense, respectively, never consume foal meat. These results support the importance of promotion strategies of foal meat, as "Quality Label" or similar, as it has been aforementioned, to help publish this meat in a defined way. The relation among consumer and trained panel's answers and chemical composition is unclear and shows a high variability that takes place in foal meat. The meat description by the trained panel and the

meat evaluation by consumers are not in concordance Thus, further sensory studies are needed in order to reduce these differences and know the perception of consumers.

At present, most of the techniques employed in meat determination are destructive, slow and complex to use. During the last two decades, the development of fast, accurate, easy to use, non-destructive, and environmentally sustainable techniques is increasing more and more as alternative to the conventional techniques (de Oliveira et al., 2014; Lozano et al., 2017; Sarriés et al., 2015). In this sense, MIR spectroscopy is one of the most interesting techniques as it provides information on a very large number of analytes, and the absorption bands are sensitive to the physical and chemical states of individual constituents (Al-Jowder et al., 1999). Among all the kind of food products, the application of MIR spectroscopy has been very limited in meat. The spectrum obtained for the samples from the young and adult foals, and supplemented with standard and linseed concentrate let see some remarkable differences in the ranges 2960-2830 cm^{-1} and 1800-1680 cm^{-1}. Nevertheless, peaks related to proteins (amino vibrations) did not shown any relevant difference. Chemometric analyses were performance and moisture and total lipids content were well predicted with coefficient of validation (R_v^2) of 82 and 66 %, respectively. Nevertheless, the ratio of prediction to deviation for chemical composition was generally poor with regard to validation results, except for moisture and total lipids content and total collagen. Several fatty acids were also well predicted with R_v^2 values over 65 % (arachidonic, vaccenic, DPA and DHA acids). Regarding quality parameters, just grease, red, green, blue coordinates were well predicted (R_c^2 = 90 %; R_v^2 > 60 %). Low prediction coefficients were also obtained for sensory attributes (juiciness, tenderness and overall appraisal. Andrés et al. (2007) and Ripoll et al. (2008) stated that it is hard to estimate sensory traits as they are subjective judgements and their correlations to other instrumental determinations are weak. The wavelength range 2198-1118 cm^{-1} was the best to classify samples according to slaughter age and finishing diet. Regarding slaughter age, samples were classified with an accuracy around

78.3 % whereas regarding finishing diet, samples were classified with accuracy around 71.8 %. From these results, it could be said that MIR spectroscopy shows a great potential, at least for distinguishing different kinds of meat from the same breed, but with different characteristics. It is worth, therefore, going on researching the employment of spectroscopy techniques as sustainable alternatives.

Nowadays, much of the research activity is focused on the production of sustainable and safe food to offer consumers quality products. In this sense, foal meat has a number of characteristics that meet the quality standards of a product. Nevertheless, the great diversity of animal management and the social perception towards its consumption make it necessary to promote studies to answer these questions. In this line of improvement and progress in the sector, this PhD thesis has studied the foal meat production chain and has made it available to consumers for evaluation. In general terms, the inclusion of linseed (5%) in the finishing diet of the foals has shown a minor effect throughout the whole study and the slaughter age has been the most relevant factor both in the quality of the carcass and the meat and in the assessment of the consumers. Foal meat is a quality and sustainable product, whose disclosure would favour the enhancement of the equine sector. The results shown in this work bring to light the necessity of developing an integrate quality system based on a quality categorization and the future implementation of a standardised and competitive production (Figure 9).

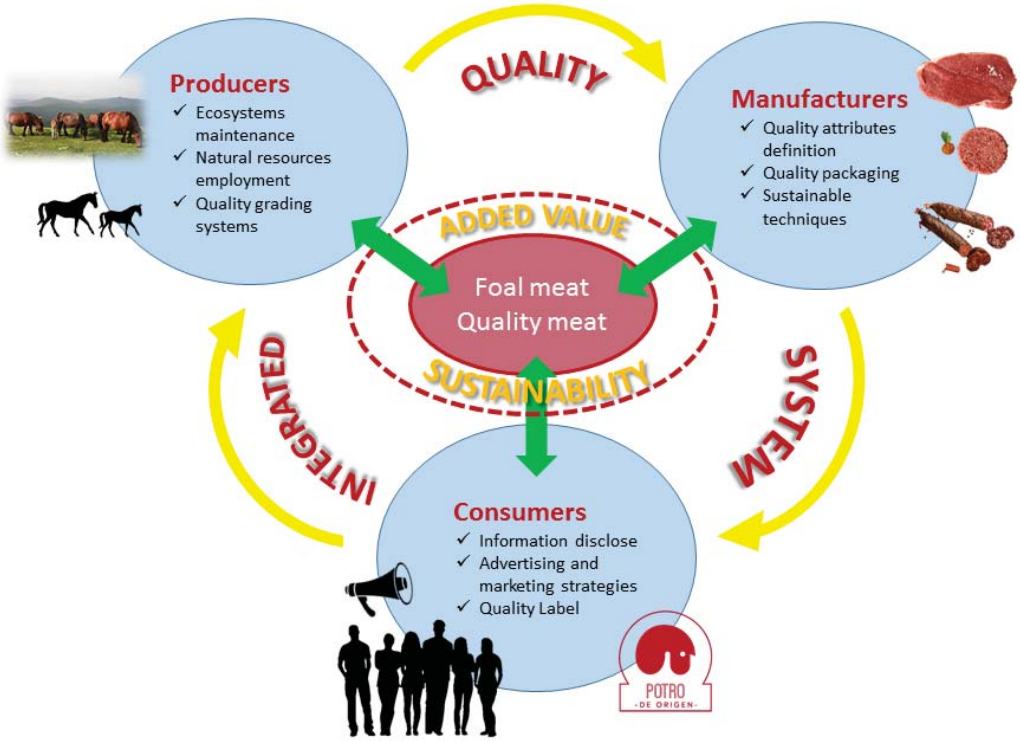

Figure 9. *Future purpose achievement for chain production of foal meat*

Finally, despite the fact that both production and consumption of foal meat are still in the minority today, the world population growth estimated for 2050 will exceed 9,000 million, and therefore the increase in protein demand. For this reason, it should be noted that foal meat production may look very interesting for the future as a source of choice for obtaining high biological value protein of animal origin. This can give added value to the equine aptitude for meat production as well as to the transformation, commercialization and finally to the consumption. In this respect, it is necessary to encourage consumer acceptance of this product in order to enrich the supply of meat on the markets with a healthy and sustainable food product.

CHAPTER 6. CONCLUSIONS

From this research conducted on the quality chain of foal meat production under the applied experimental conditions, the following conclusions are drawn:

1) According to the carcass classification, it is recommended to slaughter the foals at the age of 26 instead of 13 months, since the carcasses conformation and degree of fatness are higher classified in adults. However, this has not been followed by an improvement in the aptitude for meat production since the M:B ratios were similar between both groups of animals, despite the slight improvement of the dressing percentages achieved (2.5%). The inclusion of a 5% linseed-enriched concentrate in the finishing diet would not be an alternative to the standard concentrate in terms of carcass quality. For futures studies a higher amount of linseed or a longer fattening period should be recommended.

2) Taking into account the prediction models that showed the high relationship between the carcass grading scores and the tissue composition percentage, the improvement of grading systems and its accurate employment would be highly recommended for industries in order to predict the carcass quality and benefit, therefore, the value of the production chain of foal meat.

3) Regarding foal meat quality from Burguete foals, an ageing process of the loin in whole piece could not be necessary due to the suitable meat tenderness 24 hours *post-mortem*. Although after 7 days of ageing the loin piece, the meat goes on maintaining their quality properties. This fact means that once the loin piece is provided to the sales' points, the meat endures, at least, 7 days. Nevertheless, once the loin is sliced and the steaks are preserved under overwrapping conditions, the storage time is limited up to 3 days because its colour begins to be rejected from that moment on, due to an intense

lipid and myoglobin oxidation, resulting in the meat degradation. Even though, new studies dealing with ageing time are necessary, as this process is still not well defined.

4) In terms of slaughter age, the meat from 26-month old Galician Mountain x Burguete crossbred foals is darker (lower L* values) and redder (higher a* values) and, shows a intramuscular fat content 3 times higher than that from 13-month old foals. Moreover, the higher shear force (WBSF) obtained in meat from adult foals, supports the characteristic consistency of meat typical of matured animals. Besides, this fact would remind of the important role played by the collagen, especially the soluble fraction, which, in this case is 40% reduced in the meat of adult foals. Regarding finishing diet, the inclusion of 5% linseed did not reflect so many differences. Only the redness (a*) tended to increase in the meat from animals supplemented with linseed concentrate while it tended to decrease in the meat from foals supplemented with standard concentrate. It would be necessary to develop new works with an increase in the percentage of linseed and to check whether its effect produces an improvement in the commercial value of the meat.

5) Taking into account the sensory description of the trained panel, the foal meat from adult foals had a more intense smell and was juicier than that from young foals. Only the fibrousness was conversely evaluated in adults and young depending on the finishing diet, as it decreased in young foals and increased in adult foals due to the linseed concentrate. The similarity perceived for the rest of the sensory attributes (characteristic flavour, fat odour and flavour, tenderness, greasiness) could be related to the low intramuscular fat content shown in meat from both 13 and 26-month old foals (0.56, 1.72%, respectively), that are far from the fat percentage from which sensory differences begin to be perceived.

6) Regarding storage time, the meat from Galician Mountain x Burguete foals could be preserved, at least, during 12 days under vacuum conditions as the lipid and protein oxidation levels were below the degradation states, and consequently, colour and texture maintained their quality properties. Thus, vacuum packaging would be a good system to extend the commercial life of foal meat. Even so, it would be interesting to develop new studies dealing with this commercial life of foal meat once the vacuum is broken. The study of other alternatives as active packaging would be also worthwhile.

7) According to consumers' evaluation, the meat from young foals was perceived more tender and juicier, resulting in a higher acceptability than that from adult foals. In contrast, the finishing diet did not contribute to its differentiation. Nevertheless, the parameters for quality evaluation in the value chain of foal meat are different from producers and consumers. For the first group, quality increases with slaughter age, but for the second group, meat tends to be of higher quality in the group of young animals. Therefore, this fact highlights the controversy between commercial and consumer interests.

8) Regarding consumers' behaviour, the information disclosure about the animals' origin and breeding influenced the evaluation of foal meat in a different way according to the region; the consumers of Pamplona better evaluated the meat when the information was provided to them, whereas this information was indifferent to the assessment for the consumers of Ourense. This may be related to the different geographical location of the regions and frequency of consumption in them. These factors should be taken into account for future commercial strategies, and a broader knowledge of this field will be very useful for the different links in the foal production chain (seller, manufacturer, producer).

9) Mid-infrared spectroscopy has made it possible to classify groups of meat with different characteristics from the Galician Mountain x Burguete crossbred foals, especially due to the spectral measurements of fatty acids and the wavelength range comprising the colour parameters after the development of the prediction models. This is an important achieve as it opens up the possibility of future applications in the meat sector. Nevertheless, further studies with a higher number of samples, should be necessary to obtain more accurate estimation models.

10) As a general conclusion and from the commercial point of view, a huge part of the work to promote the production of foal meat depends on advertising and marketing strategies. One of the future commercial strategies might be linked to a "Quality Mark or Label". A Quality Mark is always associated with a quality product that meets with healthy and safe characteristics. Foal meat meets the requirements and, what is more, it offers an added value: sustainability. For this purpose, it would be very useful the development of quality systems in which all quality parameters are taken into account, throughout the whole production chain of foal meat

CONCLUSIONES

A partir de esta investigación realizada sobre la cadena de calidad de la producción de carne de potro Y bajo las condiciones experimentales utilizadas, se obtienen las siguientes conclusiones:

1) Según la clasificación de las canales, se recomendaría sacrificar a los potros con 26 en lugar de con 13 meses de edad, ya que la conformación y el grado de engrasamiento de las canales es mayor en los adultos. Sin embargo, esto no ha conllevado una mejora en la aptitud cárnica, ya que la relación carne:hueso fue similar entre ambos grupos de animales, a pesar de la ligera mejora en el rendimiento de la canal (2,5%). La inclusión de un concentrado enriquecido con lino al 5% en la dieta de acabado no sería una alternativa al concentrado estándar en términos de calidad de la canal. Para estudios futuros se recomendaría una mayor cantidad de lino o un período de cebo más largo.

2) Teniendo en cuenta los modelos de predicción y la alta relación mostrada entre los valores de clasificación de la canal y el porcentaje de composición tisular, la mejora de los sistemas de clasificación y su empleo preciso sería especialmente recomendable para las industrias con el fin de definir la calidad de la canal y beneficiar, por tanto, la cadena de valor de la producción de la carne de potro.

3) En cuanto a la calidad de la carne procedente de potros Burguete, un proceso de maduración del lomo en pieza entera podría no ser necesario debido a la adecuada terneza de la carne 24 horas post-mortem; si bien tras 7 días de maduración de la pieza de lomo, la carne sigue manteniendo sus propiedades de calidad. Esto significa que una vez que la pieza de lomo es distribuida a los puntos de venta, la carne tendría una

durabilidad mínima de 7 días. No obstante, una vez cortado el lomo y conservados los filetes en condiciones de envoltura, el tiempo de conservación se acorta a 3 días, ya que a partir de ese momento su color empieza a ser rechazado, debido a una intensa oxidación de lípidos y mioglobina que provocan la degradación de la carne. No obstante, son necesarios nuevos estudios sobre el tiempo de maduración, ya que este proceso todavía no está bien definido.

4) En cuanto a la edad de sacrificio, la carne procedente de los potros cruzados Gallego de Monte x Burguete de 26 meses de edad es más oscura (valores L* inferiores) y más roja (valores a* superiores) y presenta un contenido de grasa intramuscular 3 veces superior al de los potros de 13 meses de edad. Además, la mayor fuerza de corte (WBSF) obtenida en la carne de potros adultos, apoya la consistencia característica de la carne, típica de los animales maduros. Además, este hecho nos recuerda el importante papel que juega el colágeno, especialmente la fracción soluble, que en este caso se reduce en un 40% en la carne de los potros adultos. En cuanto a la dieta de acabado, la inclusión del 5% de lino no reflejó tantas diferencias. Sólo el enrojecimiento (a*) tendió a aumentar en la carne de los animales suplementados con concentrado de lino mientras que tendió a disminuir en la carne de los potros suplementados con concentrado estándar. Sería necesario desarrollar nuevos estudios con un aumento del porcentaje de lino y comprobar si su efecto produce una mejora en el valor comercial de la carne.

5) Teniendo en cuenta la descripción sensorial del panel entrenado, la carne procedente de los potros adultos tuvo un olor más intenso y fue más jugosa que la de los potros jóvenes. Sólo la fibrosidad fue evaluada de manera inversa en adultos y jóvenes dependiendo de la dieta de acabado, ya que disminuyó en potros jóvenes y aumentó en potros adultos debido al concentrado de lino. La similitud observada para el resto de los atributos sensoriales (sabor característico, olor y sabor de la grasa, terneza, untuosidad)

podría estar relacionada con el bajo contenido de grasa intramuscular que presenta la carne tanto de potros de 13 meses como de 26 meses de edad (0,56, 1,72%, respectivamente), que están muy alejados del porcentaje de grasa a partir del cual se empiezan a percibir las diferencias sensoriales.

6) En cuanto al tiempo de conservación, la carne procedente de los potros cruzados Gallego de Monte x Burguete pudo ser conservada, al menos, durante 12 días al vacío, ya que los niveles de oxidación de lípidos y proteínas se hallaban por debajo de los estados de degradación, y por lo tanto el color y la textura mantuvieron sus propiedades de calidad. Es por ello que el envasado al vacío sería un buen sistema para prolongar la vida comercial de la carne de potro. Aun así, sería interesante desarrollar nuevos estudios sobre dicha vida comercial una vez roto el vacío. También sería de interés el estudio de otras alternativas como los envasados activos.

7) Según la evaluación de los consumidores, la carne procedente de potros jóvenes se percibió más tierna y jugosa, lo que resultó en una aceptabilidad mayor que la carne de los potros adultos. Por el contrario, la dieta de acabado no contribuyó a su diferenciación. No obstante, los parámetros para la evaluación de la calidad en la cadena de valor de la carne de potro son muy distintos entre los productores y los consumidores. Para el primer grupo, la calidad aumenta con la edad de sacrificio, pero para el segundo grupo, la carne tiende a ser de mayor calidad en el grupo de animales jóvenes. Por lo tanto, este hecho pone de manifiesto la controversia entre los intereses comerciales y los de los consumidores.

8) En cuanto al comportamiento de los consumidores, la difusión de información sobre el origen y la cría de los animales influyó en la valoración de la carne de potro de forma diferente según las regiones; los consumidores de Pamplona evaluaron mejor la carne cuando se les proporcionó la información, mientras que esta información fue indiferente

en la valoración de los consumidores de Ourense. Esto puede estar relacionado con la diferente ubicación geográfica de las regiones y la frecuencia del consumo en las mismas. Estos factores deben tenerse en cuenta para futuras estrategias comerciales, y un conocimiento más amplio de este campo sería muy conveniente para los diferentes eslabones de la cadena de producción de carne de potro (vendedor, transformador, productor).

9) La espectroscopia de infrarrojo medio ha permitido clasificar grupos de carne con características diferentes, procedente de los potros cruzados Gallego de Monte x Burguete, especialmente debido a las determinaciones espectrales de ácidos grasos y al rango de longitud de onda que comprenden los parámetros de color tras el desarrollo de los modelos de predicción. Éste es un avance importante, ya que abre la posibilidad de futuras aplicaciones en el sector cárnico. Sin embargo, serán necesarios estudios posteriores con un mayor número de muestras para obtener modelos de estimación más precisos.

10) Como conclusión general y desde un punto de vista comercial, gran parte del trabajo para fomentar la producción de carne de potro depende de estrategias de publicidad y marketing. Una de las futuras estrategias comerciales podría estar vinculada a una "Marca de calidad". Una Marca de calidad siempre se asocia con un producto de calidad que reúne características saludables y seguras. La carne de potro cumple con los requisitos y, además, ofrece un valor añadido: la sostenibilidad. Por este motivo, sería muy útil el desarrollo de sistemas de calidad en los que se tengan en cuenta todos los parámetros de calidad, a lo largo de toda la cadena de producción de la carne de potro.

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